

Yearbook of Paediatric Endocrinology 2019

Editors

Ken Ong

Ze'ev Hochberg



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Preface

This Yearbook provides the 2018–19 highlights in paediatric endocrine science. As of last year, we are online at <http://www.espeyearbook.org/> and continue to experiment with the best way to publish the Yearbook. Those of you who prefer to read a hard copy can now purchase the book at Amazon for a nominal fee.

The enormous advances in modern medical science are based on new knowledge and concepts in the diverse fields of clinical diagnosis and treatment, genetics and genomics, innate immunology, molecular biology, systems biology, population genetics, proteomics and metabolomics, evolutionary biology – all of these require our attention. Understanding of the nature and mechanisms of disease, improved management and new therapies depend on such multi-disciplinary considerations.

Among other highlights, the Yearbook 2019 describes:

- **New treatments:** long-term methimazole in Graves' disease; FGF23 antibody treatment in XLH; oestrogen versus androgen in complete androgen insensitivity syndrome; Letrozole in boys with constitutional delay of growth and puberty; psychosocial benefits of insulin pump therapy; liraglutide in adolescents with T2DM; orlistat in Type 1 hyperlipoproteinemia; a novel non-steroidal androgen-receptor antagonist;
- **New genes:** high prevalence of growth plate gene variants in familial short stature; genetics of human thinness; *KDM6A* in congenital hyperinsulinism; *IRS4* in central hypothyroidism; microRNA-140 in skeletal dysplasia; *DNMT3A* in microcephalic dwarfism; *ESR2* in ovarian and pubertal failure;
- **New mechanisms:** primary cilia in thyroid follicle function; stem cells in the growth plate; RANKL reverse signaling; epigenetic repression of the androgen receptor; *BRC42* in ovary development and function; SIRT1 and AMPK link obesity to puberty timing; genome amplification and cellular senescence in placental development; late-pregnancy dysglycemia in obese mothers;
- **Other insights:** single-cell profiling the hypothalamus; vitamin D-binding protein deficiency; prevalence of cranial MRI findings in girls with precocious puberty; adrenarche hormones associated with anxiety; excess mortality and cardiovascular disease in type 1 diabetes; clinician personality influences T1DM outcomes; improved infant nutrition and adult cardiometabolic disease in rural Guatemala; copeptin to diagnose diabetes insipidus.

Among important therapeutic developments this year is Onpatro (Patisiran) that became the first RNA interference drug to win US FDA approval. The drug works by silencing the gene that is responsible for hereditary transthyretin-mediated amyloidosis, which cause problems with heart and nerve function (see paper 14.2). Orilissa (Elagolix) became the first oral gonadotropin-releasing hormone (GnRH) antagonist to win FDA approval (see 10.1530/ey.15.15.6). An oral medication reduced liver fat in a phase 2 trial in non-alcoholic steatohepatitis NASH patients; MGL-3196 is a thyroid hormone receptor β -selective agonist and works by reducing cholesterol levels.

We are grateful to our 13 Associate Editors and their coauthors, who have done an enormous work to discern this year's advances and provide their chapters in a timely fashion. We thank Outi Mäkitie and Olle Söder, who have been with us for 9 and 11 years, respectively, and their teams for their excellent previous contributions. We welcome Ola Nilsson and Christa Flück as new Associate Editors. We are grateful to ESPE for their continuing endorsement and support of the Yearbook series and to Bioscientifica for their professional and efficient work to produce this edition.

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1. Pituitary and Neuroendocrinology

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Preface

This year, many new mechanisms and new concepts have been described in neuroendocrinology. Investigating physiology or pathogenic mechanisms but also developing biological markers of neuroendocrine dysfunctions in humans is very challenging. Several strategies are needed: dosage of neuropeptides in the cerebrospinal fluid, genome-wide association studies in very large population studies, classical genetic investigation in mice but also studies on invertebrates to understand how neuroendocrine mechanisms have been conserved. All of these approaches contribute to the understanding of neuroendocrine regulations. We also report in this chapter an update on Kisspeptin neurons. With the improvement of techniques to investigate neuronal activity, and also to control cell activity in hypothalamic neurons, it is now possible to precisely dissect a group of neurons at different stages of development or after birth. Kisspeptin neurons represent one example of the power of these approaches.

1.1. Genome-wide association analyses of chronotype in 697,828 individuals provides insights into circadian rhythms

Jones SE, Lane JM, Wood AR, van Hees VT, Tyrrell J, Beaumont RN, Jeffries AR, Dashti HS, Hillsdon M, Ruth KS, Tuke MA, Yaghoobkar H, Sharp SA, Jie Y, Thompson WD, Harrison JW, Dawes A, Byrne EM, Tiemeier H, Allebrandt KV, Bowden J, Ray DW, Freathy RM, Murray A, Mazzotti DR, Gehrman PR, Lawlor DA, Frayling TM, Rutter MK, Hinds DA, Saxena R, Weedon MN

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Nat Commun 2019;10:343

<https://www.ncbi.nlm.nih.gov/pubmed/?term=30696823>

This paper reports many loci associated with chronotype (i.e. being a ‘morning person’ or ‘evening person’) in a genome-wide association study (GWAS) of 697,828 participants from the UK Biobank and 23andMe cohorts. The link between chronotype and sleep timing and quality is well known. It was therefore interesting to assess whether these genes were also associated to sleep timing and/or quality.

Interestingly, the 351 loci identified for sleep timing were not also associated with sleep duration or quality. A detailed analysis of biological pathways enriched for genes at associated loci revealed a strong enrichment in circadian rhythm and circadian clock pathways. Interestingly, these genes also participate in pathways involved in neuronal connections and neurogenesis. For instance, among the genes that influence chronotype was vasoactive-intestinal peptide, a gene expressed in the suprachiasmatic nucleus which is a specific region of the hypothalamus involved in the control of the circadian rhythm. Also MADD, which regulates activation control of the Rab3 pathway, involved in the control of synaptic homeostasis, a fundamental neuronal process during sleep, which seems to be crucial for memory in mice.

In the last part of this complex study, the authors sought to define genetic loci which overlap between chronotype and other traits and diseases. The idea behind was to propose new hypothesis to understand the known association between alterations to circadian timing and disease development such as metabolic and psychiatric diseases. The strongest genetic correlation was observed with subjective well-being. Furthermore, the evening chronotype was positively correlated with schizophrenia, depressive symptoms, major depressive disorder and intelligence. There was no evidence for a direct effect of schizophrenia on chronotype, but rather the genetic correlation indicated that these two conditions share common biological pathways.

This GWAS approach represents one way to propose new hypotheses to understand the physiological control of circadian rhythm. In addition to confirming that clock genes are involved in being a ‘morning’ or an ‘evening’ person, as previously reported, it also defined many additional genes in the control of circadian rhythm. Finally, the association between certain chronotypes and psychiatric diseases was well known by epidemiological studies. This work brings new insights to understand this association at the molecular level.

1.2. Cerebrospinal fluid vasopressin and symptom severity in children with autism

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Ann Neurol 2018;84:611–615
<https://www.ncbi.nlm.nih.gov/pubmed/30152888>

This paper examined the concentrations of arginine vasopressin (AVP) and oxytocin (OXY) in the cerebrospinal fluid (CSF) of autistic children (n=36) compared to controls children without psychiatric disease who needed lumbar puncture for some medical reason (n=36). The authors hypothesized that if these neuropeptides are involved in autism development, differences in levels would be more apparent in CSF than in plasma.

The results showed lower concentrations of CSF AVP in autistic children compared to controls, but no significant difference in OXT. Furthermore, CSF AVP concentrations predicted greater symptom severity in males, but not in females. This work partially confirmed the initial hypothesis: AVP concentrations in CSF are more sensitive to detect a pathologic process within the brain than concentrations in peripheral blood. The lack of association with OXT is surprising as OXT deficit has been widely implicated in the social deficit of autism. In neuroendocrine experimental research, assessment of neuropeptide concentration in CSF in domestic animals is considered as a valuable marker of its concentration in the brain. For instance, this approach has been used to study the effect of Kisspeptin on GnRH secretion in sheep. CSF is also considered as a way to deliver neuropeptides at distance of their synthesis. These results are in accord with recent results showing CSF AVP level is a marker of sociality in nonhuman primates¹.

From a clinical perspective, CSF AVP concentrations performed poorly to correctly assign individuals into one or other group, hence, low AVP CSF cannot be used in the diagnosis of autism. This study has other limitations. As recognised by the authors, in the control group CSF was collected because of some clinical indication and they may not be representative of normal children. Also, for autistic children, lumbar puncture was performed while fasted and under anesthesia, clearly very different than in the control group. However, it is unlikely that these differences explain the lower CSF AVP levels in the autism group.

Oxytocin administration has been proposed to rescue social deficit in both mice and humans. By a similar concept, these results suggest AVP administration as a potential a new way to treat social deficit in autism.

Reference

1. Parker KJ, Garner JP, Oztan O, Tarara ER, Li J, Sclafani V, Del Rosso LA, Chun K, Berquist SW, Chez MG, Partap S, Hardan AY, Sherr EH, Capitanio JP Arginine vasopressin in cerebrospinal fluid is a marker of sociality in nonhuman primates. *Sci Transl Med.* 2018;10(439):

New Mechanisms

1.3. AstA signaling functions as an evolutionary conserved mechanism timing juvenile to adult transition

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<https://www.ncbi.nlm.nih.gov/pubmed/30799245>

In vertebrates, puberty is the critical transitional step which leads to major somatic changes and the acquisition of the reproduction function. At the molecular level, puberty equates to the reactivation of the gonadotropic axis after a period of childhood quiescence, and hence increases sex steroid production. This reactivation is controlled by many neuropeptides and neurotransmitters, with kisspeptin as a major actor. In *Drosophila*, the transition from juvenile to adult corresponds to the metamorphosis, which is marked by an increase in ecdysone secretion by the prothoracic gland under the control of the prothoracicotropic hormone (PTTH). This pathway strongly contributes to the juvenile-maturation transition (JMT).

Here, the authors described AstA as a neuropeptide which acts in the fruit fly through a specific receptor (AstAR1) expressed in PTTH producing neurons. AstAR1 controls PTTH secretion and thereby regulates the JMT. Interestingly, AstA is also expressed in brain insulin-producing cells (IPCs) to promote insulin secretion and systemic growth. AstA/AstAR1 signaling is maximal prior to maturation, indicating a specific activation of this pathway at this developmental stage.

Interestingly, AstA and AstAR1 are homologous to Kiss1 and Kiss1R, respectively and many biological features reported for AstA and AstAR1 here in the fruit fly (increased activity at the end of the juvenile period, expression in insulin secreting cells, control of sexual hormone synthesis and secretion) have also been described for Kiss1 and Kiss1R in mammals. This work therefore demonstrates that JMT and puberty are under a similar ancestral mechanism, which relatively well conserved during evolution. Similar questions remain for both JMT and puberty. Which signal triggers the increase in AstA/AstAR1 or Kiss1/Kiss1R signaling at the end of the juvenile period?

1.4. Estrogen signaling in arcuate Kiss1 neurons suppresses a sex-dependent female circuit promoting dense strong bones

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Nat Commun 2019;10:163.
<https://www.ncbi.nlm.nih.gov/pubmed/30635563>

Central estrogen signaling via estrogen receptor alpha ($ER\alpha$) coordinates energy expenditure, reproduction and, in concert with peripheral estrogen, impacts skeletal homeostasis. Here, the authors showed that eliminating $ER\alpha$ in kisspeptin arcuate nucleus neurons resulted in high bone mass phenotype in female mice only.

To show these interesting results, the authors first studied bone mass in mice with a deletion of $ER\alpha$ in medio-basal hypothalamus (MBH) neurons (*Esr1^{Nkx2-1Cre}* mice). They observed a change in energy balance only in females. In addition, they observed an elevated bone density only in females *Esr1^{Nkx2-1Cre}* which persisted five weeks after ovariectomy. Hormonal homeostasis was not disturbed in these mice.

To define which MBH neurons are involved in the central control of bone mass, the authors eliminated $ER\alpha$ in the ventro-medial hypothalamus or the arcuate nucleus (ARC), and observed the increase of bone mass only in the second model. Furthermore, they show that the central control of bone mass by $ER\alpha$ is mediated through ARC kisspeptin neurons.

Altogether, this work establishes a brain-bone axis in female, but not in male, mice. The mechanism of this central control is probably humoral, by molecules that remain to be discovered. The second question asked by the authors is how the $ER\alpha$ brain-bone pathway counteracts the positive peripheral effects of estrogen on bone remodeling. It would therefore be interesting to analyze whether this brain-bone axis is active in juvenile mice.

1.5. CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency

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Science 2019;363(6424).

<https://www.ncbi.nlm.nih.gov/pubmed/30545847>

A wide range of human diseases result from haploinsufficiency, where gene expression is decreased as compared to normal conditions. Haploinsufficiency is a typical mechanism in autosomal dominant disorders. Rare mutations in *Sim1* or *Mc4r* are models of haploinsufficiency causing severe early-onset obesity with hyperphagia. Gene therapy by adenovirus vectors has been developed to rescue such disorders, by expressing the normal allele in place of the mutant. However, this strategy is limited by the size of the DNA that can be packaged in this virus, and also by the ectopic insertion of the transgene leading to off-target side-effects.

Another way to circumvent these problems is to increase the expression level of the normal allele. The authors did this by coming up with a derivative of the CRISPR-Cas9 genome editing tool. 'CRISPR-mediated activation' (CRISPRa) is a method which combines a deficient Cas9 enzyme (dCas9) fused to an activator protein. When this protein complex is guided to a specific regulatory region, for instance, a promoter, which controls gene expression, the genome is not edited but rather the expression level of the targeted gene is increased.

Using this novel approach, they increased the expression levels of *Sim1* and *Mc4r* specifically in the hypothalamus, by targeting hypothalamus-specific enhancers. They first confirmed that CRISPRa was able to regulate *Sim1* expression in cells. They then adapted the method in CRISPRa transgenic mice or by adenovirus administration of dCas9 specifically to the hypothalamus. By both approaches, the severe obesity phenotype was rescued in *Sim1*^{-/+} obese with very few off-target effects. Similar results were observed for *Mc4r*.

One great potential of this strategy is the possibility to rescue a complex phenotype, such as obesity, by increasing the expression of the normal allele. It is therefore unnecessary to define specific approaches for each genetic variant. The authors propose that up-regulation of endogenous genes could be a potential strategy to treat various altered gene dosage diseases.

1.6. Regulation of feeding by somatostatin neurons in the tuberal nucleus

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Science 2018;361:76–81.

<https://www.ncbi.nlm.nih.gov/pubmed/29976824>

The tuberal nucleus (TN) is a hypothalamic region which is well described in humans but remains poorly defined in rodents. In this paper, the authors demonstrated by specific and sophisticated techniques the role of somatostatin expressing TN (^{TN}SST) neurons in the control of food intake in mice. Interestingly, their results showed the specific role of ^{TN}SST neurons in food intake as compared to SST neurons located in the arcuate nucleus. Furthermore, the authors showed that the appetite-promoting hormone, ghrelin, induced a robust activation of ^{TN}SST neurons in both in-vivo and in hypothalamic slices. They then confirmed that specific activation of those neurons by chemogenetic or optogenetic effectors indeed promoted a dramatic food consumption. ^{TN}SST neurons project to regions in the hypothalamus which are known to be involved in the control of feeding such as the paraventricular nucleus (PVN). These ^{TN}SST neuron promoted feeding by inhibiting downstream neurons.

Besides its inhibitory action on GH secretion, SST has many extra-pituitary functions. It also influences behaviour, such grooming, locomotor activity and anxiety. The central role of SST in feeding behavior had been suggested for many years by pharmacological approaches. The interest of this work was to confirm the role of central SST in feeding behavior in-vivo but also to describe a physiological function of neurons located in the TN. SST neurons located in other regions of the hypothalamus are also involved in the control of feeding.

Although, somatostatin was already known as a regulator of food intake, for example by suppressing insulin secretion, this work adds a new central orexigenic pathway.

Updates on Kisspeptin

1.7. Genetic dissection of the different roles of hypothalamic kisspeptin neurons in regulating female reproduction

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Elife 2019;8:e43999
<https://www.ncbi.nlm.nih.gov/pubmed/30946012>

Two populations of Kisspeptin neurons are described in the hypothalamus. One population is located in the rostral part of the hypothalamus (the anteroventral periventricular nucleus, AVPV); the other is in the arcuate nucleus (ARC). The major difference between these populations is the opposing effects of 17 β -estradiol (E2) on *Kiss1* expression. E2 increases *Kiss1* expression in the AVPV, but inhibits *Kiss1* expression in the ARC. In this paper, the authors used a specific and temporally-controlled method to invalidate the estradiol receptor in Kisspeptin neurons. They show that E2 regulates LH surge generation through AVPV kisspeptin neurons, and maintains cyclicity through ARC kisspeptin neurons. This paper is interesting for two main reasons:

- 1) It describes an innovative CRISPR-Cas9 technique to investigate the role of E2 in different kisspeptin neurons. The hypothalamus is a complex region of the brain with many nuclei which interact with each other. These nuclei are defined by their anatomical location but also by their specific neuroendocrine functions. The specific deletion of one gene in specific hypothalamic population is therefore highly challenging and this technique may be used to investigate neuroendocrine functions at other specific regions.
- 2) It shows different reproductive cycle contributions of AVPV and ARC kisspeptin neurons. Surprisingly, specific deletion of ER α in ARC kisspeptin neurons did not change LH pulse frequency whereas these neurons are established as the GnRH-pulse generator (see paper 1.8). Additional experiments are needed to explain this unexpected result.

Reference

1. Han SY, Kane G, Cheong I, Herbison AE Characterization of GnRH Pulse Generator Activity in Male Mice Using GCaMP Fiber Photometry. *Endocrinology*. 2019;160(3):557–567.

1.8. Characterization of GnRH Pulse generator activity in male mice using GCaMP fiber photometry

Han SY, Kane G, Cheong I, Herbison AE
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Endocrinology 2019;160:557–567.
<https://www.ncbi.nlm.nih.gov/pubmed/30649269>

There is now no doubt that arcuate nucleus (ARC) kisspeptin neurons are the GnRH pulse generator. The authors analyzed the activity of ARC kisspeptin neurons by using a very powerful method based on the recording of calcium concentration within cells.

ARC kisspeptin neurons activity was analyzed in male mice over a period of 24 hours. Synchronous activation of ARC kisspeptin neurons was defined as a synchronization episode (SE), and each SE was associated with a LH pulse. SE's occurred on average every 166 minutes (range: 43 to 347 minutes). The inter-SE interval showed no difference between day and night, but decreased to on average 16 minutes in gonadectomized (GDX) mice, with the same 1:1 link between SEs and LH pulses as in intact mice. The wide range of inter-SE intervals and the

perfect correlation between SE and LH pulses throughout the 24 hours period in both intact and GDX mice indicate that the GnRH pulse generator operates in a stochastic manner.

This analysis in male mice improves our understanding of the activity of the GnRH pulse generator. Additional studies are needed to understand the mechanism that synchronizes the activity of ARC kisspeptin neurons altogether, and also how this frequency is accelerated in GDX mice.

1.9. GnRH pulse generator activity across the estrous cycle of female mice

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Endocrinology 2019;160:1480–1491.

<https://www.ncbi.nlm.nih.gov/pubmed/31083714>

There is now no doubt that arcuate nucleus (ARC) kisspeptin neurons are the GnRH pulse generator. (With paper 1.8) this second paper describes the activity of the GnRH pulse generator across the estrous cycle. In this work, the variable level of E2 represented an additional complexity. The second interest was to understand how the GnRH pulse generator could be involved in the LH surge.

The results show that the GnRH pulse generator operates at a constant rate across the estrous cycle. In addition, the GnRH-pulse frequency remains unchanged during the LH surge, which indicates that additional mechanisms provoke the large LH release at the transition between the proestrus and the estrus. Paper 1.7 shows that E2 regulates LH surge generation through AVPV (rather than ARC) kisspeptin neurons. The current model confirms that the LH surge is independent from the ARC kisspeptin neuron pathway. Finally, this work confirmed the major inhibitory role of progesterone on GnRH pulse generator activity in female mice.

1.10. Acute suppression of LH secretion by prolactin in female mice is mediated by kisspeptin neurons in the arcuate nucleus

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Endocrinology 2019;160:1323–1332.

<https://www.ncbi.nlm.nih.gov/pubmed/30901026>

Elevated prolactin levels suppress pulsatile release of GnRH from the hypothalamus, with a consequent reduction in pulsatile LH secretion from the pituitary. In this paper, the authors showed in mice that kisspeptin neurons located in the arcuate nucleus (ARC) respond to prolactin and conditional knockout of the prolactin receptor specifically in ARC kisspeptin neurons prevented prolactin-induced suppression of LH secretion.

This paper is interesting for several reasons:

- It shows that acute prolactin administration in mice suppresses LH pulse frequency and reduces circulating LH concentrations.
- Using a specific fluorescent marker, it shows that ARC kisspeptin neurons respond to prolactin, whereas periventricular preoptic kisspeptin neurons do not.
- It confirms the functional difference between these two types of kisspeptin neurons. Their innovative *Kiss1-cre* model knocked down the prolactin receptor only in ARC kisspeptin neurons but not in periventricular preoptic kisspeptin neurons.

In this model, high prolactin levels act via the prolactin receptor on ARC kisspeptin neurons (the GnRH pulse generator) to suppress GnRH pulses. The effect of a chronic increase in prolactin must now be investigated.

1.11. Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region

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<https://www.ncbi.nlm.nih.gov/pubmed/30385464>

The hypothalamus is a highly complex region, which receives many inputs from several other brain regions and also from the periphery. It is the site of synthesis of many neuropeptides that act as neurotransmitters or via the systemic circulation (in blood). Hypothalamic nuclei are typically defined by their exact anatomical location but also by their functions. The aim of this work was to create an annotated and spatially resolved atlas of the mouse hypothalamic preoptic region.

The authors combined two different but complementary approaches: single cell RNA sequencing (scRNA-seq) and a multiplexed fluorescence *in situ* hybridization (MERFISH) which detects individual RNA molecules with single-molecule FISH enabling transcriptome-scale RNA of individual cells *in situ*. In other words, the first method allows the classification of cells based on their gene expression profiles, whereas the second method defines cell populations based on their spatial organization *in situ*.

By profiling ~1 million cells, this work defined 70 neuronal clusters. Genes that discriminated between these neuronal clusters were enriched for neuropeptides and proteins involved in neuromodulator synthesis and transport. These clusters were enriched in genes previously reported to be involved in many physiological functions controlled by the preoptic region. One interesting feature was the confirmation that individual nuclei of the preoptic region were composed of multiple neuronal clusters. An intriguing result was the demonstration that GnRH neurons express *Esr1* which encodes estradiol receptor alpha. This result contradicts many studies indicating that E1 acts on the GnRH neuronal network through Kisspeptin neurons. This work also confirmed the expression of aromatase in specific type of nuclei which leads to a cell-autonomous action of E2 or a paracrine effect.

Finally, the authors showed the sexual-dimorphism of many genes involved in specific behavioral traits.

2. Antenatal and Neonatal Endocrinology

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Neonatal Hypoglycemia

2.1. Congenital hyperinsulinism in infants with Turner syndrome: possible association with monosomy X and *KDM6A* haploinsufficiency

Gibson CE, Boodhansingh KE, Li C, Conlin L, Chen P, Becker SA, Bhatti T, Bamba V, Adzick NS, De Leon DD, Ganguly A, Stanley CA

Horm Res Paediatr. 2018;89(6):413–422.

<https://www.ncbi.nlm.nih.gov/pubmed/29902804>

This study examined the clinical and molecular aspects of girls with Turner syndrome and hyperinsulinaemic hypoglycemia (HH). Records of girls with hyperinsulinism and Turner syndrome were reviewed.

The findings expand on previous observations suggesting a link between Turner syndrome and hyperinsulinaemic hypoglycemia (HH). Some of the patients described in this study had severe HH which was unresponsive to treatment with diazoxide and required pancreatectomy despite having no mutations in the pancreatic K_{ATP} channel genes. All the cases in this study were found to have monosomy X, suggesting that haploinsufficiency for an X chromosome gene, rather than an over-dosage of X chromosome material due to the presence of a ring X chromosome, may be responsible for the HH in Turner syndrome.

The authors suggest that haploinsufficiency for the gene *KDM6A* located on the X chromosome is an attractive explanation for the HH in Turner syndrome. *KDM6A* escapes X-inactivation and is associated with Kabuki syndrome. *KDM6A* regulates transcriptionally active chromatin through epigenetic modification of histone H3. *KDM6A* is a lysine-specific histone H3 demethylase that controls tissue specific expression of genes involved in development as well as the cell cycle. The proportion of beta cells with monosomy X in different girls with Turner syndrome is likely to be highly variable and could affect the severity of their HH.

In one patient who underwent pancreatectomy, the authors were able to study the dynamics of insulin secretion. The islets from this patient showed altered fuel sensing, with increased sensitivity to amino acids and elevated basal cytosolic calcium concentrations. These findings are similar to patients with pancreatic K_{ATP} channel defects. Studies of mouse islets exposed to an inhibitor of *KDM6A* reproduced some of the islet phenotype seen in the case of Turner syndrome, supporting the suggestion that haploinsufficiency of *KDM6A* might be responsible for HH in Turner syndrome. The mechanisms involved in dysregulated insulin secretion in Turner syndrome and in *KDM6A* deficiency warrant further investigation and should give further insights into the role of *KDM6A* in the pancreatic beta-cell. Clearly, Turner syndrome patients have an increased frequency of HH.

2.2. Congenital hyperinsulinism as the presenting feature of Kabuki syndrome: clinical and molecular characterization of 9 affected individuals

Yap KL, Johnson AEK, Fischer D, Kandikatla P, Deml J, Nelakuditi V, Halbach S, Jeha GS, Burrage LC, Bodamer O, Benavides VC, Lewis AM, Ellard S, Shah P, Cody D, Diaz A, Devarajan A, Truong L, Greeley SAW, De Leon DD, Edmondson AC, Das S, Thornton P, Waggoner D, Del Gaudio D

Genet Med. 2019 Jan;21(1):233–242.

<https://www.ncbi.nlm.nih.gov/pubmed/29907798>

This study documented the clinical features and molecular diagnoses of 9 infants with persistent hyperinsulinism and Kabuki syndrome via a combination of sequencing and copy-number profiling methodologies.

KS is characterized by typical facial features (long palpebral fissures with eversion of the lateral third of the lower eyelid; arched and broad eyebrows; short columella with depressed nasal tip; large, prominent, or cupped ears), minor skeletal anomalies, persistence of fetal fingertip pads, mild-to-moderate intellectual disability, and postnatal growth deficiency. Recognition of the dysmorphic features in the neonatal period may be difficult.

More than 75% of patients with KS harbor pathogenic variants in *KMT2D* located at 12q13.13 that typically occur *de novo* and in rare cases may be inherited (autosomal dominant Kabuki syndrome 1, KS1), and 5–8% have pathogenic variants in *KDM6A* located at Xp.11.3 that are most frequently *de novo* (X-linked dominant Kabuki syndrome 2, KS2) (Adam *et al.*). KS patients can present with hypoglycemia in the newborn period due to various causes, including hyperinsulinism. In this study on 11 patients with KS and HH, five pathogenic variants (45.5%) were found in *KDM6A* suggesting that patients with KS harboring pathogenic *KDM6A* variants (KS2) may have an increased likelihood of presenting with HH as compared with patients with KS who harbor pathogenic variants in *KMT2D* (KS1). KS patients with a history of HH did not appear to have any additional specific phenotypic features that would differentiate them from patients without HH.

The authors recommend an increased awareness among clinicians of KS in patients with HH in the newborn period as the dysmorphic features may be subtle. KS should be considered in the differential diagnosis of persistent HH and also include comprehensive evaluation (sequencing and deletion/duplication analysis) of the KS-associated genes, *KMT2D* and *KDM6A*, during genetic testing for HH. Early diagnosis may inform appropriate follow-up and treatment of the hypoglycemic events in patients with KS, since these patients are likely to respond to diazoxide therapy. As with Soto syndrome discussed in 2.3, patients with KS have an increased incidence of HH and should have full genetic evaluation. The roles of *KMT2D* and *KDM6A* in pancreatic beta-cell physiology need further study.

Reference

Adam MP, Banka S, Bjornsson HT, Bodamer O, Chudley AE, Harris J, Kawame H, Lanpher BC, Lindsley AW, Merla G, Miyake N, Okamoto N, Stumpel CT, Niikawa N. Kabuki Syndrome Medical Advisory Board. Kabuki syndrome: international consensus diagnostic criteria. *J Med Genet*. 2019 Feb;56(2):89–95.

2.3. Hyperinsulinemic hypoglycemia in seven patients with *de novo* NSD1 mutations

Grand K, Gonzalez-Gandolfi C, Ackermann AM, Aljeaid D, Bedoukian E, Bird LM, De Leon DD, Diaz J, Hopkin RJ, Kadakia SP, Keena B, Klein K, Krantz I, Leon E, Lord K, McDougall C, Medne L, Skraban CM, Stanley CA, Tarpinian J, Zackai E, Deardorff MA, Kalish JM
Am J Med Genet A. 2019 Apr;179(4):542–551.
<https://www.ncbi.nlm.nih.gov/pubmed/30719864>

This study describes 7 individuals with hyperinsulinemic hypoglycemia caused by *NSD1* gene mutations with 3 having persistent hyperinsulinemic hypoglycemia.

The underlying mechanisms that lead to hyperinsulinaemic hypoglycemia in Sotos syndrome are not known. Since most of the previous reported cases of Sotos syndrome are due to microdeletions in the 5q35 region it is assumed that deletion of specific genes in this region lead to HH. In this study, the authors report 7 patients with point mutations in the *NSD1* gene and either transient or persistent HH. These observations suggest that the *NSD1* gene has a role in glucose and insulin homeostasis and that other genes might not be involved in the microdeletions around 5q35.

The functions of *NSD1* have not been fully elucidated. It is thought to act as a transcriptional intermediary factor capable of both negatively and positively influencing transcription depending on the cellular context. *NSD1* is expressed in human pancreatic beta-cells as shown by single cell RNA analysis and bulk islet analysis. *NSD1* is a regulator of the chromatin structure and gene expression as it catalyzes specific types of histone methylation, and contributes to the initiation, maintenance, or termination of gene activation or repression. The *NSD1* molecule has no DNA-binding domain, but binds to both cofactors and methylated histones, suggesting that it exhibits features of a cofactor complex. It is possible that the disrupted interaction between *NSD1* and

histones or cofactors may directly cause the abnormal expression of insulin. *NSDI* may also be associated with beta cell-specific transcription factor(s) to suppress the expression of the insulin gene. Another explanation of the hyperinsulinemia in patients with Sotos syndrome includes the involvement of factors that influence the biological function of insulin in vivo (Matsuo *et al.*).

The important message from this study is that patients with Sotos can present with atypical features and blood glucose levels should be checked in these patients. Further research on the role of *NSDI* in beta-cell physiology should shed some insights into the mechanisms of unregulated insulin secretion.

Reference

Matsuo T, Ihara K, Ochiai M, Kinjo T, Yoshikawa Y, Kojima-Ishil K, Noda M, Mizumoto H, Misaki M, Minagawa K, Tomiyama K, Hara T. Hyperinsulinemic hypoglycemia of infancy in Sotos syndrome. *Am J Med Genet A* 2013 Jan;161A(1):34–7.

2.4. Sirolimus: efficacy and complications in children with hyperinsulinemic hypoglycemia: A 5-year follow-up study

Maria G, Antonia D, Michael A, Kate M, Sian E, Sarah FE, Mehul D, Pratik S
J Endocr Soc. 2019 Feb 7;3(4):699–713.
<https://www.ncbi.nlm.nih.gov/pubmed/30882046>

This paper describes a retrospective study of patients with congenital hyperinsulinism (CHI) who were treated with mammalian target of rapamycin (mTOR) inhibitor, sirolimus, in a tertiary service, and reviews the 15 publications reporting CHI patients treated with sirolimus.

The diffuse forms of CHI can present with severe hypoglycemia usually unresponsive to therapy with diazoxide and needing a near total pancreatectomy. These patients are a real challenge to manage clinically as the hypoglycemia can be so severe. The therapeutic options for these patients with diffuse forms of hyperinsulinism are limited.

Sirolimus has been successfully used in patients with severe diffuse HH, unresponsive to maximum doses of diazoxide and octreotide. However, the therapeutic response to sirolimus has been variable with some studies reporting good responses and other studies reporting poor responses. In this largest study to date, 22 patients who were treated with sirolimus, virtually all showed some response with the best responses observed in those patients with either compound or heterozygous *ABCC8/KCNJ11* gene mutations. As expected, the limiting factors for continuation of treatment were the side effects of sirolimus. However, it is difficult to know if all the side effects reported can be attributed solely to treatment with sirolimus. For example, newborns with severe HH have multiple other risk factors (such as central venous lines for delivering concentrated dextrose infusions) for infections.

The exact mechanism/s how sirolimus ameliorates HH is unclear. In clinical practice, sirolimus should be used as a last resort in patients with diffuse HH to avoid pancreatectomy. In some patients, the combination of sirolimus, diazoxide and or octreotide might be more effective in preventing a near total pancreatectomy. Sirolimus can also be used as a short-term treatment until the underlying HH becomes milder and then an alternative form of treatment can be started. Patients on sirolimus should be carefully monitored for possible side effects. Understanding the impact of mTOR inhibitors on beta-cell function in patients with diffuse HH might give further insights into their action. There is an urgent need to develop novel therapies for the diffuse form of CHI so that a near total pancreatectomy can be avoided.

2.5. Diagnosis and management of hyperinsulinaemic hypoglycaemia

Galcheva S, Al-Khawaga S, Hussain K
Best Pract Res Clin Endocrinol Metab. 2018 Aug;32(4):551–573.
<https://www.ncbi.nlm.nih.gov/pubmed/30086874>

This review provides a state of the art in the field of hyperinsulinaemic hypoglycemia (HH). The physiology of insulin secretion is discussed followed by the classification of the different subtypes of HH and then a detailed description of all the monogenic forms of HH. Novel forms of HH, such as due to mutations in the genes hexokinase 1 (*HK1*), Phosphoglucosyltransferase 1 (*PGM1*), Phosphomannosyltransferase 2 (*PPM2*) and *FOXA2* are described. The importance of a timely diagnosis and management are emphasized and an algorithm for the key steps in the management of these complex patients is suggested.

2.6. Trisomy 21 is a cause of permanent neonatal diabetes that is autoimmune but not HLA associated

Johnson MB, De Franco E, Atma W, Greeley S, Letourneau LR, Gillespie K, International DS-PNDM consortium, Wakeling MN, Ellard S, Flanagan SE, Patel KA, Hattersley AT
Diabetes. 2019 Apr 8. pii: db190045. doi: 10.2337/db19-0045.
<https://www.ncbi.nlm.nih.gov/pubmed/30962220>

This study assessed the incidence of permanent neonatal diabetes mellitus (PNDM) in patients with Trisomy 21.

Patients with Trisomy 21 have an increased prevalence of autoimmune conditions, such as Type 1 diabetes, celiac disease, alopecia, vitiligo and autoimmune thyroid disorders (Hashimoto's thyroiditis and Graves' disease). Childhood onset autoimmune diabetes is 4-times more common in patients with Trisomy 21 than the general population. Diabetes in patients with Trisomy 21 is usually autoimmune and associated with the presence of autoantibodies and Human Leucocyte Antigen (HLA) haplotypes which increase the risk of diabetes. It is not known if Trisomy 21 can present with permanent neonatal diabetes mellitus (PNDM).

In this study, the authors looked at a large cohort of patients with PNDM. 13 patients with Trisomy 21 were identified as having developed PNDM in the first 6 months of life and in about 44% of these patients, one or more autoantibodies were positive. However interestingly none of these patients had the HLA haplotype associated (HLA DR3/DR4) with the increase risk of developing diabetes, suggesting that the PNDM in Trisomy 21 is not HLA associated. These data imply that there is prenatal onset of beta cell dysfunction in patients with Trisomy 21 and PNDM. Previous post mortem studies on pancreatic tissue from non-diabetic infants with Trisomy 21 have shown that there is no deficiency of pancreatic beta-cells (Butler AE *et al.*). Trisomy 21 is a cause of PNDM, and the underlying mechanism of the diabetes is likely to involve autoimmunity against the beta cells. A complex interaction between multiple genes (*AIRE*, *UBASH3A*, *IFNAR1*, *IFNAR2*, *IFNGR2* and *IL1ORB*) on chromosome 21 may be responsible for the increased autoimmunity in patients with Trisomy 21.

Diabetes in patients with Trisomy 21 seems to be a heterogenous condition and consists of a subgroup diagnosed very young (including PNDM) which is autoimmune but not HLA mediated and a second group, usually presenting beyond infancy, that is similar to Type 1 diabetes in the non-Trisomy population and has a strong HLA association.

Reference

Butler AE, Sacks W, Rizza RA, Butler PC. Down Syndrome-Associated Diabetes Is Not Due To a Congenital Deficiency in β -Cells. *J Endocr Soc*. 2017 Jan 1;1(1):39–45.

2.7. A specific CNOT1 mutation results in a novel syndrome of pancreatic agenesis and holoprosencephaly through impaired pancreatic and neurological development

De Franco E, Watson RA, Weninger WJ, Wong CC, Flanagan SE, Caswell R, Green A, Tudor C, Lelliott CJ, Geyer SH, Maurer-Gesek B, Reissig LF, Lango Allen H, Caliebe A, Siebert R, Holterhus PM, Deeb A, Prin F, Hilbrands R, Heimberg H, Ellard S, Hattersley AT, Barroso I
Am J Hum Genet. 2019 May 2;104(5):985–989.
<https://www.ncbi.nlm.nih.gov/pubmed/31006513>

This study reports the identification of a novel gene that is involved in the regulation of the pancreatic development.

Understanding the molecular mechanisms of pancreatic development is important, for example to guide the progress of beta-cell replacement therapy for patients with Type 1 diabetes mellitus. Prior to this study six different genetic causes (*PDX1*, *PTF1A*, *GATA4*, *GATA6*, *HNF1B* and *RFX6*) of pancreatic agenesis/hypoplasia were known. This study identified heterozygous missense mutations in a novel gene (*CNOT1*) in 3 patients with pancreatic agenesis and holoprosencephaly. The CNOT1 protein has not previously been suggested to have a role in pancreatic development (it is known to act both as scaffold of the

CCR4-NOT complex and as an independent factor). It is thought to mediate transcriptional repression and is expressed extremely early during embryonic development (E3.5 in the inner cell mass in mice).

In vitro studies have suggested that CNOT1 plays a critical role in maintaining human and mice embryonic stem cells in a pluripotent state by inhibiting primitive endoderm factors. *CNOT1* expression peaks in undifferentiated human induced pluripotent stem (iPS) cells compared to subsequent stages of *in vitro* differentiation toward pancreatic endocrine cells, supporting its fundamental role in stem cells. Using CRISPR, CNOT1 knockout mice were generated to try and understand the molecular basis of the pancreatic agenesis observed in these patients. Heterozygous mice were born at a lower than expected frequency, but without an obvious phenotype. However, the homozygous mice were embryonically lethal. At E14.5, embryos were still alive and present at expected Mendelian ratios and their phenotype was studied further.

The pancreas was smaller in these embryos due to dorsal pancreatic agenesis. RNA analysis showed increased expression of Sonic hedgehog (Shh) and decreased expression of *Pdx1*, *Ins*, *Hnf1b*, and *Ptf1a* and no difference in *GATA6* or *Rxra*. The *CNOT1* mutation seems to increase the expression of Shh which prevents embryonic stem cells from differentiating into brain and pancreatic tissue. Shh is a key developmental factor that is known to be crucial for pancreatic and brain development. Heterozygous loss-of-function mutations in Shh cause holoprosencephaly and studies in both mouse and human embryos have shown that Shh expression needs to be repressed in the dorsal foregut endoderm for successful differentiation toward dorsal pancreas.

This is the 7th gene to be described that leads to pancreatic agenesis when mutated and further expands the knowledge on developmental biology of the pancreas. This has important implications for developing stem cell regenerative therapies for type 1 diabetes.

2.8. Effectiveness and safety of long-term treatment with sulfonylureas in patients with neonatal diabetes due to *KCNJ11* mutations: an international cohort study

Bowman P, Sulen Å, Barbetti F, Beltrand J, Svalastoga P, Codner E, Tessmann EH, Juliusson PB, Skriverhaug T, Pearson ER, Flanagan SE, Babiker T, Thomas NJ, Shepherd MH, Ellard S, Klimes I, Szopa M, Polak M, lafusco D, Hattersley AT, Njølstad PR, Neonatal Diabetes International Collaborative Group. Collaborators (113)

Lancet Diabetes Endocrinol. 2018 Aug;6(8)

<https://www.ncbi.nlm.nih.gov/pubmed/29880308>

This study describes a 10-year follow-up of a large international multicenter cohort of patients with *KCNJ11* permanent neonatal diabetes. It addresses key questions relating to long-term efficacy and safety of sulfonylureas in these patients.

The discovery that mutations in the *KCNJ11* gene lead to neonatal diabetes mellitus in some patients transformed the care of these patients by switching from subcutaneous insulin injections to oral sulphonylureas. Although most patients respond to sulphonylureas in the short-term, there are no data on long-term follow up and whether these patients show decreasing responses to sulphonylureas over the long-term, as happens for example in patients with Type 2 diabetes.

In this international multicenter study, all patients who were diagnosed with neonatal diabetes mellitus due to *KCNJ11* mutations and switched over to oral sulphonylureas before 30th of November 2006 were recruited and their long-term glycemic control and sulphonylurea safety were assessed over 10 years. The study showed that sulphonylurea failure, which is commonly seen in Type 2 diabetes, is not a feature in neonatal diabetes patients with mutations due to *KCNJ11*. Sulfonylureas were found to be safe in the long term, even in high doses, in this unique group of patients and there was excellent glycemic control maintained over the 10 year follow up period. In the short term there was some improvement in the neurological features, but unfortunately this did not continue in the long-term.

The key message from this study is that all infants diagnosed with neonatal diabetes aged less than 6 months old should undergo rapid genetic testing to facilitate early transfer of those with *KCNJ11* mutations to sulfonylureas as first-line treatment. This action should result in safe and long-lasting excellent glycemic control for at least 10 years. Neurological features might show initial improvement but are likely to persist. Further research is needed to establish the effect of very early transfer and high-dose sulphonylurea therapy on neurological features.

2.9. Diabetes and obesity during pregnancy are associated with oxidative stress genotoxicity in newborns

Castilla-Peon MF, Medina Bravo PG, Sánchez-Urbina R, Gallardo-Montoya JM, Soriano-López LC, Coronel Cruz FM
J Perinat Med. 2019 Apr 24;47(3):347-353. doi: [10.1515/jpm-2018-0201](https://doi.org/10.1515/jpm-2018-0201).

This study measured 8-hydroxy-deoxyguanosine (8-OH-dG), a marker of DNA oxidative damage, in venous umbilical cord plasma from newborns of mothers with and without a diabetes diagnosis during pregnancy.

Adult offspring born to mothers with gestational diabetes mellitus (GDM) have an increased frequency of conditions associated with high cardiovascular risk, such as obesity, insulin resistance, hypertension and other pro-atherosclerotic risk factors. However, the mechanisms underlying these observations are unknown. Oxidative stress is the imbalance between the production of reactive oxygen species (ROS) and the endogenous antioxidant mechanisms. Oxidative stress is increased in individuals with all types of diabetes including GDM. Oxidative stress leads to deoxynucleic acid (DNA) damage (genotoxicity) a phenomenon that has been linked to the development of some cardiovascular risk conditions and other age-related diseases. Fetuses of women with GDM are exposed to high oxidative stress and animal studies suggest that oxidative stress genotoxicity might be increased as well. Adult offspring of mothers with type 2 diabetes mellitus exhibit higher levels of the genotoxicity biomarker, 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OH-dG) than controls without a family history of DM. 8-OH-dG has been extensively used as a biomarker of oxidative stress genotoxicity in diabetes and other diseases and 8-OH-dG from umbilical cord venous blood has been used as a biomarker of fetal oxidative stress.

In this study, the levels of 8-OH-dG were higher in the offspring born to mothers with diabetes diagnosed during pregnancy than in women without diabetes, regardless of other confounding variables, such as maternal pre-gestational weight, HbA1c levels at the end of pregnancy, and age of the mother, and mode of delivery. It is possible that oxidative DNA damage may be a mechanism that increases the risk for age-related conditions in the offspring of women with GDM and/or obesity. The cross-sectional design of this study did not allow for inferring the causal relations between diabetes and/or overweight and oxidative stress or genotoxicity in the newborns. If more evidence from longitudinal studies supports this cause-effect theory, interventions aimed at reducing oxidative stress or promoting DNA repair could be investigated to evaluate their effect on the health of offspring. The authors suggest that following up newborns with high 8-OH-dG levels at birth could be useful to monitor the long-term development of cardiovascular diseases.

2.10. Association of gestational diabetes with maternal disorders of glucose metabolism and childhood adiposity

Lowe WL Jr, Scholtens DM, Lowe LP, Kuang A, Nodzenski M, Talbot O, Catalano PM, Linder B, Brickman WJ, Clayton P, Deerochanawong C, Hamilton J, Josefson JL, Lashley M, Lawrence JM, Lebenthal Y, Ma R, Maresh M, McCance D, Tam WH, Sacks DA, Dyer AR, Metzger BE, HAPO Follow-up Study Cooperative Research Group
JAMA. 2018 Sep 11;320(10):1005–1016. doi: [10.1001/jama.2018.11628](https://doi.org/10.1001/jama.2018.11628).

The aims of this study were to assess whether in utero exposure to untreated gestational diabetes (using the IADPSG criteria) is associated with long-term risk of a disorder of glucose metabolism among mothers and greater adiposity among their children at 10 to 14 years post-partum.

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study was undertaken to identify risks of adverse outcomes associated with degrees of maternal glucose intolerance less severe than overt diabetes mellitus (Metzger BE *et al.*). It found associations between increasing levels of fasting, 1-hour, and 2-hour plasma glucose obtained on oral glucose-tolerance testing and birth weight above the 90th percentile and cord-blood serum C-peptide levels above the 90th percentile, with weaker associations between glucose levels and primary cesarean delivery and clinical neonatal hypoglycemia (Metzger BE *et al.*). Positive associations were also found between increasing plasma glucose levels and each of the five secondary outcomes examined: premature delivery, shoulder dystocia or birth injury, intensive neonatal care, hyperbilirubinemia, and preeclampsia. Based on the HAPO Study and other studies, new criteria for the diagnosis of gestational

diabetes were proposed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) and adopted by the World Health Organization and others.

The primary aims of this current study were to assess whether in utero exposure to untreated gestational diabetes (using the IADPSG criteria) was associated with long-term risk of a disorder of glucose metabolism among mothers and greater adiposity among their children at 10 to 14 years post-partum. This study demonstrated that untreated gestational diabetes was associated with a higher risk for a maternal disorder of glucose metabolism at 10 to 14 years postpartum. The association of gestational diabetes with childhood overweight or obesity was not statistically significant. Gestational diabetes is a risk factor for type 2 diabetes and a meta-analysis of 20 studies that were performed prior to the development of the IADPSG criteria and that used varied criteria to diagnose gestational diabetes demonstrated that women with a history of gestational diabetes had a higher risk of developing type 2 diabetes compared with women with a normoglycemic pregnancy. The risk factors for progression to type 2 diabetes included maternal age, pre-partum and postpartum BMI, family history of type 2 diabetes, insulin treatment for gestational diabetes, and fasting glucose level during pregnancy. In the current study a larger population of women with lesser degrees of hyperglycemia (IADPSG-defined gestational diabetes) were at risk for progression to prediabetes and type 2 diabetes. The higher prevalence of obesity among women with gestational diabetes confounds the association of gestational diabetes with childhood obesity. Higher maternal BMI might be associated with higher childhood adiposity through shared genetics, familial lifestyle and environmental factors, and the intrauterine environment. Adjusting for maternal BMI attenuated the associations between gestational diabetes and childhood obesity in earlier studies, therefore raising the question whether the association of gestational diabetes with childhood adiposity is independent of maternal BMI. Further studies assessing the independent effects of maternal obesity/BMI and GDM on childhood obesity might help to explain these observations.

Reference

Metzger BE, Lowe LP, Dyer AR, *et al*; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*. 2008; 358(19):1991–2002.

Maternal Obesity and Long-term Infant Consequences

2.11. Metformin for women who are overweight or obese during pregnancy for improving maternal and infant outcomes

Dodd JM, Grivell RM, Deussen AR, Hague WM
Cochrane Database Syst Rev. 2018 Jul 24;7:CD010564.
<https://www.ncbi.nlm.nih.gov/pubmed/30039871>

This review evaluated the role of metformin in pregnant women with obesity or who are overweight, on maternal and infant outcomes, including adverse effects of treatment and costs.

Obesity and being overweight in pregnancy affect approximately 50% of women across low-income nations and is associated with a range of well recognized maternal and infant health complications. Maternal risks include gestational hypertension, pre-eclampsia and gestational diabetes; women are more likely to have their labour induced, and give to birth by caesarean section. Infants born to women with obesity or who are overweight in pregnancy have a higher risk of being of high birthweight or being large-for-gestational age, and of associated complications, including shoulder dystocia, jaundice and hypoglycaemia. It has been estimated that the costs of providing antenatal and postpartum care for women who are overweight are increased by 23% when compared with women of normal BMI, increasing further to 37% for women with obesity. Given these issues there has been considerable interest in providing antenatal dietary and lifestyle advice for women with obesity or who are overweight during pregnancy, as a strategy to limit gestational weight gain and improve maternal and infant health.

This review examined whether metformin has a role in improving health outcomes for pregnant women with obesity or who are overweight, and their babies. The review considered possible benefits, adverse effects and healthcare system costs. Three randomised controlled studies (1099 pregnant women) were found comparing metformin tablets with placebo taken by mouth from 10–20 weeks of pregnancy until birth (Chiswick C *et al*. ×3). The studies involved women with obesity (but not overweight). Women who were given metformin or placebo

during pregnancy had a similar risk of a baby being born large-for-gestational age. Metformin probably makes little or no difference in the risk of women developing gestational diabetes. Metformin may also have little or no difference in the risk of women developing gestational hypertension or pre-eclampsia. Women who were given metformin gained slightly less weight during pregnancy, but were more likely to experience diarrhoea. There were no other important differences identified for other maternal outcomes including, caesarean birth, giving birth before 37 weeks of pregnancy, shoulder dystocia perineal trauma or heavy bleeding after the baby has been born. Babies of women who were given metformin had similar birthweight to babies of women who were given placebo. No other important differences were identified for other infant outcomes of interest: hypoglycaemia, hyperbilirubinaemia, Apgar score at five minutes or death of the baby before or after being born.

Thus, there is insufficient evidence to support the use of metformin for women with obesity in pregnancy for improving outcomes for the mother and her baby. Metformin was associated with increased risk of adverse effects, particularly diarrhoea. More research is needed to evaluate the role of metformin in pregnant women with obesity or who are overweight, as a strategy for improving maternal and infant health, either alone or as an additional intervention.

References

Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, *et al.* Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled trial. *Lancet. Diabetes & Endocrinology* 2015;3(10):778–86.

Chiswick CA, Reynolds RM, Denison FC, Drake AJ, Forbes S, Newby DE, *et al.* Does metformin reduce excess birthweight in offspring of obese pregnant women? A randomised controlled trial of efficacy, exploration of mechanisms and evaluation of other pregnancy complications. *Efficacy and Mechanism Evaluation* 2016; Vol. 3, issue 7.

Chiswick CA, Reynolds RM, Denison FC, Whyte SA, Drake AJ, Newby DE, *et al.* Efficacy of metformin in pregnant obese women: a randomised controlled trial. *BMJ Open* 2015;5(1):e006854.

2.12. Maternal obesity impairs skeletal development in adult offspring

Chen JR, Lazarenko OP, Zhao H, Alund AW, Shankar K

J Endocrinol. 2018 Jul 26.

<https://www.ncbi.nlm.nih.gov/pubmed/30307152>

This study, using a mouse model, investigated the effects of high fat diet-induced maternal obesity on both fetal and adult offspring skeletal development.

Maternal nutrition appears to influence epigenetic alterations in the offspring and to program gene expression in key metabolic pathways, such as fatty acids and glucose metabolism. Maternal obesity caused by excessive consumption of a high-calorie, high-fat diet (HFD) has a profound influence on the health of cardiovascular and skeletal system of the offspring during fetal development and infancy, as well as later on in childhood and into adulthood. The relevance of epigenetic control in bone-related gene expression, bone development and organ homeostasis, as well as in the onset and progression of musculoskeletal diseases, has also been increasingly shown. Maternal obesity is known to program fetal pre-osteoblastic cell senescence signaling and glucose metabolism (Chen JR *et al.*).

This study, using a mouse model combined with analysis of osteoprogenitors from human obese mothers, provides evidence that maternal obesity regulates fetal osteoblastic cell senescence signaling and fetal and adult offspring skeletal development. Regardless of postnatal HFD challenge, adult offspring from HFD obese dams had suppressed bone formation. This phenomenon appeared to be much more robust in males compared to females. Such suppressed bone formation in adult offspring from HFD obese dams is a phenomenon of early bone involution/degeneration, and may be in part due to histone acetylation, (epigenetic regulation of genes involved in cell senescence signaling in pre-osteoblasts). The molecular basis of these observations seems complex and involves activation of CBP/p300 (co-activating proteins that interact with numerous transcription factors to increase the expression of their target genes) which in turn activates H3K27 acetylation (histone modification marker). This then leads to increases in cell senescence-related genes and PPAR γ expression (PPAR γ phosphorylation has a role in controlling bone mass) in osteogenic calvarial cells from HFD-obese dams, and in human umbilical cord mesenchymal stem cells isolated following delivery by obese and lean mothers. Thus, maternal high fat diet in mice has epigenetic effects on offspring bone formation and development.

Reference

Chen JR, Lazarenko OP, Blacbburn ML, Rose S, Frye RE, Badger TM, Andres A, Shankar K. Maternal Obesity Programs Senescence Signaling and Glucose Metabolism in Osteo-Progenitors From Rat and Human. *Endocrinology* 2016 Nov;157(11):4172–4183. Epub 2016 Sep 21.

2.13. Maternal obesity and the human milk metabolome: associations with infant body composition and postnatal weight gain

Isganaitis E, Venditti S, Matthews TJ, Lerin C, Demerath EW, Fields DA

Am J Clin Nutr. 2019 Apr 4

<https://www.ncbi.nlm.nih.gov/pubmed/30968129>

The study analyzed relationships between maternal obesity and human milk metabolites, infant body composition, and postnatal weight gain.

Maternal obesity is one of the strongest predictors of childhood obesity. Although the mechanism/s by which this obesity risk is transmitted to the offspring are not known differences in infant feeding and milk composition may play a role. This study used a metabolomics approach to analyze metabolites, nutrients and small molecules in human milk that differ according to both maternal and infant weight status. Infant body composition and milk composition were analyzed in parallel at 1 and 6 months postpartum.

The study found that maternal obesity is associated with modest differences in human milk metabolome. Levels of human milk metabolites were associated with infant weight status and identify a subset of metabolites correlating with maternal BMI and infant adiposity. These data suggest that obesity associated differences in human milk composition might contribute to early childhood obesity, although this needs further evaluation. Maternal obesity was associated with metabolomic signatures in human milk. At 1-month postpartum maternal BMI was modestly associated with the abundance of human milk oligosaccharides which are known to function as prebiotics, raising the possibility that obesity associated changes in maternal milk composition may modulate infant microbiome acquisition. At 6 months postpartum maternal BMI was associated with acylcarnitines, sugar alcohols and amino acid metabolites in human milk, a pattern reminiscent of the plasma metabolomics signature in obesity and type 2 diabetes.

The association between milk adenine and both maternal and infant weight status raises the possibility that some milk constituents might play a role in the mother-to-child transmission of obesity. Thus, maternal obesity leads to changes in the milk metabolome and this might have a role in transmission of obesity from mother to infant. Further studies in this area will help in understanding this interesting observation.

2.14. Association between maternal diabetes, being large for gestational age and breast-feeding on being overweight or obese in childhood

Kaul P, Bowker SL, Savu A, Yeung RO, Donovan LE, Ryan EA

Diabetologia 2019 Feb;62(2):249–258. doi: 10.1007/s00125-018-4758-0.

This is the first population-based study to examine the relative contribution of maternal diabetes, excess birthweight and breast-feeding on the risk of being overweight and obese in childhood.

Being large for gestational at birth is a potentially modifiable factor and this study highlights the need to better understand the factors associated with its incidence in order to develop strategies to reduce the number of children who are overweight/obese. Children who are large at birth are more likely to be obese in early childhood and maternal diabetes during pregnancy is associated with excess weight in the offspring during childhood. Breast feeding is associated with a lower risk of excess weight in childhood. Both large for gestational age and maternal diabetes during pregnancy are associated with an increased risk of the offspring being overweight/obese in early childhood. Large for gestational age is a stronger marker for risk of being overweight/obese in early childhood than maternal diabetes. Breast feeding is associated with a lower risk of being overweight/obese in a majority of children, however this association is not maintained in large for gestational age children of mothers with diabetes.

This study establishes that a larger proportion of excess weight in childhood can be attributed to being born large for gestational age than maternal diabetes during pregnancy. The findings should reinforce public health advice for women who are planning to get pregnant that, just like smoking, alcohol consumption and other lifestyle choices, their weight prior to, and weight gain and glycaemic control during, pregnancy may have a significant impact on the future health of their children.

Impact of Maternal Diet on Hypothalamic-Pituitary-Adrenal Function

2.15. Maternal high fat diet programs hypothalamic-pituitary-adrenal function in adult rat offspring

Niu X, Wu X, Ying A, Shao B, Li X, Zhang W, Lin C, Lin Y
Psychoneuroendocrinology. 2018 Dec 6;102:128–138.
<https://www.ncbi.nlm.nih.gov/pubmed/30544004>

This study investigated whether maternal high fat diet (HFD) exposure during rat pregnancy and lactation can alter the hypothalamic-pituitary-adrenal (HPA) in adult male offspring.

Maternal diet and metabolic status are important factors which influence the intrauterine and early postnatal environment that offspring experiences in early life. There is accumulating evidence supporting that perinatal overnutrition or high fat HFD consumption may result in developmental and health problems, such as metabolic syndrome, hypertension, cardiovascular remodeling and cerebrovascular dysfunction. Moreover, maternal HFD exposure also confers offspring susceptibility to behavioral disorders and mental problems, including anxiety, depression, cognitive deficit, impairments in social behavior and reward-based behaviors. As the core mediator of the neuroendocrine stress response, HPA axis is subject to programming by early life challenges.

This study provides further evidence for the long-lasting influence of maternal diet exposure on the development of the HPA axis in adult offspring. The experiments support the hypothesis that a fat rich diet during pregnancy and lactation fundamentally alters the activity of the HPA axis in adult offspring, under both stress-free and stressful conditions. The maternal HFD-induced remodeling of the HPA axis would ultimately not only affect the adult responsiveness to the stressful challenge, but also give rise to an offspring phenotype predisposed to the development of behavior disorders and other health problems in adult. The present study demonstrated, for the first time that a maternal diet affects the HPA response in a stressor-specific manner, with alternations of the neuroendocrine response to psychological and systemic but not metabolic stressors.

Moreover, the medial and central regions of the amygdala play an important role in the hyperresponsiveness of the HPA axis to psychological and systemic stress in maternal HFD offspring, respectively, suggesting that maternal HFD exposure may selectively modulate the HPA response to different incoming signals through differential neural pathways. Identification of the mechanisms and pathways that produce long-term vulnerability in response to perinatal environmental factors will facilitate development of clinical intervention and prevention strategies to reduce the incidence and severity of disease. Therefore, more studies need to be conducted to characterize the pathways and mechanisms by which maternal HFD consumption influences the HPA axis activity.

Fetal and Neonatal Cortisol Physiology

2.16. Characterization of human adrenal steroidogenesis during fetal development

Melau C, Nielsen JE, Frederiksen H, Kilcoyne K, Perlman S, Lundvall L, Thuesen LL, Juul Hare K, Andersson AM, Mitchell RT, Juul A, Jørgensen A
J Clin Endocrinol Metab. 2018 Dec 21. [Epub ahead of print]. doi: [10.1210/jc.2018-01759](https://doi.org/10.1210/jc.2018-01759).

This study aimed to investigate Human fetal adrenal (HFA) steroidogenesis by analyzing adrenal glands from 1st and 2nd trimester. Steroidogenesis in the HFA is tightly regulated throughout the first and second trimesters, which is crucial because the adrenal steroid hormones affect the overall intrauterine endocrine environment from early fetal development. Elevated levels of HFA androgens can be a consequence of dysregulated adrenal steroid pathways (for example in congenital adrenal hyperplasia). HFA steroidogenesis is not well characterized during fetal development, as previous studies investigating the steroidogenic function have focused on either the first or the second/third trimester only, characterizing selected adrenal steroidogenic enzymes.

This study therefore aimed to collect detailed expression data of all the classic steroidogenic enzymes and determine the intra-adrenal steroid levels from the first- and second-trimester HFAs in one inclusive study. This study provides detailed characterizations of both male and female adrenal endocrine functions during the first and second trimesters of human fetal development. It is evident from the gene and protein expression patterns of steroidogenic enzymes, as well as the steroid measurements in tissues, that the HFA functions as an active steroidogenic organ from early development by producing high levels of mineralocorticoids, glucocorticoids, and androgens. Even from 8 weeks of gestation, a distinct expression pattern for the investigated adrenal steroidogenic enzymes was noted, with a major increase in gene expression in second-trimester samples for the majority of steroidogenic enzymes, with the exception of the unaltered expression of *3β-HSD2* and *ARK1C3*. On the basis of the intra-adrenal steroid hormone concentrations, the study found that androstenedione was the most abundant adrenal androgen synthesized via the classic steroidogenic pathway throughout the first and second trimesters. Serum cortisol seems to be produced throughout the first and second trimesters.

Vitamin D Supplementation in Pregnancy and Fetal and Infant Growth

2.17. Vitamin D supplementation in pregnancy and lactation and infant growth

Roth DE, Morris SK, Zlotkin S, Gernand AD, Ahmed T, Shanta SS, Papp E, Korsiak J, Shi J, Islam MM, Jahan I, Keya FK, Willan AR, Weksberg R, Mohsin M, Rahman QS, Shah PS, Murphy KE, Stimec J, Pell LG, Qamar H, Al Mahmud A
N Engl J Med. 2018 Aug 9;379(6):535–546.
<https://www.ncbi.nlm.nih.gov/pubmed/30089075>

This study tested whether vitamin D supplementation from mid pregnancy to delivery or 6 months post-partum had an effect on length-for-age z scores at 1 year or on other anthropometric measures from birth to 1 year.

Bangladesh is a resource poor country where approximately 30% of newborns are small for gestational age, and the growth of 36% of children younger than 5 years of age is stunted (height-for-age z score, < -2). Vitamin D deficiency or insufficiency is thought to be common among pregnant women especially in Bangladesh and supplementation with vitamin D is given frequently to women of reproductive age. Vitamin D supplementation of 4000 IU/d for pregnant women is safe and most effective in achieving sufficiency in all women and their neonates regardless of race. Some previous studies have shown that prenatal vitamin D supplementation increased infant linear growth (Roth DE *et al.*, Brooke OG *et al.*). However, these previous studies were small, each involving fewer than 135 participants, and included postnatal growth as a post hoc outcome, and the between-group differences may have been due to chance. A meta-analysis of six trials of prenatal, multiple-micronutrient supplementation that included relatively low doses of vitamin D (200–400 IU per day) in low- and middle-income countries showed no effect on height at 2–8.5 years of age (Devakumar D *et al.*).

The findings of this large randomized, double-blind, placebo-controlled trial conducted in Bangladesh reports that vitamin D supplementation from mid pregnancy to delivery or 6 months post-partum had no significant effect on length-for-age z scores at 1 year or on other anthropometric measures from birth to 1 year. Similarly, vitamin D supplementation had no significant effect on numerous clinical outcomes during pregnancy or infancy. Vitamin D supplementation had no effect on length-for-age z scores at 1 year of age despite normalizing the maternal and infant serum concentrations of 25-hydroxyvitamin D, parathyroid hormone levels, and calcium and the urinary calcium: creatinine ratio.

Currently, the World Health Organization (WHO) does not recommend routine vitamin D supplementation during pregnancy. The findings of this study support that recommendation even in populations where vitamin D deficiency is relatively common.

References

Roth DE, Perumal N, Al Mahmud A, Baqui AH. Maternal vitamin D3 supplementation during the third trimester of pregnancy: effects on infant growth in a longitudinal follow-up study in Bangladesh. *J Pediatr* 2013; 163(6): 1605–1611.e3.

Brooke OG, Butters F, Wood C. Intrauterine vitamin D nutrition and postnatal growth in Asian infants. *Br Med J (Clin Res Ed)* 1981; 283: 1024.

Devakumar D, Fall CH, Sachdev HS, *et al.* Maternal antenatal multiple micronutrient supplementation for long-term health benefits in children: a systematic review and meta-analysis. *BMC Med* 2016; 14: 90.

3. Thyroid

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Preface

This year's THYROID chapter aims at summarizing important new knowledge on all aspects of the thyroid axis from fundamental and clinical research relevant for scientists and clinicians, ranging from studies on the function of primary cilia within the thyroid follicle over new genetic defects along the thyroid axis to new immunomodulatory therapeutic approaches for Graves' disease. These new insights provide better molecular understanding of thyroid hormone regulation and action in health and disease eventually opening new avenues of research and will hopefully add to improved clinical diagnosis and more stratified management of children affected by thyroid diseases.

Mechanism of the Year

3.1. Primary cilium in the human thyrocyte: changes in frequency and length in relation to the functional pathology of the thyroid gland

Fernández-Santos JM, Utrilla JC, Vázquez-Román V, Villar-Rodríguez JL, Gutiérrez-Avilés L, Martín-Lacave I

Thyroid. 2019;29:595–606.

<https://www.ncbi.nlm.nih.gov/pubmed/30767621>

Here, ultrastructural analysis of primary cilia in human thyroid follicles revealed a direct relationship between frequency and length of primary cilia and the functional state of the individual follicle in normal compared to hyperfunctioning thyroid tissues.

Primary cilia are present in many different tissues. They are involved in key processes of cell physiology during development and homeostasis, e.g. mediating intraocular pressure sensation. Human diseases associated with defective cilia function are called ciliopathies, affecting a broad range of organs such as eye, brain, kidney, liver, and many more, reviewed in detail by Reiter and Leroux [1]. Although primary cilia in the human thyroid have been described decades ago, their putative role for the angio-follicular unit of the thyroid has not been investigated so far. This study provides a detailed morphometric and electron microscopy study of normal thyroid tissues compared to nodular hyperplasia and Graves' disease tissues to describe quantitatively the changes of primary cilia in different conditions of thyroid activity.

The key result was that the number and length of primary cilia was significantly negatively associated with the functional level of the thyroid tissues. Analyzing > 1300 thyroid follicles and 43,000 thyrocytes, they showed lower number and shorter cilia length in hyperfunctioning tissues (nodular hyperplasia and Graves' disease) in contrast to normal thyroid tissues. The authors hypothesize that in analogy with other organs, where cilia have sensing functions for key physiological processes, thyroid follicular cell cilia might be involved in intrafollicular sensing of iodinated thyroglobulin and could have regulatory function for thyroid hormonogenesis for the thyrocyte. Recent work in the mouse model provide first molecular evidence for such regulatory mechanisms [2]. The question arises whether defects in thyroidal ciliogenesis could cause genetic hypothyroidism.

References

1. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol* 2017;18:533–47.
2. Qatato M, Szumska J, Skripnik V, Rijntjes E, Köhrle J, Brix K. Canonical TSH regulation of cathepsin-mediated thyroglobulin processing in the thyroid gland of male mice requires Taar1 expression. *Front Pharmacol*. 2018;9:221.PMID: 29615904.

3.2. Thyroid hormone signaling specifies cone subtypes in human retinal organoids

Eldred KC, Hadyniak SE, Hussey KA, Brenerman B, Zhang PW, Chamling X, Sluch VM, Welsbie DS, Hattar S, Taylor J, Wahlin K, Zack DJ, Johnston RJ Jr

Science. 2018;362(6411).

<https://www.ncbi.nlm.nih.gov/pubmed/30309916>

Here, developmental regulation of deiodinases DIO2 and DIO3 were identified as main regulators of thyroid hormone dependent sequential cone subtype specification in human retinal organoids. Either complete loss of thyroid hormone signaling or non-physiologically high thyroid hormone levels completely suppressed either medium and long wavelength cones or short wavelength cones, respectively.

Cone differentiation develops in a timely regulated manner. First, short wavelength cones develop in a low thyroid hormone context. By increasing thyroid hormone levels, short wavelength cone specification is blocked, while medium/long wavelength cone specification is induced. This article is a proof of concept of thyroid hormone dependent retinal cone differentiation in the human described earlier in the mouse model [1] and provides molecular evidence for retinal regulation of thyroid hormone concentrations by dynamic *DIO2* and *DIO3* gene expression during development of the human retina. It is further providing molecular evidence for direct effect of *in utero* hypothyroidism on visual abilities in the fetus that have been described in children exposed to maternal hypothyroidism *in utero*, children with congenital hypothyroidism, and preterm with hypothyroxinemia. Systematic studies revealed decreased contrast sensitivity and color vision processing [2, 3].

From this paper, we learn as clinicians to keep an eye on the vision of children exposed to hypo- or hyperthyroidism *in utero*.

References

1. Roberts MR, Srinivas M, Forrest D, Morreale de Escobar G, Reh TA. Making the gradient: thyroid hormone regulates cone opsin expression in the developing mouse retina. *Proc Natl Acad Sci U S A*. 2006;103:6218–23. PMID: 16606843.
2. Mirabella G, Westall CA, Asztalos E, Perlman K, Koren G, Rovet J. Development of contrast sensitivity in infants with prenatal and neonatal thyroid hormone insufficiencies. *Pediatr Res*. 2005;57:902–7. PMID: 15774837.
3. Simic N, Westall C, Asztalos EV, Rovet J. Visual abilities at 6 months in preterm infants: impact of thyroid hormone deficiency and neonatal medical morbidity. *Thyroid*. 2010;20:309–15. PMID: 20144040.

3.3. Genome-wide analyses identify a role for SLC17A4 and AADAT in thyroid hormone regulation

Teumer A, Chaker L, Groeneweg S, Li Y, Di Munno C, Barbieri C *et al*.

Nat Commun. 2018;9:4455.

<https://www.ncbi.nlm.nih.gov/pubmed/30367059>

Here, the largest genome-wide association study (GWAS) to date on thyroid function and dysfunction, in 72,167 individuals testing 8 million genetic variants, substantially increased the number of loci that are involved in the regulation of thyroid function. It provides functional evidence that two newly identified genes are involved in thyroid hormone transport and metabolism.

This extensive study adds to the understanding of our current knowledge in three ways: first, previous GWAS identified about 30 loci for thyroid function, explaining <9% of heritability in TSH and FT4 variation. The current study increased this number to 109 loci associated with thyroid function, replicating all known loci from earlier studies. Second, the authors calculated a genetic risk score based on combined effects of variants for TSH and FT4 levels, and showed significant associations with risk for overt hyperthyroidism and subclinical hypothyroidism. Finally, based on newly identified loci, the authors identified two new interesting genes for thyroid hormone action in target tissues: 1) *SLC17A4*, encoding a transport protein of the solute carrier family, and 2) *AADAT*, encoding a mitochondrial aminotransferase with broad substrate specificity. By *in vitro* studies, they provide functional evidence that *SLC17A4* is a yet unknown high-affinity transmembrane transporter of T3 and T4, and that *AADAT* effectively converts T3 and T4 to their pyruvic acid metabolites TK3 and TK4 by oxidative deamination of the

alanine side-chain of thyroid hormones. This pathway was described in 1957 by Wilkinson, but the responsible enzyme was not known until now [1, 2]. T3 and T4 conversion to TK3 and TK4 represents an alternative synthetic route of triiodothyroacetic acid (Triac) and tetraiodothyroacetic acid (Tetrac) [2].

Finally, as an outlook, the authors suggest future studies to investigate the use of the known markers to predict individual hypothalamo-pituitary-thyroid axis set points for more accurate individualized treatment of patients with thyroid diseases. In summary, these results clearly confirm the value of GWAS in the general population to identify new genes of interest, extend current knowledge of thyroid hormone physiology and open avenues for personalized thyroid disease treatment.

References

1. Wilkinson JH. Recent work on thyroid hormones. *Postgrad Med J* 1957;33:333–7.
2. Groeneweg S, Peeters RP, Visser TJ, Visser WE. Triiodothyroacetic acid in health and disease. *J Endocrinol* 2017;234:R99–121.

3.4. Non-thyroidal illness syndrome in critically ill children: prognostic value and impact of nutritional management

Jacobs A, Derese I, Vander Perre S, van Puffelen E, Verstraete S, Pauwels L, Verbruggen S, Wouters P, Langouche L, Garcia Guerra G, Joosten K, Vanhorebeek I, Van den Berghe G

Thyroid. 2019;29:480–492.

<https://www.ncbi.nlm.nih.gov/pubmed/30760183>

The thyroid axis is particularly responsive to critical illness. Adaptation processes of the thyroid axis to critical illness and prolonged fasting are well described as non-thyroidal illness syndrome. Non-thyroidal illness is mainly explained by two mechanisms: 1) peripheral inactivation of T4 to rT3 by decreased type-1 deiodinase (DIO1) and increased type-3 deiodinase (DIO3) and 2) if critical illness persists, by central suppression of TSH secretion and resulting decrease of T4.

The current publication was a preplanned secondary analysis of a randomized controlled multicenter study of early *versus* late parenteral nutritional in infants and children admitted to a pediatric ICU [1]. In this secondary analysis, the authors examined the effect of nutrition on thyroid hormone metabolism during critical illness, including 402 infants and 580 children. First, the authors investigated the prognostic value of non-thyroidal illness at ICU admission.

In multivariable analyses, including thyroid parameters (TSH, T4, T3, rT3 and T3/rT3 ratio) and baseline risk factors (risk of malnutrition, severity of illness by standardized scores), T4 was negatively associated with 90-day mortality and acquisition of new infection during ICU. Second, late parenteral nutrition (starting day 8 after admission) compared to early parenteral nutrition (starting within 24 hours after admission) had beneficial effects on the parameters of peripheral T4 inactivation (fT3 and T3/rT3 ratio) but adverse effects on the parameters of central thyroid regulation (TSH, T4) partly neutralizing the protective effect of late parenteral nutrition.

These data provide new insights into the pathophysiology of non-thyroidal illness and nutrition. As an outlook, the authors suggest to evaluate the effect of TRH to treat the central component of non-thyroidal illness in a randomized controlled study based on their current results and previous data in adults [2].

References

1. Fizez T, Kerklaan D, Mesotten D, Verbruggen S, Wouters PJ, Vanhorebeek I, Debaveye Y, Vlasselaers D, Desmt L, Caesaer MP, Garcia Guerra G, Hanot J, Joffe A, Tibboel D, Joosten K, van den Berghe G. Early *versus* late parenteral nutrition in critically ill children. *N Engl J Med* 2016;374:1111–1122.
2. Van den Berghe G, Wouters P, Weekers F, Mohan S, Baxter RC, Veldhuis JD, Bowers CY, Bouillon R. Reactivation of pituitary hormone release and metabolic improvement by infusion of growth-hormone-releasing peptide and thyrotropin-releasing hormone in patients with protracted critical illness. *J Clin Endocrinol Metab* 1999;84:1311–1323.

3.5. Levothyroxine in women with thyroid peroxidase antibodies before conception

Dhillon-Smith RK, Middleton LJ, Sunner KK, Cheed V, Baker K, Farrell-Carver S, Bender-Atik R, Agrawal R, Bhatia K, Edi-Osagie E, Ghobara T, Gupta P, Jurkovic D, Khalaf Y, MacLean M, McCabe C, Mulbagal K, Nunes N, Overton C, Quenby S, Rai R, Raine-Fenning N, Robinson L, Ross J, Sizer A, Small R, Tan A, Underwood M, Kilby MD, Boelaert K, Daniels J, Thangaratinam S, Chan SY, Coomarasamy A

N Engl J Med. 2019;380:1316–1325.

<https://www.ncbi.nlm.nih.gov/pubmed/30907987>

This large multicenter randomized double-blind placebo-controlled trial, in euthyroid women with thyroid peroxidase antibodies and a history of miscarriage or infertility, found no effect of levothyroxine substitution from before conception to the end of pregnancy on likelihood of live birth.

In 2011, Thangaratinam (co-author of this study) *et al.* reported a strong association between thyroid peroxidase antibodies with miscarriage and preterm birth, as discussed in the Yearbook 2012 thyroid chapter [1]. The study rationale was further supported by a few small trials indicating apparent benefits of levothyroxine substitution on higher live birth rates in TPO antibody positive women. However, the evidence was insufficient for the 2017 American Thyroid Association guidelines to recommend levothyroxine substitution in these women [2].

The presented data from this large randomized controlled trial, which screened almost 20,000 women for eligibility and included 470 women each in the control and the treatment arm, provide robust evidence for no advantage of levothyroxine treatment of TPO antibody positive women for the primary end point of number of live births. These results are important for counseling of women positive for TPO antibodies concerning miscarriage risk and suggest that non-thyroid hormone mechanisms are involved in TPO antibody associated miscarriages.

References

1. Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J, Coomarasamy A. Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. *BMJ.* 2011;342:d2616. PMID: 21558126.
2. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ, Peeters RP, Sullivan S. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid.* 2017;27:315–389. PMID: 28056690.

3.6. Association of maternal iodine status with child IQ: a meta-analysis of individual-participant data

Levie D, Korevaar TIM, Bath SC, Murcia M, Dineva M, Llop S, Espada M, van Herwaarden AE, de Rijke YB, Ibarluzea JM, Sunyer J, Tiemeier H, Rayman MP, Guxens M, Peeters RP

J Clin Endocrinol Metab. 2019 Mar 28. pii: jc.2018-02559. [Epub ahead of print]

<https://www.ncbi.nlm.nih.gov/pubmed/30920622>

This meta-analysis aimed to define the effect of mild to moderate iodine deficiency on child neurological outcome. The authors combined data from three large prospective national studies on 6180 mother child pairs with available urinary iodine and creatinine concentrations in pregnancy and child IQ.

Iodine status differed between the three cohorts, from sufficient (Generation R, The Netherlands [1]), to mildly deficient (INMA, Spain [2]) and moderately deficient (ALSPAC, UK [3]). Importantly, to provide robust results, the authors used statistical methods to harmonize laboratory results, and defined a set of possible confounding factors and approaches to avoid selection bias for drop-outs in the three cohorts.

The main result was a significant curvilinear association of urinary iodine/creatinine concentration with verbal but not for non-verbal IQ. However, the overall effect on verbal IQ was dependent on gestational age at measurement of urinary iodine, and ranged from -5 IQ points below 12th gestational week to -3 IQ points between 12th – 14th gestational weeks in the offspring. This association was present beyond the 14th gestational week.

This study adds to current knowledge that even mild to moderate iodine deficiency is associated with adverse neurodevelopmental outcome in offspring, if present during the first trimester. In view of these results, future

randomized controlled studies should start with screening for iodine deficiency and supplementation ideally before pregnancy.

References

1. Korevaar TI, Muetzel R, Medici M, Chaker L, Jaddoe VW, de Rijke YB, Steegers EA, Visser TJ, White T, Tiemeier H, Peeters RP. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study. *Lancet Diabetes Endocrinol.* 2016;4:35–43. PMID: 26497402.
2. Kooijman MN, Kruijthof CJ, van Duijn CM, Duijts L, Franco OH, van IJendoorn MH, de Jongste JC, Klaver CC, van der Lugt A, Mackenbach JP, Moll HA, Peeters RP, Raat H, Rings EH, Rivadeneira F, van der Schroeff MP, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius E, Felix JF, Jaddoe VW. The Generation R Study: design and cohort update 2017. *Eur J Epidemiol.* 2016;31:1243–64. PMID: 28070760.
3. Guxens M, Ballester F, Espada M, Fernández MF, Grimalt JO, Ibarluzea J, Olea N, Rebagliato M, Tardón A, Torrent M, Vioque J, Vrijheid M, Sunyer J; INMA Project. Cohort Profile: the INMA–Infancia y Medio Ambiente–(Environment and Childhood) project. *Int J Epidemiol.* 2012;41:930–40. PMID: 21471022.
4. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: the ‘children of the 90s’–the index offspring of the Avon Longitudinal Study of parents and children. *Int J Epidemiol.* 2013;42:111–27. PMID: 22507743.

Congenital Hypothyroidism

3.7. Incidence of congenital hypothyroidism over 37 Years in Ireland

McGrath N, Hawkes CP, McDonnell CM, Cody D, O’Connell SM, Mayne PD, Murphy NP

Pediatrics. 2018;142(4). pii: e20181199.

<https://www.ncbi.nlm.nih.gov/pubmed/30242075>

This unique study investigated the incidence of congenital hypothyroidism from the start of the newborn screening in 1979 to 2016. The authors report a marked increase in incidence from 0.27 cases per 1000 live births in 1979–1991 to 0.41 in 1992–2004 and 0.65 in 2005–2016.

A major strength of this study is that over the complete study period, there was no change in TSH cut-offs, TSH whole blood laboratory measurement technique, or population ethnicity, which were typical confounders in previous studies [1, 2, 3]. Further, diagnostic imaging and confirmatory thyroid hormone results were available in >80% of the whole patient cohort (n=1063).

There was a stable incidence of thyroid dysgenesis (athyreosis, ectopy, hypoplasia), but a significant increase of patients with gland *in situ* congenital hypothyroidism. The majority of these patients with gland *in situ* had initial TSH values between 20–100 mU/L, thus not just borderline results, in accordance with studies from France and Italy [1, 2]. When severity was categorized by FT4 according current definitions, the significant increase was observed in cases of mild congenital hypothyroidism (FT4 10–15 pmol/L). Finally, the authors provide data on the ratio of permanent (52%) *versus* transient (48%) forms of congenital hypothyroidism in patients with gland *in situ* after standardized reevaluation. The authors point out the only weakness of the study: missing data on iodine sufficiency in the general population of Ireland. However, assuming mild iodine deficiency, one would rather expect a gradual increase of severity of disease with the highest percentage in the lowest TSH range of 8–20 mU/L.

In summary, as clinicians we need to face increasing evidence of a shift of congenital hypothyroidism phenotype away from the dysgenetic forms to a structurally and functionally milder entity, however with clear indication for substitutive treatment at birth and more than 50% even after reevaluation in this study. The cause of this shift however remains unknown, opening avenues for further research.

References

1. Deladoëy J, Ruel J, Guiguère Y, van Vliet G. Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Québec. *J Clin Endocrinol Metab* 2011;96:2422–29.
2. Olivieri A, Fazzini C, Medda E; Italian Study Group for Congenital Hypothyroidism. Multiple factors influencing the incidence of congenital hypothyroidism detected by neonatal screening. *Horm Res Paediatr* 2015;83:86–93.

3. Barry Y, Bonaldi C, Goulet V, Coutant R, Léger J, Paty AC, Delmas D, Cheillan D, Roussey M. Increased incidence of congenital hypothyroidism in France from 1982 to 2012: a nationwide multicenter analysis. *Ann Epidemiol* 2016;26:100105.e4.

3.8. Newborn screening for primary congenital hypothyroidism: estimating test performance at different TSH thresholds

Knowles RL, Oerton J, Cheetham T, Butler G, Cavanagh C, Tetlow L, Dezateux C

J Clin Endocrinol Metab. 2018;103:3720–28.

<https://www.ncbi.nlm.nih.gov/pubmed/30113641>

This nationwide prospective surveillance study aimed to estimate the performance of the current UK recommended TSH threshold (10 mU/L on day 5 after birth) for newborn blood spot screening compared to lower thresholds: 8 mU/L and 6 mU/L. Over a 12-month period, the authors included all patients with positive TSH based newborn screening (n = 629) or suspected congenital hypothyroidism on clinical grounds (n = 21). Further, a three year follow-up period was added to exclude not confirmed cases and those who successfully discontinued levothyroxine (n = 214). Based on this data set, incremental changes in the detection rate, false-positive rate, and relative likelihood ratios were assessed to compare the three thresholds. The optimal TSH threshold was 8 mU/L for newborns screened at day 5 of life.

As clinicians, we learn that such threshold analyses, adapted to nation-specific practices, are important means of quality control and should help to optimize detection rates of newborn screening programs.

3.9. Early determinants of thyroid function outcomes in children with congenital hypothyroidism and a normally located thyroid gland: a regional Cohort Study

Saba C, Guilmin-Crepon S, Zénaty D, Martinerie L, Paulsen A, Simon D, Storey C, Dos Santos S, Haignere J, Mohamed D, Carel JC, Léger J

Thyroid. 2018;28:959–67.

<https://www.ncbi.nlm.nih.gov/pubmed/29968521>

This observational single center study aimed to identify predictors of transient *versus* permanent congenital hypothyroidism in patients with thyroid gland *in situ* diagnosed at birth. Strengths of the study are the prospectively documented clinical, biochemical and radiological parameters, the high inclusion rate, and the detailed outcome data during follow-up.

In a multivariate analysis, the authors identified one main predictor of transient congenital hypothyroidism: levothyroxine dose at 6 months <3.2 microgram/kg/d had a sensitivity of 71% and a specificity of 79% for predicting transient hypothyroidism. These results are the first to provide discriminative levothyroxine doses under treatment at such an early time point of life to distinguish between patients with transient forms *versus* permanent congenital hypothyroidism. Further, the findings show no evidence to support other often reported predictors, such as initial biochemical severity of congenital hypothyroidism, and thyroid morphology.

If validated prospectively, we as clinicians will get a new tool to trial-off patients from levothyroxine treatment much earlier than currently recommended. However, as stated by the authors, the long-term outcome of the transient disease group after ‘apparent’ successful discontinuation of substitutive therapy remains to be ascertained.

New Genes

3.10. Mutations in *IRS4* are associated with central hypothyroidism

Heinen CA, de Vries EM, Alders M, Bikker H, Zwaveling-Soonawala N, van den Akker ELT, Bakker B, Hoorweg-Nijman G, Roelfsema F, Hennekam RC, Boelen A, van Trotsenburg ASP, Fliers E

J Med Genet. 2018;55:693–700

<https://www.ncbi.nlm.nih.gov/pubmed/30061370>

This genetic study identified, by whole exome sequencing, mutations in the insulin receptor substrate 4 gene (*IRS4*) in 5 families with isolated central congenital hypothyroidism. Thus, the authors add a fifth genetic cause of isolated congenital hypothyroidism to the previously known genes: *TSHB*, *TRHR*, *IGSF1*, and *TBLX1*. Interestingly, as for *IGSF1* and *TBLX1*, *IRS4* is also located in the X-chromosome, resulting in X-linked inheritance. While detailed clinical phenotyping of affected male patients revealed all elements of central hypothyroidism, such as mildly decreased FT4 in the context of inadequately normal TSH, decreased basal, pulsatile and total TSH secretion over 24 hours, the phenotype could not be reproduced in *IRS4* knock-out mice. The general molecular role of the IRS family of proteins (IRS1-6) is to interact with tyrosine kinase receptors such as insulin, leptin, and insulin-like growth factor 1 (IGF-1) receptors and it is well known that starvation modifies (e.g. by leptin levels) the activity of the central regulation of the thyroid axis. However, the detailed mechanism how *IRS4* regulates the hypothalamo-pituitary-thyroid axis remains to be described.

3.11. Homozygous loss-of-function mutations in *SLC26A7* cause goitrous congenital hypothyroidism

Cangul H, Liao XH, Schoenmakers E, Kero J, Barone S, Srichomkwan P, Iwayama H, Serra EG, Saglam H, Eren E, Tarim O, Nicholas AK, Zvetkova I, Anderson CA, Frankl FEK, Boelaert K, Ojaniemi M, Jääskeläinen J, Patyra K, Löf C, Williams ED, UK10K Consortium, Soleimani M, Barrett T, Maher ER, Chatterjee VK, Refetoff S, Schoenmakers N
JCI Insight. 2018 Oct 18;3(20). pii: 99631.
<https://www.ncbi.nlm.nih.gov/pubmed/30333321>

This paper describes a new form of goitrous congenital hypothyroidism associated with mutations in the solute carrier family 26 member 7 gene (*SLC26A7*) in 6 unrelated families. In patients, a partial iodide organification defect (PIOD) with normal iodide uptake was observed, hence these mutations cause a new form of thyroid dysmorphogenesis. All patients were detected by neonatal screening, presenting with moderate to severe congenital hypothyroidism, while goiter was detected in only 8 of 15 patients, possibly due to differences in nutritional iodine uptake.

Additionally, *Slc26a7* knockout studies in mice reproduced the phenotype of thyroid dysmorphogenesis with goiter. However, *in vitro* studies showed reduced iodine uptake rather than an organification defect as the consequence of defective *Slc26a7* function. Thus, the molecular aspects could not be ultimately clarified and will need further investigations. Nevertheless, the authors provide robust genetic and clinical evidence for a novel form of thyroid dysmorphogenesis, in accordance with another independent publication on *SLC26A7* mutations [1].

Reference

1. Zou M, Alzahrani AS, Al-Odaib A, Alqahtani MA, Babiker O, I-Rijjal RA, BinEssa HA, Kattan WE, Al-Enezi AF, Al Qarni A, Al-Faham MSA, Baitei EY, Alsagheir A, Meyer BF, Shi Y. Molecular analysis of congenital hypothyroidism in Saudi Arabia: *SLC26A7* mutation is a novel defect in thyroid dysmorphogenesis. *J Clin Endocrinol Metab* 2018;103:1889–98.

3.12. *TUBB1* mutations cause thyroid dysgenesis associated with abnormal platelet physiology

Stoupa A, Adam F, Kariyawasam D, Strassel C, Gawade S, Szinnai G, Kauskot A, Lasne D, Janke C, Natarajan K, Schmitt A, Bole-Feysot C, Nitschke P, Léger J, Jabot-Hanin F, Tores F, Michel A, Munnich A, Besmond C, Scharfmann R, Lanza F, Borgel D, Poalk M, Carré A
EMBO Mol Med 2018;e9569:1–18
<https://www.ncbi.nlm.nih.gov/pubmed/30446499>

TUBB1 encodes a member of the beta-tubulin protein family. Beta-tubulins and alpha-tubulins form dimers, which assemble into microtubules belonging to the intracellular cytoskeleton structure.

The authors identified three different mutations in *TUBB1* by whole exome sequencing in a large cohort of patients with congenital hypothyroidism due to thyroid dysgenesis. The thyroid phenotype in the three families ranged from hemiagenesis/hypoplasia to ectopy. The biochemical phenotype was variable even within families, some patients detected by neonatal screening presented with mild to moderate congenital hypothyroidism at confirmatory testing, while some only being diagnosed by systematic familial screening after *TUBB1* mutation detection in family members. Interestingly, the patients also showed

macrothrombocytes as extrathyroidal pathology, a phenotype previously described for *TUBB1* mutations [1]. Extensive functional and developmental studies in mice provided evidence that the three described *TUBB1* defects resulted in non-functional alpha/beta tubulin dimers, which could not be assembled to microtubules and ultimately leading to thyroid dysgenesis and macrothrombocytes.

These findings extend the spectrum of proteins involved in normal thyroid development to members of the cytoskeleton and provides evidence for microtubule dysfunction resulting in macrothrombocytes and thyroid dysgenesis. Recently, *TUBB1* mutations were also identified in familial thyrombocytopenia, however thyroid function in those cases was not detailed [2]. Thus, further studies are necessary to completely elucidate the role of tubulins in thyroid and platelet development and function.

References

1. Kunishima S, Kobayashi R, Itoh TJ, Hamaguchi M, Saito H. Mutation of the beta1-tubulin gene associated with congenital macrothrombocytopenia affecting microtubule assembly. *Blood* 2009;113:458–61.
2. Matsumura T, Nakamura-Ishizu A, Takaoka K, Maki H, Muddineni SSNA, Wang CQ, Suzushima H, Kawakita M, Asou N, Matsuoka M, Kurokawa M, Osato M, Suda T. TUBB1 dysfunction in inherited thyrombocytopenia causes genome instability. *Br J Haematol* 2019;185:888–902.

Graves' Disease

3.13. Adjuvant rituximab, a potential treatment for the young patient with Graves' hyperthyroidism (RiGD): study protocol for a single-arm, single-stage, phase II trial

Cole M, Hynes AM, Howel D, Hall L, Abinun M, Allahabadia A, Barrett T, Boelaert K, Drake AJ, Dimitri P, Kirk J, Zammitt N, Pearce S, Cheetham T
BMJ Open. 2019;9:e024705.

<https://www.ncbi.nlm.nih.gov/pubmed/30670519>

This protocol paper describes an innovative phase II trial to study the effect of a single low dose of adjuvant rituximab (a chimeric anti-B-cell monoclonal antibody targeting the surface molecule CD20) compared to classical carbimazole therapy in adolescents and young adults with Graves' disease. Carbimazole treatment will be stopped after 12 months and the primary endpoint remission of Graves' disease will be analysed at 24 months.

This paper describes in detail the aims, background and expected outcome of the adjuvant administration of a single low dose of rituximab at diagnosis. The immunomodulatory approach in Graves' disease in children is convincing, based first on current knowledge on the efficacy and safety of rituximab in children with other autoimmune diseases, and second on its effects in adults with Graves' disease with or without Graves' orbitopathy.

The results of this proof of concept study will be important before planning phase III controlled randomized trials in larger cohorts. The following limitations are inherent to this study design: small patient number, inclusion of pubertal and post-pubertal patients with potentially different disease course than pre-pubertal children, and short follow-up period. Nevertheless, this combined treatment approach for Graves' disease could result in a higher rate of remission in children and adolescents due to possible synergistic immunomodulatory effects of rituximab and carbimazole, potentially reduced cumulative carbimazole dose lowering the rate of side effects of carbimazole and, last but not least, fewer patients who will need total thyroidectomy or radioiodine ablation.

We look forward to the results of this first, and hopefully not the last, study investigating this promising new therapeutic approach for children with Graves' disease.

3.14. Long-term methimazole therapy in juvenile Graves' disease: a randomized trial

Azizi F, Takyar M, Madreseh E, Amouzegar A
Pediatrics. 2019;143(5).
<https://www.ncbi.nlm.nih.gov/pubmed/31040197>

This randomized prospective long-term study investigated the effect of long-term (8–10 years) *versus* short-term (18–24 months) methimazole therapy for Graves' disease in 56 Iranian adolescents. The primary endpoint was disease remission at 48 months after stopping methimazole.

The authors observed a significantly higher rate of ongoing remission in the long-term treatment group than in the short-term treatment group after 4 years of methimazole weaning: 88% of patients treated for at least 8 years with slowly decreasing doses were in remission compared to 33% in the short-term treatment group. There were no side effects during the long-term treatment observed.

The strength of this study are its randomized prospective design with a long follow-up. The promising results of efficacy and safety are in accordance with a recent meta-analysis of long-term anti-thyroid drugs for Graves' diseases in adults and children with reduction of relapse of 19% and 14% of every year of anti-thyroid drug treatment in adults and children [1] and a pediatric study from 1987 [2].

However, by design, the 2 study groups differed substantially in disease duration at final follow-up (up to 8 years longer in the long-term treatment group). Furthermore, as the study cohort consisted of adolescent patients (mean age 15 years) longer treatment periods might be necessary for children diagnosed before 10 years of age to achieve comparable efficacy. Nevertheless, these data provide further evidence to offer adolescents an alternative to total thyroidectomy or radioiodine ablation in case of relapse after the standard treatment period of 2 years anti-thyroid medication.

References

1. Azizi F, Malboosbaf R. Long-term anti-thyroid drug treatment: a systematic review and meta-analysis. *Thyroid* 2017;27:1223–31.
2. Lippe BM, Landaw EM, Kaplan SA. Hyperthyroidism in children treated with long term medical therapy: twenty-five percent remission every two years. *J Clin Endocrinol Metab* 1987;64:1241–45.

Thyroid Cancer

3.15. Pediatric thyroid cancer incidence and mortality trends in the United States, 1973–2013

Qian ZJ, Jin MC, Meister KD, Megwalu UC
JAMA Otolaryngol Head Neck Surg. 2019 Jul 1;145(7):617–623
<https://www.ncbi.nlm.nih.gov/pubmed/31120475>

Differentiated thyroid cancer represents 2–4% of all pediatric malignancies. In adults, increasing incidence rates have been reported over the last decades. Data on trends in pediatric patients are scarce. This paper reports the 40-year incidence rate of differentiated thyroid cancer in patients younger than 20 years and calculated annual % changes to detect trends most accurately.

The key finding was a marked change in annual % change of differentiated thyroid cancer from 1.1% (1973–2006) to 9.56% (2006–2013). This change was evident for small (<20 mm) and large (>20 mm) tumours as well as for extended and localized disease, suggesting that enhanced diagnosis of thyroid cancer was not the only reason for this trend. In contrast to the steep rise in rates of papillary histology, the incidence of follicular histology remained at low and stable rates over 40 years. There was a higher incidence in female than male patients. White patients represented 83.2% and the age group of 15–20 years represented 76.6% of all affected patients. Mortality was very low.

In summary, the authors describe a significant increase in incidence rate of differentiated thyroid cancer from before *versus* after 2006. These results are in accordance with a second study of trends of pediatric thyroid cancer incidence from 1998–2013 published in 2019 [1]. However, neither study provides any explanation for this trend.

As a consequence of these data, and despite the discussion of over-diagnosis of thyroid cancer in adults and children, we as clinicians need to develop an appropriate clinical awareness and suspicion for thyroid cancer in pediatric patients.

Reference

1. Bernier MO, Withrow DR, Berrington de Gonzalez A, Lam CJK, Linet MS, Kitahara CM, Shiels MS. Trends in pediatric thyroid cancer incidence in the United States, 1998–2013. *Cancer* 2019; doi:10.1002/encr.32125 [Epub ahead of print].

New Guidelines

3.16. 2018 European Thyroid Association (ETA) Guidelines on the diagnosis and management of central hypothyroidism

Persani L, Brabant G, Dattani M, Bonomi M, Feldt-Rasmussen U, Fliers E, Gruters A, Maiter D, Schoenmakers N, van Trotsenburg ASP

Eur Thyroid J. 2018;7:225-37. doi: 10.1159/000491388. Epub 2018 Jul 19

<https://www.ncbi.nlm.nih.gov/pubmed/30374425>

Diagnosis and treatment of central hypothyroidism are much more complex than for primary hypothyroidism. For the first time, guidelines summarize all the available evidence to provide a thorough state of the art summary of current knowledge for this condition. Based on this review of the literature, the authors make 34 recommendations for optimal care of affected patients. These range from diagnostic criteria, indication for genetic testing, starting dose and dose adaptations of levothyroxine in the context of different conditions, and treatment monitoring during follow-up. For example, recommendation 3 suggests to consider the diagnosis of central congenital hypothyroidism in the context of low FT4 and slightly increased TSH (<10 mU/l, or inappropriately lower than expected from the FT4 level), stressing the clinical experience of even slightly increased TSH levels in this context. Recommendation 11 states that this biochemical constellation should be confirmed at least on two separate occasions for a definitive diagnosis.

The strength of these guidelines is that it comprises recommendations for adults and pediatric patients, and precisely defines specific needs of patients with congenital central hypothyroidism. However, the authors stress that all of their recommendations were developed in the complete absence of randomized controlled trial evidence on the diagnosis and treatment of central hypothyroidism, an important aspect for future studies.

4. Growth and Growth Factors

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Preface

This selection of articles in the field of growth and growth factors is characterized by a number of papers unravelling novel pathophysiological mechanisms underlying some forms of severe short stature. Growth plate gene variants have been described in a high proportion of children with severe familial short stature and/or born small for gestational age. Besides, some studies have enriched the knowledge of IGF system physiology showing the key role of IGF-1 receptor in health and life span, the involvement of IGF-I and its receptor in the development and maintenance of non-alcoholic fatty liver diseases and the capacity of IGF-II to modulate the innate immune memory of macrophages. Promising clinical trials have been published in the last year reporting the short-term efficacy of GH therapy in patients with Temple syndrome, the effectiveness of GH in improving mental and motor development in young children with Prader-Willi syndrome, the effect of GH treatment on growth of children with mutations of IGF-I receptor, the impact of combined GH/GnRH analogs on adult height of children with idiopathic short stature and of rhIGF-I/rhIGFBP-3 administration on the health outcomes of very preterm infants. We aimed to provide a balanced mixture of clinical practice papers and food for thought reports paving the way for further understanding of the fascinating physiology of growth and growth factors.

Important for Clinical Practice

4.1. Growth hormone improves short-term growth in patients with temple syndrome

Brightman DS, Lokulo-Sodipe O, Searle B, Mackay DJG, Davies JH, Temple IK, Dauber A
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Horm Res Paediatr. 2018;90:407–413.

<https://www.ncbi.nlm.nih.gov/pubmed/30836360>

Temple syndrome (TS) is a rare imprinting disorder caused by the dysregulation of imprinted genes in the chromosomal region 14q32 [1]. Most cases (approximately 70–80%) are caused by maternal uniparental disomy of chromosome 14. Paternal deletions and primary imprinting defects involving chromosomal region 14q32 can be, though rarely, implicated. Patients typically have impaired pre- and postnatal growth, associated with heterogeneous clinical features including hypotonia, facial dysmorphic characteristics, motor delay, feeding difficulties in infancy, early puberty, small hands and feet and truncal obesity.

Short stature is a common feature of these patients and many children with Temple syndrome undergo growth hormone (GH) treatment with the indication of short stature secondary to being born small for gestational age (SGA).

This retrospective study reviewed the clinical characteristics of 14 patients (aged 2.5–28 years) with molecular diagnosis of TS, seven of whom were treated with GH (median dose 0.04 mg/kg/day) for 12 months.

The response to GH treatment was available in only six patients. Mean height gain was 1.31 SDS after 1 year of treatment and the median increase of height velocity was 5.3 cm/year. The results show the efficacy of GH treatment in the short-term and suggest that patients with TS could be considered for GH treatment, independently of being born SGA.

Due to the rareness of this condition, whose prevalence is probably underestimated, only few sporadic data on the efficacy of GH therapy were previously reported. On the basis of their results, the authors strongly support the use of GH in short children with TS. However, the conclusions should be taken with caution for the small number of treated patients and the potential accelerator effect of GH therapy on bone maturation [2] even more worrying in TS children who often develop precocious puberty. Further controlled studies on larger cohorts and longer observations, up to the achievement of adult height, are needed to evaluate GH efficacy and safety in this condition.

References

1. Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. *J Med Genet* 2014;51:495–501.
2. Kagami M, Nagasaki K, Kosaki R, Horikawa R, Naiki Y, Saitoh S, Tajima T, Yorifuji T, Numakura C, Mizuno S, Nakamura A, Matsubara K, Fukami M, Ogata T. Temple syndrome: comprehensive molecular and clinical findings in 32 Japanese patients. *Genet Med* 2017;19:1356–1366.

4.2. Improved mental and motor development during 3 years of GH treatment in very young children with Prader-Willi syndrome

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<https://www.ncbi.nlm.nih.gov/pubmed/30113638>

Prader-Willi syndrome (PWS) is a rare genetic disorder secondary to absent expression of the paternal active genes in the PWS critical region of chromosome 15. 70% of patients have a microdeletion, 28% a uniparental disomy (UPD) and 1% an imprinting defect. PWS has an estimated incidence rate of 1 in 25,000 live births and represents the most common form of genetic obesity. The clinical phenotype of PWS patients includes neonatal hypotonia, endocrine defects, scoliosis, developmental delay, cognitive impairment, and characteristic facial appearance, besides severe obesity [1,2]. GH therapy in PWS patients contributes to improve growth, body composition, resting energy expenditure, motor development, muscle strength, exercise tolerance, bone health and lipid profiles [3]. It has recently been reported that short-term GH therapy may improve neurological and motor skills in children with PWS [4].

This study, a large prospective patient cohort, for the first time reports the long-term effectiveness of GH therapy in improving motor and mental development in children affected by genetically confirmed PWS. 63 children and toddlers with PWS (median age 1 year) were evaluated by Bayley Scales of Infant Development II (BSIDII) tests over the 3 years of GH treatment.

The results showed a significant improvement of mental and motor skills during treatment, reducing the performance gap between PWS children and healthy control children. In particular baseline mental and motor development were 58.1% and 41.9% vs. healthy reference subjects ($P < 0.001$) and increased to 79.6 and 78.2%, respectively, after three years of treatment. A younger age at start of GH treatment was associated with a greater improvement in both mental and motor development. The head circumference increased from -1.0 SDS at baseline to 0.1 SDS after 3 years ($P < 0.01$) but this change was not associated with the course of mental and motor development. At least part of the observed improvement of mental and motor development may be explained by spontaneous improvement of hypotonia with age and early start of physical therapy. Since IGF-I is involved in brain development and myelinization, the observed effect of GH treatment is probably mediated by the neurotrophic actions of IGF-I [5].

The lack of an untreated group is the major weakness of this study, however, a prospective randomized control trial is unethical since GH therapy is started in all patients with genetic confirmation of PWS. On the basis of these results showing a better psychomotor development when GH treatment is started at a younger age, the initiation of GH treatment in young infants with PWS should be encouraged.

References

1. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genet Med* 2012; 14: 10–26.
2. Grugni G, Crinò A, Bosio L et al. The Italian National Survey for Prader-Willi syndrome. An epidemiologic study. *Am J Med Genet A* 2008; 146: 861–872.
3. Deal CL et al. Growth Hormone Research Society Workshop Summary. Consensus Guidelines for Recombinant Human Growth Hormone Therapy in Prader-Willi Syndrome. *J Clin Endocrinol Metab* 2013;98:E1072–87.
4. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Willem Pilon J, Lunshof LB, Hokken-Koelega AC. Beneficial effects of growth hormone treatment on cognition in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study. *J Clin Endocrinol Metab*. 2012;97:2307–2314.
5. Nyberg F, Hallberg M. Growth hormone and cognitive function. *Nat Rev Endocrinol*. 2013;9:357–365.

4.3. High prevalence of growth plate gene variants in children with familial short stature treated with growth hormone

Plachy L, Strakova V, Elblova L, Obermannova B, Kolouskova S, Snajderova M, Zemkova D, Dusatkova P, Sumnik Z, Lebl J, Pruhova S

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Short stature is the most common reason for referral to pediatric endocrinologists. Familial short stature (FSS) is used to describe a child with a stature below the normal but within the parental target range and with at least one short parent.

In the last years, there has been a widespread use of genetic analysis to identify the etiology of short stature and a high number of loci, accounting for approximately 30% of adult height variation, have been identified [1]. Milder forms of FSS are likely related to a polygenic inheritance whereas severe forms more often depend on a single gene anomaly. Monogenic causes involve either growth plate-related genes such as SHOX, ACAN, FGFR3, NPR2, COL11A1, COL9A2, COL2A2 or GH/IGF-I axis genes [2]. The current workup for short stature usually identifies the etiology in 1-40% of subjects [3] and most FSS cases remain without a definite diagnosis.

In this study, 33 children with severe familial short stature (FSS), treated with GH for SGA or GHD indications, underwent whole-exome sequencing (WES). This identified the underlying genetic cause in half of the patients (17/33 subjects, 52%), with a high prevalence of growth plate single-gene variants, especially in SGA subjects.

By applying next generation sequencing to FSS subjects, this study shows for the first time that monogenic conditions are a frequent cause of FSS and suggests that growth plate-related gene variants, usually involved in bone/cartilage dysplasias [4], should be investigated in subjects with severe FSS born SGA, even without disproportionate short stature. Furthermore, this study has also the merit to clearly demonstrate the pitfalls associated with the workup of short stature often leading to the erroneous diagnosis of GHD. Indeed, only 1/23 children with clinically diagnosed GHD was shown to carry a genetic variant affecting GH secretion whereas 7 GHD patients were shown to carry growth plate-related, IGF-related or Noonan syndrome-related gene variants [5]. The major limitation of the study is the lack of functional studies. Nevertheless, this study paves the way for a novel approach to the child with FSS based on the molecular characterization, thus favoring a tailored monitoring and management.

References

1. Murray PG, Clayton PE, Chernausk SD. A genetic approach to evaluation of short stature of undetermined cause. *Lancet Diabetes Endocrinol* 2018;6:564–574.
2. Hattori A, Katoh-Fukui Y, Nakamura A, Matsubara K, Kamimaki T, Tanaka H, Dateki S, Adachi M, Muroya K, Yoshida S, Ida S, Mitani M, Nagasaki K, Ogata T, Suzuki E, Hata K, Nakabayashi K, Matsubara Y, Narumi S, Tanaka T, Fukami M. Next generation sequencing-based mutation screening of 86 patients with idiopathic short stature. *Endocr J* 2017;64:947–954.
3. Dauber A, Rosenfeld RG, Hirschhorn JN. Genetic evaluation of short stature. *J Clin Endocrinol Metab* 2014;99:3080–3092.

- Zhang H, Yang R, Wang Y, Ye J, Han L, Qiu W, Gu X. A pilot study of gene testing of genetic bone dysplasia using targeted next-generation sequencing. *J Hum Genet* 2015;60:769–776.
- Baron J, Savendahl L, De Luca F, Dauber A, Phillip M, Wit JM, Nilsson O. Short and tall stature: a new paradigm emerges. *Nat Rev Endocrinol* 2015;11:735–746.

4.4. Phenotypic features and response to growth hormone treatment of patients with a molecular defect of the IGF-1 receptor

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The IGF receptor gene *IGF1R* is located at 15q26.3 locus and encodes for a tyrosine kinase receptor which mediates the IGF-I biological actions. The key role of IGF-IR in growth and development was proved in *IGF1R* null mice that had severely impaired prenatal growth and invariably died at birth from respiratory insufficiency [1]. Consistently, only two patients with homozygous mutations have been reported so far [2], suggesting that in humans only mild homozygous mutations of *IGF1R* are compatible with survival. The cases reported with a lack of *IGF1R* are either heterozygous carriers [3,4] or, in only three patients, compound heterozygous carriers [5].

IGF1R defects are associated with both intrauterine and postnatal growth failure, microcephaly, and IGF-I levels above the reference range, although IGF-I levels can be initially low for feeding problems. *IGF1R* defects are recognized in SGA subjects [3, 6]. Interestingly, terminal deletions of chromosomal region 15q, including the *IGF1R* locus, have been reported in patients with impairment of growth and development and abnormalities in the skeleton and heart. The diagnosis of *IGF1R* defects can be challenging due to the broad phenotypic variability. Conflicting data on the efficacy of GH therapy in children with *IGF1R* heterozygous mutations have been reported so far.

This retrospective study based on the clinical data of 32 patients with *IGF1R* defects, proposed a novel clinical score for the diagnosis of *IGF1R* mutations, inspired by the score used to diagnose Silver-Russell syndrome. This *IGF1R* score is based on birth size, height (Ht) and head circumference (HC). A score ≥ 3 (birth weight and/or length SDS < -1 ; Ht at presentation < -2.5 SDS; HC at presentation < -2 SDS; and IGF-I > 0 SDS) had a sensitivity of 76% in identifying patients harboring *IGF1R* defects. The score was then applied to a large cohort ($n=372$) of patients born SGA, with sensitivity and specificity of 75% and 69%, respectively.

The 19 children treated with GH were stratified into two groups: group 1 with *IGF1R* pathogenic mutations and group 2 with 15q deletions including *IGF1R*. Overall, responses to GH therapy were moderate. Patients from group 1 gained an average of 0.50, 0.65 and 0.91 SDS in height during the 1, 2 and 3 first years of therapy, respectively. For patients from group 2 this was 0.75, 1.10 and 1.30 SDS, respectively. In children born SGA, height gain was 0.90, 1.45 and 1.82 SDS, respectively. In the 6 patients for whom data on adult height were available, mean adult Ht SDS was -2.0 (range -3.5 to -0.6), compared to an initial height SDS of -3.4 SDS (range -5.5 to -1.2). The mean adult height gain was 1.0 SDS after a variable duration of treatment ranging from 1.25 to 9.58 years. These results suggest that in the first years of GH treatment, linear growth increases less than documented for patients with SGA, in spite of considerably higher serum IGF-I concentrations. However, the adult height gain may be similar.

This is the largest cohort of patients with *IGF1R* defects described so far. The merit of this study is to provide a comprehensive view of the clinical features, biochemistry, and response to long-term GH therapy according to the underlying genetic defect. Moreover, the proposed novel clinical score will be extremely helpful in assisting the physician to select patients for genetic testing.

References

- Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 1993;75:59–72.

2. Gannage-Yared MH, Klammt J, Chouery E, Corbani S, Megarbane H, Abou Ghoch J, Choucair N, Pfaffle R, Megarbane A. Homozygous mutation of the IGF1 receptor gene in a patient with severe pre- and postnatal growth failure and congenital malformations. *Eur J Endocrinol* 2013;168:K1–7.
3. Leal AC, Montenegro LR, Saito RF, Ribeiro TC, Coutinho DC, Mendonca BB, Arnhold IJ, Jorge AA. Analysis of the insulin-like growth factor 1 receptor gene in children born small for gestational age: *in vitro* characterization of a novel mutation (p.Arg511Trp). *Clin Endocrinol* 2013;78:558–563.
4. Harmel EM, Binder G, Barnikol-Oettler A, Caliebe J, Kiess W, Losekoot M, Ranke MB, Rappold GA, Schlicke M, Stobbe H, Wit JM, Pfaffle R, Klammt J. Alu-mediated recombination defect in IGF1R: haploinsufficiency in a patient with short stature. *Horm Res Paediatr* 2013;80:431–442.
5. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, Kiess W, Klammt J, Kratzsch J, Osgood D, Pfaffle R, Raile K, Seidel B, Smith RJ, Chernausk SD. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med* 2003;349:2211–2222.
6. Klammt J, Kiess W, Pfaffle R. IGF1R mutations as cause of SGA. *Best Pract Res Clin Endocrinol Metab* 2011;25:191–206.

New Therapeutic Options

4.5. The beneficial effect of combined GH/GnRHa therapy in increasing adult height outcome in children with ISS

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The current definition of idiopathic short stature (ISS) refers to a heterogeneous group of short children, in the absence of any underlying detectable cause, including both normal variants of growth and pathological conditions. ISS subjects have been reported to reach an average final height of -1.5 SDS in boys and -1.6 SDS in girls [1,2], but with wide variability due to the different underlying conditions. In 2003, the FDA approved the use of GH in ISS children in USA, but its efficacy seems modest [3]. By slowing bone maturation and pubertal progression, gonadotropin-releasing hormone analogues (GnRHa) may prolong the time available for growth and there could be a rationale for their use as therapeutic agents in children with ISS. Studies on the efficacy of GnRHa in ISS subjects have provided conflicting results [4,5,6]. The effects of combination therapy with GnRHa and rhGH are variable [8,9] and not all studies have assessed data on adult height. Consequently, the combined treatment of GnRHa and rhGH is not currently recommended for ISS children [7].

This retrospective observational study reports the efficacy of combined GH + GnRHa therapy in a cohort of 192 ISS subjects treated either with GH alone (70%) or with the combined therapy (30%). Combined therapy was administered for 1.5 to 3 years in the prepubertal group ($n=31$), to children with relatively early pubertal onset (chronological age: boys >9.5 and <11 ; girls >8.5 and <10) and fast transition (<1 year) from Tanner stage 2 to 3; and in the pubertal group ($n=27$), to adolescents who were already in mid-puberty (Tanner stage 3–4) at referral. Both GH alone and combined treatment were effective in improving adult height compared to predicted adult and target height; the benefits were more pronounced in children who were prepubertal at baseline. However, the adult height achieved by GH-treated patients was within the normal population range irrespective of the treatment regimen and most of the children, whether treated by GH alone or by combined GH/GnRHa, reached an adult height within their mid-parental height range.

These findings strongly suggest that most of the children enrolled in the trial had a constitutional delay of growth and puberty (CDGP), a normal variant of growth which does not affect the achievement of a normal adult height. Indeed, the current definition of ISS encompasses many children with CDGP who do not need any treatment for achieving a normal adult height. In this respect, this study lacks a control group of untreated ISS children. Another major limitation lies in the retrospective observational design and the consequent absence of a close matching between the study groups. Finally, a recent randomized controlled study has raised a safety issue for the potential negative impact of such combined therapy on the incidence of bone fractures [10].

References

1. Ranke MB, Grauer ML, Kistner K, Blum WF, Wollmann HA. Spontaneous adult height in idiopathic short stature. *Horm Res* 1995;44:152–157.
2. Rekers-Mombarg LT, Wit JM, Massa GG, Ranke MB, Buckler JM, Butenandt O, Chaussain JL, Frisch H, Leiberman E. Spontaneous growth in idiopathic short stature. European Study Group. *Arch Dis Child* 1996;75:175–180.
3. Deodati A, Cianfarani S. Impact of growth hormone therapy on adult height of children with idiopathic short stature: systematic review. *Bmj* 2011;342:c7157.
4. Yanovski JA, Rose SR, Municchi G, Pescovitz OH, Hill SC, Cassorla FG, Cutler GB, Jr. Treatment with a luteinizing hormone-releasing hormone agonist in adolescents with short stature. *N Engl J Med* 2003;348:908–917.
5. Municchi G, Rose SR, Pescovitz OH, Barnes KM, Cassorla FG, Cutler GB, Jr. Effect of deslorelin-induced pubertal delay on the growth of adolescents with short stature and normally timed puberty: preliminary results. *J Clin Endocrinol Metab* 1993;77:1334–1339.
6. Carel JC, Hay F, Coutant R, Rodrigue D, Chaussain JL. Gonadotropin-releasing hormone agonist treatment of girls with constitutional short stature and normal pubertal development. *J Clin Endocrinol Metab* 1996;81:3318–3322.
7. Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR, Antoniazzi F, Berenbaum S, Bourguignon JP, Chrousos GP, Coste J, Deal S, de Vries L, Foster C, Heger S, Holland J, Jahnukainen K, Juul A, Kaplowitz P, Lahlou N, Lee MM, Lee P, Merke DP, Neely EK, Oostdijk W, Phillip M, Rosenfield RL, Shulman D, Styne D, Tauber M, Wit JM. Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics* 2009;123:e752–762.
8. Balducci R, Toscano V, Mangiantini A, Municchi G, Vaccaro F, Picone S, Di Rito A, Boscherini B. Adult height in short normal adolescent girls treated with gonadotropin-releasing hormone analog and growth hormone. *J Clin Endocrinol Metab* 1995;80:3596–3600.
9. Kamp GA, Mul D, Waelkens JJ, Jansen M, Delemarre-van de Waal HA, Verhoeven-Wind L, Frolich M, Oostdijk W, Wit JM. A randomized controlled trial of three years growth hormone and gonadotropin-releasing hormone agonist treatment in children with idiopathic short stature and intrauterine growth retardation. *J Clin Endocrinol Metab* 2001;86:2969–2975.
10. Benabbad I, Rosilio M, Tauber M, Paris E, Paulsen A, Berggren L, Patel H, Carel JC. Growth hormone in combination with leuporelin in pubertal children with idiopathic short stature. *Endocr Connect* 2018;7:708–718.

Clinical Trials

4.6. rhIGF-1/rhIGFBP-3 in preterm Infants: A phase 2 randomized controlled trial

Ley D, Hallberg B, Hansen-Pupp I, Dani C, Ramenghi LA, Marlow N, Beardsall K, Bhatti F, Dunger D, Higginson JD, Mahaveer A, Mezu-Ndubuisi OJ, Reynolds P, Giannantonio C, van Weissenbruch M, Barton N, Tocoian A, Hamdani M, Jochim E, Mangili A, Chung JK, Turner MA, Smith LEH, Hellström A

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IGF-I plays a key role in fetal growth and development [1]. IGF-I exerts pleiotropic effects including cell proliferation, survival and differentiation, but also influencing metabolism and angiogenesis.

Very preterm newborns show a rapid decline serum IGF-I concentrations that remain low for the first weeks of life relative to corresponding fetal levels in utero. Lower IGF-I levels in extremely preterm infants have been associated with an increased risk of retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD), neurodevelopmental delay and growth impairment [2]. In animal models, IGF-I administration reduces the risk of developing oxygen-induced retinopathy [3] and has protective effects on lung and brain damage. A complex of recombinant human Insulin-like Growth Factor-I and recombinant human Insulin-like Growth Factor Binding Protein-3 (rhIGF-I/rhIGFBP-3) was developed to increase the half-life of rhIGF-I and reduce the incidence of side effects, mainly hypoglycemia, associated with the use of rhIGF-I alone. In a previous trial conducted by the same Swedish team, the infusion of rhIGF-I/rhIGFBP-3 in preterm infants increased circulating levels of IGF-I without major safety concerns [4].

This phase II, randomized, multicenter trial tested the efficacy and safety of rhIGF-I/rhGFBP-3 infusion in extremely premature infants. The primary efficacy outcome measure was the incidence of ROP, the secondary efficacy outcomes were the incidence of other morbidities and the discharge time. 121 infants born from 23 to 27 weeks gestational age were enrolled and randomized to standard care ($n=60$) or to continuous intravenous infusion of rhIGF-I/rhGFBP-3 (250 mcg/kg per 24 hours, from <24 hours of birth to postmenstrual age 29 weeks) ($n=61$) with the intention of maintaining serum IGF-I levels within 28–109 mcg/l. Target exposure (based on IGF-I levels and overall infusion duration) was achieved in only 24/61 treated subjects. ROP severity and incidence as well as discharge time and growth were unaffected by rhIGF-I/rhGFBP-3. The incidence and severity of bronchopulmonary dysplasia (BPD) were significantly reduced in infants treated with rhIGF-I/rhGFBP-3, and a tendency to milder forms of intraventricular hemorrhage was also observed. The rhIGF-I/rhGFBP-3 complex was well tolerated. Fatal SAEs were reported in 19.7% of treated infants compared with 11.7% of controls, although none was considered related to therapy.

Overall, although the results of this trial are inconclusive, the potential beneficial effect on BPD deserves further studies in larger cohorts of extremely preterm infants. However, it has to be pointed out that all intervention studies in this type of population are inevitably and heavily affected by a number of confounders such as late pregnancy events, modes of delivery, transfusions, infections, antibiotic/probiotic use, nutrition and comorbidities as well as the standard care provided in different neonatology units.

References

1. Hwa V, Fang P, Derr MA, Fiegerlova E, Rosenfeld RG. IGF-I in human growth: lessons from defects in the GH-IGF-I axis. *Nestle Nutr Inst Workshop Ser* 2013;71:43–55.
2. Hellstrom A, Engstrom E, Hard AL, Albertsson-Wikland K, Carlsson B, Niklasson A, Lofqvist C, Svensson E, Holm S, Ewald U, Holmstrom G, Smith LE. Postnatal serum insulin-like growth factor I deficiency is associated with retinopathy of prematurity and other complications of premature birth. *Pediatrics* 2003;112:1016–1020.
3. Vanhaesebrouck S, Daniels H, Moons L, Vanhole C, Carmeliet P, De Zegher F. Oxygen-induced retinopathy in mice: amplification by neonatal IGF-I deficit and attenuation by IGF-I administration. *Pediatr Res* 2009;65:307–310.
4. Hansen-Pupp I, Hellstrom A, Hamdani M, Tocoian A, Kreher NC, Ley D, Hallberg B. Continuous longitudinal infusion of rhIGF-1/rhIGFBP-3 in extremely preterm infants: Evaluation of feasibility in a phase II study. *Growth Horm IGF Res* 2017;36:44–51.

New Perspectives

4.7. Idiopathic short stature and growth hormone sensitivity in prepubertal children

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Idiopathic short stature (ISS) refers to a heterogeneous population of children with a height more than 2 SDS below the mean for age, sex, and population, in presence of normal birth size and body proportions, and without evidence of any identifiable cause. The use of next generation sequencing (NGS) has shown that a high proportion of subjects with ISS have specific genetic variants. The majority of these variants are in genes related to the growth plate cartilage and in the GH/IGF-I axis. Affected patients may have mild forms of skeletal dysplasia, or subtle hormonal abnormalities suggesting hormone insensitivity.

ISS subjects show normal GH responses to pharmacological stimulation tests. The finding of subnormal levels of IGF-I [1–3] associated with reduced serum GH binding protein (GHBP), the circulating fragment of the GH receptor encompassing its extracellular domain [4], has raised the suspicion of mild forms GH resistance in some of these children.

This study evaluated a cohort of 23 ISS and 23 sex and age-matched normal stature (NS) children. All patients underwent IGF-I generation test, as an index GH sensitivity, and a wide range of biochemical parameters was analyzed. ISS and NS children showed no differences in IGF-I generation. Among ISS children, a lower birth weight was associated with a lower percentage of IGF-I increase in the generation test. After four days of rhGH administration, GHBP levels decreased in ISS subjects but increased in NS children, whereas leptin levels increased in NS but did not change in ISS subjects. A higher response of insulin to rhGH administration was noted in ISS children.

Collectively these results show no reduction in GH induced IGF-I generation in ISS compared to normal children. The correlation between lower birth size and lower response to IGF-I generation test in ISS children, indicating lower GH sensitivity, is puzzling. Fetal growth is independent of GH as the fetal liver does not express GH receptors which start to be massively expressed only after birth.

References

1. Ranke MB, Schweizer R, Elmlinger MW, Weber K, Binder G, Schwarze CP, Wollmann HA. Significance of basal IGF-I, IGFBP-3 and IGFBP-2 measurements in the diagnostics of short stature in children. *Horm Res* 2000;54:60–68.
2. Blair JC, Camacho-Hubner C, Miraki Moud F, Rosberg S, Burren C, Lim S, Clayton PE, Bjarnason R, Albertsson-Wikland K, Savage MO. Standard and low-dose IGF-I generation tests and spontaneous growth hormone secretion in children with idiopathic short stature. *Clin Endocrinol* 2004;60:163–168.
3. Clayton P, Bonnemaire M, Dutailly P, Maisonnobe P, Naudin L, Pham E, Zhang Z, Grupe A, Thiagalingam A, Deneffe P. Characterizing short stature by insulin-like growth factor axis status and genetic associations: results from the prospective, cross-sectional, epidemiogenetic EPIGROW study. *J Clin Endocrinol Metab* 2013;98:E1122–1130.
4. El Kholly M, Mella P, Rashad M, Buzi F, Meazza C, Zahra S, Elsedfy HH. Growth hormone/IGF-I axis and growth hormone receptor mutations in idiopathic short stature. *Horm Res Paediatr* 2011;76:300–306.

4.8. Multigene sequencing analysis of children born small for gestational age with isolated short stature

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A child born with birth weight and/or birth length less than 2 SDS below the mean for gestational age is defined as SGA [1]. This definition implies that rather than a specific diagnostic group, SGA children are a heterogeneous population with different etiologies, growth patterns and metabolic outcomes. Most SGA children experience postnatal catch-up growth leading to the achievement of a normal stature within the first 2 years of age. Approximately 10% remain permanently below the third centile and those with a more severe growth impairment are considered candidates for GH therapy. A broad range of causes underlie SGA birth, as fetal growth is regulated by maternal, placental and fetal factors [2]. The recent use of next generation sequencing (NGS) in SGA children with severe short stature has permitted to discover novel genetic causes of intrauterine growth impairment. These genetic variants are involved in the physiology of IGF system, cartilage growth plate, cartilage extracellular matrix and paracrine factors.

In this study, a cohort of 55 short SGA subjects was assessed by whole-exome sequencing (WES) or targeted gene panel sequencing. Eight (15%) heterozygous pathogenic or likely pathogenic variants were identified. All these variants involved genes associated with growth disorders such as growth plate genes (Indian hedgehog, HH; Natriuretic peptide receptor 2, NPR2; Short stature homeobox, SHOX; Aggrecan, ACAN) and RAS/MAPK pathway genes (Neurofibromin 1, NF1 and Protein-tyrosine phosphatase nonreceptor-type 11, PTPN11). Seven patients were SGA only for birth length, and one patient was SGA for both length and weight. Six of the patients with pathogenic variants had a family history of short stature, but none had a phenotype suggestive of the detected genetic variant. The identification of molecular etiologies is important for: a) providing the diagnosis to the patients and parents, b) driving genetic counselling, c) leading to the right therapeutic strategy and d) for the advancement of science.

References

1. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *J Clin Endocrinol Metab* 2007;92:804–810.
2. Finken MJ, van der Steen M, Smeets CCJ, Walenkamp MJE, de Bruin C, Hokken-Koelega ACS, Wit JM. Children Born Small for Gestational Age: Differential Diagnosis, Molecular Genetic Evaluation, and Implications. *Endocr Rev* 2018;39:851–894.

4.9. Low IGF-I bioavailability impairs growth and glucose metabolism in a mouse model of human PAPP2 p.Ala1033Val mutation

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Pregnancy-associated plasma protein A2 (PAPP-A2) is a metalloproteinase which, by cleaving IGFBP-3 and IGFBP-5, releases free IGF-I from the ternary complexes and regulates its bioavailability. *PAPP2* gene mutations (p.D643fs25* and p.Ala1033Val) have recently been described in various members of two unrelated families. Affected patients have short stature, moderate microcephaly, thin long bones, mildly decreased bone density, insulin resistance, elevated total IGF-I and IGFBP-3 but low free IGF-I [1].

In this study, a mouse model harboring the human *Pappa2* p.Ala1033V mutation was generated using a knock-in strategy, leading to detectable protein levels of PAPP-A2 but without protease activities. Animals underwent a thorough characterization including anthropometry, glucose and insulin tolerance test, hormonal assessments (total IGF-I, free IGF-I, intact IGFBP-3, GH, insulin, ALS and PAPP2), bone morphology and bone mineral density evaluation.

Pappa2 mutation homozygous mice showed clinical features resembling those of patients: reduced body length and weight, higher liver weight associated with elevated IGFALS levels, higher fat mass percentage, slender bones and decreased bone length, and insulin resistance. Consistent with human data, although total IGF-I levels were increased, free IGF-I was reduced, thus explaining the impaired growth. The presence of insulin resistance may be secondary to GH hypersecretion, caused by the impaired IGF-I (i.e. less free IGF-I) negative feedback on pituitary. An alternative or complementary explanation for insulin resistance is the impaired IGF-I signaling secondary to reduced IGF-I bioavailability. Human IGF-I has ~50% homology with pro-insulin and can bind to both the IGF-I receptor (IGF1R) and, with reduced affinity, the insulin receptor (IR). Free IGF-I can signal via IGF-IR, insulin receptor or an insulin/IGF-IR hybrid receptor, stimulating the glucose transport into the muscle. Therefore, low free IGF-I levels in PAPP-A2 deficiency could per se contribute to insulin resistance.

Overall, these findings clearly show a close similarity between *Pappa2* mutation knock-in animals and the clinical picture of patients with *PAPP2* mutations. This animal model could be used to test new therapeutic options such as PAPP-A2 administration to increase free IGF-I levels in the cases of PAPP-A2 deficiency and, potentially, in patients with other conditions associated with severe short stature [2].

References

1. Dauber A, Munoz-Calvo MT, Barrios V, Domene HM, Kloverpris S, Serra-Juhe C, Desikan V, Pozo J, Muzumdar R, Martos-Moreno GA, Hawkins F, Jasper HG, Conover CA, Frystyk J, Yakar S, Hwa V, Chowen JA, Oxvig C, Rosenfeld RG, Perez-Jurado LA, Argente J. Mutations in pregnancy-associated plasma protein A2 cause short stature due to low IGF-I availability. *EMBO Mol Med* 2016;8:363–374.
2. Andrew M, Liao L, Fujimoto M, Khoury J, Hwa V, Dauber A. PAPP2 as a Therapeutic Modulator of IGF-I Bioavailability: in vivo and in vitro Evidence. *J Endocr Soc* 2018;2:646–656.

4.10. Associations of protein intake in early childhood with body composition, height, and insulin-like growth factor I in mid-childhood and early adolescence

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Early life nutrition has long-term effects on body composition and obesity risk. Breastfed infants have a lower risk of obesity, which may be related to the lower protein intake in human milk compared to infant formula. Recent studies have confirmed the association between high-protein intake during the first years of life and rapid growth rate and later obesity [1]. Avoiding high-protein intake in early life may thus represent a strategy to prevent childhood obesity [2]. The insulin-like growth factor (IGF) axis is sensitive to the nutritional status [3] and may represent the link between early nutrition and later body composition and growth. For instance, IGF-I levels during infancy are higher in non-breastfed infants and are related to later increase of linear growth [4].

This study included 1,165 US children of the Boston-area Project Viva (NCT02820402), a prospective cohort study of mother–child pairs examining associations of prenatal, perinatal, and early-life exposures with pregnancy and child health outcomes. The aim of this study was to investigate the association between protein intake early after infancy and later growth and body composition. The protein intake in early childhood (median 3.2 years) was tested for association with anthropometry, body composition and IGF-I concentrations in mid-childhood (median 7.7 years) and early adolescence (median 13 years). Several potential confounders (i.e. race, sociodemographic factors, parental and birth size, breastfeeding, physical activity and fast food intake) were included in the analysis.

Early protein intake was not associated with any of the mid-childhood parameters. Only in males, each 10-g increase in animal protein intake in early childhood was associated with higher BMI z-score (+0.12), lean mass (+1.34%) and IGF-I (+5.67%) in adolescence, suggesting that early protein intake may affect to a certain extent the puberty-related growth in boys. No association was observed in girls. A reasonable explanation for this sex dimorphism is that these early adolescent features may be driven primarily by pubertal sex steroids in girls. In boys, who have less advanced puberty in early adolescence, these features may be more strongly influenced by early life factors such as protein intake.

The major limitation of the study is the evaluation of dietary intake by Food Frequency Questionnaires that may have led to a possible over-reporting of protein intake. Furthermore, no information about child diets after early childhood was available and hence included in the analysis, thus leaving open the possibility of a major effect of mid-childhood and early-adolescence protein intake on the measured outcomes.

References

1. Weber M, Grote V, Closa-Monasterolo R, Escribano J, Langhendries JP, Dain E, Giovannini M, Verduci E, Gruszfeld D, Socha P, Koletzko B. Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial. *Am J Clin Nutr* 2014;99:1041–1051.
2. Putet G, Labaune JM, Mace K, Steenhout P, Grathwohl D, Raverot V, Morel Y, Picaud JC. Effect of dietary protein on plasma insulin-like growth factor-1, growth, and body composition in healthy term infants: a randomised, double-blind, controlled trial (Early Protein and Obesity in Childhood (EPOCH) study). *Br J Nutr* 2016;115:271–284.
3. Hawkes CP, Grimberg A. Insulin-Like Growth Factor-I is a Marker for the Nutritional State. *Pediatr Endocrinol Rev* 2015;13:499–511.
4. Ong KK, Langkamp M, Ranke MB, Whitehead K, Hughes IA, Acerini CL, Dunger DB. Insulin-like growth factor I concentrations in infancy predict differential gains in body length and adiposity: the Cambridge Baby Growth Study. *Am J Clin Nutr* 2009;90:156–161.

4.11. Late-life targeting of the IGF-1 receptor improves healthspan and lifespan in female mice

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“Somatopause” causes the physiological decline over time in GH secretion leading to low IGF-I levels in aging subjects. GH has been proposed as an anti-aging therapy, but with no evidence of beneficial effects and with some potential risks [1,2]. Therefore, current guidelines do not recommend GH therapy as anti-aging treatment [3].

Conversely, the possibility of slowing aging by negatively affecting GH signaling has been demonstrated in many animal models. Mutations that reduce GH activity were associated with increased longevity [2] as well as with reduced cancer and diabetes incidence. IGF-IR plays a key role in modulating mammalian lifespan [4] and its mutations have been described in centenarians [5]. Low IGF-I levels predict longer survival in females with exceptional longevity [6]. The mechanism by which reduced IGF-I signaling influences lifespan is unknown, both improved stress defenses and lower cancer susceptibility are likely involved. Collectively, these data suggest that the pharmacological modulation of GH pathway may be used to prolong lifespan.

This elegant study evaluated the effects of IGF-IR signaling inhibition on longevity. 18 months old CB6F1 male and female mice, weekly treated with IGF-IR monoclonal antibodies (L2-Cmu, Amgen Inc; 20 mg/kg) until 24 months of age or until natural death were investigated to determine the effects on aging outcomes. The authors showed that targeting IGF-IR signaling in late life of these mice improves aging. Consistently with previous reports, the major positive effects occurred in female mice, which experienced a 9% increase of lifespan, a reduced incidence of cancer and systemic inflammation, with restoration of some cytokines to a more youthful profile. No difference in weight or body composition was observed in females, whereas male animals showed decreased weight and lean mass. Insulin levels were unaffected in all tissues in both sexes. Endurance performance and strength as well as improvement in cardiac function were observed in female mice but not in males. The sex dimorphism may depend on a different hormonal milieu or on differences in drug metabolism. Interestingly, these effects on ageing occurred even though the treatment was initiated later in life when IGF-IR modulation is likely safer than in younger ages.

This study has the merit to experimentally prove the efficacy of selective targeting of IGF-IR in improving ageing. However, before planning to translate this evidence in clinical trials the protective effect of IGF-I towards osteoporosis, type 2 diabetes, cerebrovascular and cognitive decline has to be taken into account [6].

References

1. Liu H, Bravata DM, Olkin I, Nayak S, Roberts B, Garber AM, Hoffman AR. Systematic review: the safety and efficacy of growth hormone in the healthy elderly. *Ann Intern Med* 2007;146:104–115.
2. Junnila RK, List EO, Berryman DE, Murrey JW, Kopchick JJ. The GH/IGF-1 axis in ageing and longevity. *Nat Rev Endocrinol* 2013;9:366–376.
3. Thorer MO. Statement by the Growth Hormone Research Society on the GH/IGF-I axis in extending health span. *J Gerontol A Biol Sci Med Sci* 2009;64:1039–1044.
4. Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloën A, Even PC, Cervera P, Le Bouc Y. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 2003;421:182–187.
5. Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilai N, Cohen P. Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci U S A* 2008;105:3438–3442.
6. Milman S, Atzmon G, Huffman DM, Wan J, Crandall JP, Cohen P, Barzilai N. Low insulin-like growth factor-1 level predicts survival in humans with exceptional longevity. *Aging Cell* 2014;13:769–771.

4.12. Expression of insulin-like growth factor I and its receptor in the liver of children with biopsy-proven NAFLD

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NAFLD is a multifactorial disease characterized by an abnormal accumulation of fat in the liver without a history of significant alcohol intake. NAFLD is the most common form of chronic liver disease, affecting 30% of adults and 10% of children in the USA [1]. Alarmingly, its prevalence is increasing worldwide paralleling to the increased incidence of obesity in both adults and children. Insulin resistance, oxidative stress and inflammation are involved in the development of NAFLD. No pharmacological therapy is available for this condition, diet and lifestyle representing the main targets for effective interventions.

There is evidence that the IGF system is involved in NAFLD pathogenesis. GHD adults have a higher prevalence of NAFLD/NASH, and GH replacement therapy reduces liver steatosis [2]. Reduced IGF-I expression is associated with NAFLD development and progression [3], and both adults and children with NAFLD have lower circulating IGF-I levels [4,5]. Systemic IGF-I administration improves steatosis, inflammation, and fibrosis, thus suggesting that overall the IGF system exerts a hepatoprotective effect [6]. In physiological conditions, hepatocytes lack IGF-IR [2] which, on the contrary, is abundantly expressed in liver diseases such as cancer, hepatitis and cirrhosis.

This study was performed in a large population of 45 children with biopsy-proven nonalcoholic fatty liver disease (NAFLD), and demonstrated that both IGF-I and its receptor (IGF-IR) expression correlate with the severity of liver fibrosis, being highly expressed in the advanced stages of liver disease. The expression of IGF-I and IGF-IR was particularly increased in hepatic stellate cells that play a key role in fibrosis. These results support the concept of a beneficial effect of IGF-I on the liver, the local release of IGF-I coupled with IGF-IR expression representing a response to the damage to favor tissue repair.

References

1. Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE. From NAFLD to NASH to cirrhosis-new insights into disease mechanisms. *Nat Rev Gastroenterol Hepatol*. 2013;10:627–36.
2. Takahashi Y. The Role of Growth Hormone and Insulin-Like Growth Factor-I in the Liver. *Int J Mol Sci*. 2017;18(7).
3. Hribal ML, Procopio T, Petta S, Sciacqua A, Grimaudo S, Pipitone RM, et al. Insulin-like growth factor-I, inflammatory proteins, and fibrosis in subjects with nonalcoholic fatty liver disease. *J Clin Endocrinol Metab*. 2013;98:E304–8.
4. Arturi F, Succurro E, Procopio C, Pedace E, Mannino GC, Lugara M, et al. Nonalcoholic fatty liver disease is associated with low circulating levels of insulin-like growth factor-I. *J Clin Endocrinol Metab*. 2011;96:E1640–4.
5. Cianfarani S, Inzaghi E, Alisi A, Germani D, Puglianiello A, Nobili V. Insulin-like growth factor-I and -II levels are associated with the progression of nonalcoholic fatty liver disease in obese children. *J Pediatr*. 2014;165:92–8.
6. Adamek A, Kasprzak A. Insulin-Like Growth Factor (IGF) System in Liver Diseases. *Int J Mol Sci*. 2018;19(5).

4.13. Growth hormone-Insulin-like growth factor 1 axis hyperactivity on bone fibrous dysplasia in McCune-Albright Syndrome

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Clin Endocrinol. 2018;89:56–64.

<https://www.ncbi.nlm.nih.gov/pubmed/29672904>

McCune Albright syndrome (MAS) is a rare disorder caused by somatic gain-of-function mutations of the *GNAS* gene [1]. This gene encodes the α -subunit of the Gs protein and its mutations are responsible for persistent stimulation of adenylyl cyclase and dysregulated production of cyclic AMP leading to persistent

overactivity in the target tissues. The extent of the disease is determined by the proliferation, migration and survival of the cell in which the mutation spontaneously occurs during embryonic development. Therefore, patients with *GNAS* mutations show a wide range of phenotypes, differing in the degree of severity and the age at onset of the disease. The disease is characterized by a variable association of café-au-lait skin spots, hyperfunctioning endocrinopathies and skeletal lesions with fibrous dysplasia involving one (monostotic) or multiple (polyostotic) bones.

Approximately 20% of subjects with MAS have GH excess, which can worsen craniofacial bone disease leading to vision and hearing deficits related to cranial expansion [2]. Early diagnosis and management of GH excess improve clinical outcomes and decrease the incidence of optic neuropathy [3]. Treatment options include medical management, surgery, and radiotherapy. Somatostatin analogues represent the first line agents followed by Pegvisomant, alone or in combination.

This retrospective, multicentric cross-sectional study was performed in three different MAS cohorts (from Italy, USA and Australia) and included 195 patients. 37 subjects with MAS and GH excess were identified and compared to 34 subjects with MAS without GH excess, as control group. The aim was to define the impact of GH hyperactivity and its therapeutic management on the development of MAS comorbidities. All patients underwent a comprehensive characterization including baseline and dynamic hormone assessment, bone fibrous dysplasia imaging, audiology, neuro-ophthalmology and pain assessment.

GH excess was more common in males and was associated with increased head circumference, hearing defects, optic neuropathy, facial asymmetry and malignancies. Medical therapy (Octreotide alone 10–30 mg or associated with Pegvisomant 10–20 mg) was effective in lowering IGF-I levels within the treatment target (IGF-I Z-score between -2 and $+2$ SDS). Early start of treatment (below the age of 16) was associated with lower risk of optic neuropathy and reduced growth of pituitary adenomas. The clinical value of this paper is further strengthened by the diagnostic flow chart provided to drive the physicians in the management of MAS patients with suspicion of GH excess.

References

1. Javaid MK, Boyce A, Appelman-Dijkstra N, Ong J, Defabianis P, Offiah A, Arunde P, Shaw N, Pos VD, Underhill A, Portero D, Heral L, Heegaard AM, Masi L, Monsell F, Stanton R, Dijkstra PDS, Brandi ML, Chapurlat R, Hamdy NAT, Collins MT. Best practice management guidelines for fibrous dysplasia/McCune-Albright syndrome: a consensus statement from the FD/MAS international consortium. *Orphanet J Rare Dis* 2019;14:139.
2. Akintoye SO, Chebli C, Booher S, Feuillan P, Kushner H, Leroith D, Cherman N, Bianco P, Wientroub S, Robey PG, Collins MT. Characterization of gsp-mediated growth hormone excess in the context of McCune-Albright syndrome. *J Clin Endocrinol Metab* 2002;87:5104–5112.
3. Boyce AM, Glover M, Kelly MH, Brillante BA, Butman JA, Fitzgibbon EJ, Brewer CC, Zaleski CK, Cutler Peck CM, Kim HJ, Collins MT. Optic neuropathy in McCune-Albright syndrome: effects of early diagnosis and treatment of growth hormone excess. *J Clin Endocrinol Metab* 2013;98:E126–134.

Mechanism of the Year

4.14. IGF-2 preprograms maturing macrophages to acquire oxidative phosphorylation-dependent anti-inflammatory properties

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Cell Metab. 2019 4;29:1363–1375.

<https://www.ncbi.nlm.nih.gov/pubmed/30745181>

Mesenchymal stem and/or stromal cells (MSCs) regulate immune system and have been associated with inflammatory and autoimmune diseases. The IGF system is mainly known for its role in the regulation of growth, development and metabolism. A possible role of IGF-I in inflammation has recently been proposed [1, 2], whereas the effects of IGF-II are largely unknown.

This elegant study investigated the IGF-II anti-inflammatory actions in a laboratory model of autoimmune encephalomyelitis (EAE). The authors demonstrated that: (A) human MSCs exposed to low oxygen (LO-MSCs) have anti-inflammatory effects secondary to the expression and production of IGF-II. The concentrated cell culture supernatant of MSCs was administered to EAE mice and was able to lower the EAE disease scores and improve the spinal cord histological appearance. (B) IGF-II administration to EAE significantly lowered the EAE clinical scores, decreased demyelination, and reduced mononuclear cell infiltration in the CNS. (C) IGF-II was found to act on maturing macrophages by programming them towards persistent oxidative phosphorylation (OXPHOS), which was not reduced by pro-inflammatory stimulation. This trained immune phenotype in macrophages imprinted by IGF-II was associated with reduced IL-1 β production and upregulated expression of PD-L1, a well-known immunosuppressive molecule. (D) The administration of IGF-II-preprogrammed macrophages to EAE mice increased Tregs and alleviated the disease in a PD-L1 dependent way. Metabolomic and epigenomic analyses showed that IGF-II-preprogrammed macrophages underwent both epigenetic and metabolic reprogramming, strongly supporting the notion that IGF-II is able to modulate the innate immune memory of macrophages.

Overall these results reveal an unexpected and novel action of IGF-II which, by training an important component of innate immune system toward an anti-inflammatory profile, may be beneficial in the context of autoimmune diseases.

References

1. Hribal ML, Procopio T, Petta S, Sciacqua A, Grimaudo S, Pipitone RM, Perticone F, Sesti G. Insulin-like growth factor-I, inflammatory proteins, and fibrosis in subjects with nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2013;98:E304–308.
2. Bekkering S, Arts RJW, Novakovic B, Kourtzelis I, van der Heijden C, Li Y, Popa CD, Ter Horst R, van Tuijl J, Netea-Maier RT, van de Veerdonk FL, Chavakis T, Joosten LAB, van der Meer JWM, Stunnenberg H, Riksen NP, Netea MG. Metabolic Induction of Trained Immunity through the Mevalonate Pathway. *Cell* 2018;172:135–146.e139.

5. Bone, Growth Plate and Mineral Metabolism

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Preface

It has been an exciting year for life science in the skeletal research field with several seminal findings ranging from basic science and genetics to novel successful treatments. The powerful technology of cell-tracing is developing and is bringing new understanding to the identity and behaviour of skeletal stem cells. Two reports in *Nature* have identified the perichondrial stem cell pool that contribute bone cells to the periosteum as well as the behaviour of the growth plate stem cells in the resting zone.

For a number of years, the development of an FGF23 antibody (Burosumab) that binds and neutralises the excess FGF23 in XLH and the development of a bone-targeted alkaline phosphatase replacement therapy (Asfotase Alfa) for hypophosphatasia have been in focus. We now highlight: the first phase 3 study of FGF23 antibody treatment in children with XLH, demonstrating superiority over conventional treatment; outcomes of a single-arm 7-year phase 2 extension trial of Asfotase alfa for infants and children with hypophosphatasia; and additional preclinical data of ENPP1 enzyme replacement therapy, an emerging therapy for generalized arterial calcification of infancy. In addition to these areas of progress, the chapter reports several exciting findings including new genes in rare skeletal disorders, cartilage-targeted IGF1 therapy, Humanin as a potential treatment to prevent glucocorticoid-induced bone growth impairment and more.

New Therapies and Novel Therapeutic Strategies

5.1. Cartilage-targeted IGF-1 treatment to promote longitudinal bone growth

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Mol. Ther. 2019;27:673–680.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=30765323

In brief: The authors developed a fusion protein containing a cartilage-targeting antibody fragment and Insulin-like growth factor 1 (Igf1) and demonstrate that it can stimulate growth plate cartilage at lower and less frequent doses than Igf1. This is a novel approach that paves the way for the development of tissue-specific targeting of Igfs and other paracrine pathways in growth plate cartilage.

Comment: The growth hormone (GH) insulin-like growth factor-1 (IGF1) axis is a major regulator of linear growth in mammals. In addition to its endocrine functions, IGF1 also acts locally in a paracrine manner to mediate the effects of GH. Currently, there are limited strategies to specifically target paracrine pathways by systemically applied treatments.

The authors developed a cartilage-targeting single-chain human antibody fragment that targets cartilaginous tissues. They then produced a fusion protein of the antibody fragment and IGF1 in order to specifically target

IGF1 to the growth plate. In this proof-of-principle study in a GH-deficient mouse model, the authors show that cartilage-targeted IGF1 has more on-target and less off-target effects than regular IGF1.

Although clinical use of recombinant IGF1 treatment is limited to rare cases of severe growth hormone insensitivity, these authors succeeded to target a systemically applied therapeutic to growth plate cartilage for the very first time.

Similar to the bone anchor of asfotase alfa in the treatment of severe hypophosphatasia, the development of cartilage-targeted growth factors could finally target growth plate pathologies at a paracrine level. This novel approach could ultimately allow the development of therapeutic strategies to selectively modify growth plate regulation while avoiding off-target effects.

5.2. Humanin is a novel regulator of Hedgehog signaling and prevents glucocorticoid-induced bone growth impairment

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FASEB J. 2019;33:4962–4974.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=30657335

In brief: Glucocorticoid-induced growth impairment is reverted by the mitochondrial peptide humanin in dexamethasone-treated mice without attenuation of anti-inflammatory effects.

Comment: Humanin (HN) is a 24 aminoacid peptide that was originally discovered as a neuroprotective factor and later shown to have multiple modes of action including anti-inflammatory and anti-apoptotic effects (1). Glucocorticoid (GC)-induced bone disease is one of the most prevalent causes for growth cessation and secondary osteoporosis in children worldwide (2). Treatment options are limited as systemic GH treatment lacks efficacy (3) and there is no therapeutic option that specifically targets GC-induced growth impairment. The authors previously identified the proapoptotic protein Bax as an important mediator of GC-induced growth plate alterations and the neuroprotective factor humanin as a specific Bax-inhibitor (4).

Using both humanin-overexpressing mice and humanin analogue treatments, the authors show complete rescue of Dexamethasone-induced linear growth impairment *in vivo*, *in vitro* and *ex vivo*. On a molecular level, the reduced expression of Indian hedgehog (Ihh) was identified as a critical regulator of growth plate chondrocyte proliferation. The authors showed restoration of Ihh expression by cotreatment with humanin and further confirmed a rescue of GC-induced apoptosis rates in human growth plate chondrocytes. Importantly, anti-inflammatory effects of GC seemed to be unaffected in the murine model – an essential criterion for the humanin-pathway as a target for therapeutic applicability.

Humanin has been demonstrated to exert cyto-protective effects in several murine disease models. If the effect on GC-induced growth retardation without loss of anti-inflammatory potential can be reproduced in human studies, a completely new treatment strategy to avoid GC-induced growth impairment may become available.

References

1. Kim, S. J., Guerrero, N., Wassef, G., Xiao, J., Mehta, H. H., Cohen, P., Yen, K. (2016) The mitochondrial-derived peptide humanin activates the ERK1/2, AKT, and STAT3 signaling pathways and has age-dependent signaling differences in the hippocampus. *Oncotarget* 7, 46899–46912.
2. Hansen, K. E., Kleker, B., Safdar, N. & Bartels, C. M. A systematic review and meta-analysis of glucocorticoid-induced osteoporosis in children. *Semin. Arthritis Rheum.* 44, 47–54 (2014).
3. Simon, D. *et al.* Effects of recombinant human growth hormone for 1 year on body composition and muscle strength in children on long-term steroid therapy: randomized controlled, delayed-start study. *J. Clin. Endocrinol. Metab.* 98, 2746–2754 (2013).
4. Zaman, F., Chrysis, D., Huntjens, K., Fadeel, B. & Sävendahl, L. Ablation of the Pro-Apoptotic Protein Bax Protects Mice from Glucocorticoid-Induced Bone Growth Impairment. *PLOS ONE* 7, e33168 (2012).

5.3. ENPP1 enzyme replacement therapy improves blood pressure and cardiovascular function in a mouse model of generalized arterial calcification of infancy

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Dis Model Mech. 2018 Oct 1; 11(10): dmm035691.

Abstract: <http://dmm.biologists.org/cgi/pmidlookup?view=long&pmid=30158213>

In brief: Generalized arterial calcification of infancy (GACI) is a severe, rare disease characterized by excessive calcification of large and medium sized arteries caused by homozygous loss-of-function mutations in *ENPP1*. Here, in *Enpp1* deficient mice, treatment with recombinant human ENPP1 protein was more effective than bisphosphonates to resolve not only the vascular calcification, but also the hypertension that eventually leads to cardiac failure in GACI patients.

Comment: Generalized arterial calcification of infancy (GACI) is a severe, rare disease characterized by excessive calcification and stenosis of large- and medium-sized arteries. Approximately 70% of GACI cases are associated with loss-of-function mutations in *ENPP1*, an ectonucleotide pyrophosphatase/phosphodiesterase 1 that produces inorganic pyrophosphate (PPi). PPi is an inhibitor of tissue mineralization critical to prevent mineralization of non-skeletal tissues. Disease onset of GACI can vary, but the majority of patients present *in utero* through 6 months of age. GACI is associated with a high mortality rate, with death typically occurring from vascular occlusion and additional cardiovascular complications. If left untreated, GACI results in 85% lethality by 6 months of age. A retrospective case study by Rutsch et al. demonstrated that although bisphosphonate treatment reduced the mortality rate during infancy to 35%, 7/17 patients treated with bisphosphonates still died during infancy.

In this study, authors examined rhENPP1 in Asj-2J mice, which have a phenotype that is more similar to the human disease than that of previously studied mouse models. Similar to humans with GACI, Asj-2J mice spontaneously develop severe disease and exhibit elevated systemic blood pressure, which is believed to be the cause of death in GACI patients. The authors observed significant improvements in both vascular calcification and cardiovascular function in Asj-2J mice following treatment with rhENPP1. Since hypertension is the leading cause of cardiac failure in GACI patients, rhENPP1 ERT could be important in resolving the disease more effectively and in a greater number of patients than bisphosphonates.

Further studies will be necessary to examine the therapeutic window for rhENPP1 ERT in GACI patients, and whether it has capabilities beyond being used as a disease prevention tool. Calcification reversal will likely be more difficult to achieve owing to the pronounced stability and insolubility of hydroxyapatite. The role of rhENPP1 in calcification regression will need to be evaluated in the future but will likely depend on the extent of calcification and will likely require long-term treatment.

New Insight into Rare Skeletal Disorders

5.4. Vitamin D-binding protein deficiency and homozygous deletion of the GC gene

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N Engl J Med. 2019;380(12):1150–1157. PMID: 30893535.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=30893535

In brief: Homozygous deletion of the group-specific component (*GC*) gene that encodes for vitamin D-binding protein causes persistently low measurable 25-hydroxy Vitamin D concentrations with no clinical features of rickets or osteomalacia.

Comment: Vitamin D and its metabolites are bound to vitamin D-binding protein and are biologically inactive. Free vitamin D metabolites, which are in equilibrium with bound vitamin D metabolites, are available for cellular functions, as is the case with 1,25(OH)₂D binding the vitamin D receptor. Group-specific component (*GC*) gene encodes for vitamin D-binding protein and no previous study had found complete deletion or gross alterations in *GC*. In addition, analyses of vitamin D-binding protein and vitamin D metabolites had not identified any person in whom this protein was absent. Knockout mice lacking vitamin D-binding protein are not only viable and fertile but, when fed on a vitamin D-enriched diet, normal calcium levels and bone structure are maintained, despite having significantly lower plasma concentrations of 25(OH)D and 1,25(OH)₂D.

Here, the authors describe for the first time a patient with complete vitamin D-binding protein deficiency caused by homozygous deletion of the *GC* gene. They also compare the patient with her normal and heterozygous siblings. Despite a lifelong deficiency of vitamin D-binding protein, limited sun exposure, and a diet that was probably lacking sufficient vitamin D, the patient with homozygous deletion of *GC* did not have rickets or osteomalacia, but rather osteopenia and fragility fractures that first occurred only in her fifth decade of life. The disconnect between low plasma 25(OH)D concentration and her relatively mild bone disease highlights the controversy surrounding the use of total 25(OH)D to define vitamin D status. The patient's clinical course and laboratory values were similar to those of mice deficient in vitamin D-binding protein. When the mice were fed a vitamin D-replete diet, they showed significant reductions in serum 25(OH)D but maintained normal calcium, phosphate, and PTH concentrations. However, when they were fed a vitamin D-deficient diet, they remained normocalcemic while developing more pronounced secondary hyperparathyroidism, hypophosphatemia, and bone histomorphometric changes than their normal littermates.

The absence of clinical manifestations of Vitamin-D binding protein deficiency parallels that of inherited thyroid hormone binding protein deficiency, due to mutations in *SERPINA7*, where no clinical manifestations of thyroid disease are seen.

5.5. Osteoporosis and skeletal dysplasia caused by pathogenic variants in *SGMS2*

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JCI Insight. 2019; Apr 4;4(7).

Abstract: <https://www.ncbi.nlm.nih.gov/pubmed/?term=30779713>

In brief: This study describes a novel autosomal dominant form of primary osteoporosis caused by *SGMS2* mutations in six families. A recurrent mutation p.Arg50* led to primary osteoporosis in four families, whereas missense mutations p.Ile62Ser and p.Met64Arg caused a much more severe bone phenotype with spondylometaphyseal dysplasia and neonatal fractures.

Comment: Osteoporosis is characterized by low bone mineral density and deteriorated bone microstructure, leading to increased fragility fractures. Genetic factors play a major role in determining bone mass and risk of fractures, but thus far only few genes underlying familial forms of bone fragility disorders have been described (1). Most commonly such genes relate to type I collagen but other cellular pathways have also been implicated.

Here, an international group of investigators report on a novel form of dominantly inherited osteoporosis with remarkable phenotypic variability. Heterozygous mutations in *SGMS2* were identified as the cause of the disease in six unrelated families using next-generation sequencing techniques. *SGMS2* encodes sphingomyelin synthase 2, an enzyme involved in sphingolipid metabolism. Mutations led to changes in the enzyme function and disturbed bone metabolism and mineralisation through mechanisms so far partially unknown. Depending on the type of *SGMS2* mutation, affected children and adults presented with isolated osteoporosis, osteoporosis associated with sclerotic skull lesions ('calvarial doughnut lesions'), or severe spondylometaphyseal dysplasia and short stature. Some subjects also had neurological symptoms, transient facial nerve paralysis being a particularly common feature, suggesting that this may be a distinctive marker of this form of osteoporosis.

Bone tissue samples collected from three patients demonstrated significant abnormalities with disturbed bone microarchitecture and defective bone mineralisation. The changes were particularly dominant in the cortical bone, in line with an abundant expression of *SGMS2* in cortical bone. The current evidence suggests that

sphingomyelin synthase 2 plays an important role in bone matrix mineralisation, the phenotypic variability corresponds to variable enzyme activity, both intracellularly and at the plasma membrane.

These novel findings are of great interest not only in the field of rare bone diseases but also more widely in osteoporosis research, since such new discoveries may uncover novel targets for drug development. Future studies are awaited to learn more about the phenotypic variability and the shared mechanisms behind bone fragility and neurological symptoms.

Reference

1. Marini JC, Forlino A, Bächinger HP, Bishop NJ, Byers PH, Paeppe A, Fassier F, Fratzl-Zelman N, Kozloff KM, Krakow D, Montpetit K, Semler O. Osteogenesis imperfecta. *Nat Rev Dis Primers*. 2017 Aug 18;3:17052.

5.6. Gain-of-function mutation of microRNA-140 in human skeletal dysplasia

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Nat Med. 2019 Apr;25(4):583–590. PMID: 30804514

Abstract: <https://www.ncbi.nlm.nih.gov/pubmed/?term=30804514>

In brief: This study describes the first skeletal dysplasia caused by a mutation in a microRNA that is not simply inactivating, but modifies the repertoire of target genes.

Comment: MicroRNAs (miRNAs) are small (20–24 nucleotides) noncoding RNA molecules that post-transcriptionally regulate gene expression. There are about 2000 known microRNAs and each targets several genes. Therefore, microRNAs are involved in the regulation in many, if not all, cellular functions.

MicroRNA-140, miR140, is abundantly and almost exclusively expressed in chondrocytes. miR140 knockout mice exhibit dwarfism and craniofacial dysmorphism due to accelerated hypertrophic differentiation and therefore accelerated endochondral ossification.

In this study, the authors describe a novel skeletal dysplasia and show strong evidence for a causative miR-140 mutation. However, in contrast to that expected with loss of function of miR-140, the novel dysplasia exhibited delayed bone maturation. The authors found that the skeletal phenotype was replicated when the identified nucleotide substitution was introduced in mice and was distinct from the phenotype of miR-140 complete knockout mice, indicating that the nucleotide substitution does not result in a simple loss-of-function. Instead, the mutant miR-140 caused de-repression of wild-type miR-140 target genes and repression of a new set of target genes. This report thus identifies the first skeletal dysplasia that is caused by a complex combination of gain- and loss-of-function effects of a microRNA mutation and expands our understanding of the mechanisms by which mutations in non-coding micro RNAs can contribute to human disease.

5.7. Gain-of-function DNMT3A mutations cause microcephalic dwarfism and hypermethylation of Polycomb-regulated regions

Heyn P, Logan CV, Fluteau A, Challis RC, Auchynnikava T, Martin CA, Marsh JA, Tagliani F, Kilanowski F, Parry DA, Cormier-Daire V, Fong CT, Gibson K, Hwa V, Ibáñez L, Robertson SP, Sebastiani G, Rappsilber J, Allshire RC, Reijns MAM, Dauber A, Sproul D, Jackson AP

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Nat Genet. 2019 Jan;51(1):96–105.

Abstract Link: www-nature-com/articles/s41588-018-0274-x

In brief: Gain-of-function mutations altering *DNMT3A* are identified as a new cause of microcephalic dwarfism. Modelling of the disease in mice show that the mutations abrogate *DNMT3A* binding to H3K36me2 and H3K36me3 and lead to aberrant DNA methylation of Polycomb-marked regions and therefore repression of growth genes.

Comment: *DNMT3A* codes for a *de novo* DNA methyltransferase, which is particularly important for establishing DNA methylation patterns during early development. Loss-of-function mutations in *DNMT3A* have recently been shown to cause an overgrowth syndrome with macrocephaly and intellectual disability (Tatton-Brown-Rahman syndrome) (1). Interestingly, genome/exome sequencing of a large number of individuals with presumed monogenic tall stature identified mutations in several genes involved in creating and maintaining DNA and histone modifications, e.g. *NSD1*, *EZH2*, and *DNMT3A*, indicating that mutations in epigenetic modifiers are a relatively frequent cause of syndromic tall stature (2). The mechanism by which these mutations cause overgrowth is not completely clear, but likely include derepression of growth-promoting genes expressed in the growth plate (3). Interestingly, in the current study, the authors identified gain-of-function mutations in *DNMT3A* as a new cause of severe microcephalic dwarfism.

Furthermore, studies of knock-in mice with a similar (orthologous) mutation replicated the phenotype and shed light on the pathogenic mechanism. The mutations abrogated *DNMT3A* binding to Histone 3 molecules that are di- or trimethylated at Lysine 36 (H3K36me2 and H3K36me3) and also led to aberrant DNA methylation of Polycomb-marked regions and thereby repressing growth-promoting genes and/or upregulating growth-inhibiting genes.

References

1. Tatton-Brown, K. *et al.* Mutations in the DNA methyltransferase gene DNMT3A cause an overgrowth syndrome with intellectual disability. *Nat. Genet.* 46, 385–388 (2014).
2. Tatton-Brown, K. *et al.* Mutations in Epigenetic Regulation Genes Are a Major Cause of Overgrowth with Intellectual Disability. *American Journal of Human Genetics* 100, 725–736, doi:10.1016/j.ajhg.2017.03.010 (2017).
3. Lui, J. C. *et al.* EZH1 and EZH2 promote skeletal growth by repressing inhibitors of chondrocyte proliferation and hypertrophy. *Nat Commun* 7, 13685, doi:10.1038/ncomms13685 (2016).

Clinical Advances in Treatment

5.8. Burosumab versus conventional therapy in children with X-linked hypophosphataemia: a randomised, active-controlled, open-label, phase 3 trial

Imel EA, Glorieux FH, Whyte MP, Munns CF, Ward LM, Nilsson O, Simmons JH, Padidela R, Namba N, Cheong HI, Pitukcheewanont P, Sochett E, Högl W, Muroya K, Tanaka H, Gottesman GS, Biggin A, Perwad F, Mao M, Chen CY, Skrinar A, San Martin J, Portale AA

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Lancet. 2019 May 16.

Abstract: www.ncbi.nlm.nih.gov/pubmed/31104833

In brief: In a randomised, active-controlled, open-label, phase 3 trial, burosumab (an anti-FGF23 antibody) demonstrated significantly greater clinical improvements in rickets severity, growth, and biochemistries among children with X-linked hypophosphataemia compared with continuation of conventional therapy with oral phosphate and active vitamin D analogues.

Comment: Burosumab, a fully human monoclonal antibody against FGF23, received approval from the US Food and Drug Administration and from Health Canada, and conditional marketing approval by the European Medicines Agency in 2018 for the treatment of X-linked hypophosphataemia. Previously, two paediatric, single-arm, phase 2 clinical trials had shown that inhibition of FGF23 with burosumab restored phosphate homeostasis, and improved rickets, growth, and mobility in affected 1–12 year old children.

This international, randomised, active-controlled, open-label, phase 3 trial is the first to compare the efficacy and safety of switching to burosumab versus continuing conventional therapy among 1–12 year old children with X-linked hypophosphataemia. 64-weeks of burosumab treatment resulted in greater radiographic improvement of rickets and lower extremity bowing, larger decreases in serum alkaline phosphatase activity, and greater increases in serum phosphorus, growth, and mobility than continuation of conventional therapy.

This phase 3 trial presents the first comparison of conventional therapy with burosumab in children with X-linked hypophosphataemia and showed the superiority of burosumab over continuation of conventional therapy for several clinical outcomes, including the correction of renal phosphate wasting. By improving rickets, long bone deformities, and linear growth, burosumab confirms the promising results from the phase 2 trials and demonstrate that this new treatment approach for children with X-linked hypophosphataemia clearly is superior to optimised conventional treatment.

5.9. Asfotase alfa for infants and young children with hypophosphatasia: 7 year outcomes of a single-arm, open-label, phase 2 extension trial

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Lancet Diabetes Endocrinol. 2019 Feb;7(2):93–105.

Abstract: www.ncbi.nlm.nih.gov/pubmed/30558909

In brief: The study reports outcomes of a single-arm 7-year phase 2 extension trial of Asfotase alfa for infants and children with life-threatening hypophosphatasia who received a median of 6·6 years of therapy. The early improvements previously reported were sustained for up to 7 years of treatment.

Comment: Perinatal and infantile hypophosphatasia are severe, life-threatening diseases of skeletal undermineralisation due to lack of alkaline phosphatase activity and accumulation of its substrate inorganic pyrophosphate (PPi). The development of an enzyme replacement therapy with bone-targeted alkaline phosphatase (Asfotase alfa) is one of the most shining examples of novel therapies for rare disorders with dramatic effects in short-term phase 2 trials promising to effectively convert a deadly disease to a chronic condition requiring life-long injection therapy. Concerns that treatment efficacy would decrease with time or that the patients would develop neutralizing antibodies or unacceptable side-effects have lingered.

In the preceding 6 month phase 2 study, 10/11 participants completed the trial (one withdrew due to infusion reaction) and entered this extension trial. One patient died (sepsis unrelated to treatment) leaving 9 patients to complete the study receiving asfotase alfa for at least 6 years. The study found that the skeletal healing reported in the previous short-term trial was sustained over 7 years of treatment, that no patient who completed the study required respiratory support after year 4, body weight improved to within normal range from year 3 and height improved but remained below normal. Serious adverse events related to asfotase alfa occurred in three (27%) patients (severe chronic hepatitis; moderate immediate post-injection reaction; and severe craniosynostosis with severe conductive deafness). No patient developed resistance to treatment.

This study show that the dramatic result reported from asfotase alfa treatment of children with life-threatening hypophosphatasia before 3 years of age are maintained over a long period and is followed by improved weight, motor and cognitive functions. This study provides important reassurance and reinforces previous evidence for asfotase alfa as an emerging therapy for a rare and deadly skeletal disorder.

Reference

1. Whyte MP, Greenberg CR, Salman NJ, Bober MB, McAlister WH, Wenkert D, Van Sickle BJ, Simmons JH, Edgar TS, Bauer ML, Hamdan MA, Bishop N, Lutz RE, McGinn M, Craig S, Moore JN, Taylor JW, Cleveland RH, Cranley WR, Lim R, Thacher TD, Mayhew JE, Downs M, Millán JL, Skrinar AM, Crine P, Landy H. Enzyme-replacement therapy in life-threatening hypophosphatasia. *N Engl J Med.* 2012, PMID: 22397652.

5.10. Hypoparathyroidism

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N Engl J Med. 2019 May 2;380(18):1738–1747.

Abstract: <https://www.ncbi.nlm.nih.gov/pubmed/?term=31042826>

In brief: This article carefully reviews available data and provide useful hands-on tips for the management of hypoparathyroidism. It also points out areas where additional data are needed. This is mandatory reading for any aspiring endocrinologist.

Comment: Hypoparathyroidism is a rare disease resulting in hypocalcemia, which can be asymptomatic or result in symptoms ranging from paresthesia and muscle cramps to seizures and life-threatening laryngospasm. In adults, the most common cause of hypoparathyroidism is injury to or removal of the parathyroid gland during anterior neck surgery. In children, genetic and autoimmune causes are more common and may be isolated or part of a syndrome, e.g. 22q11 deletion or Autoimmune polyendocrine syndrome type 1. The goal of treatment is to maintain the blood calcium level near to the low end of the normal range, while preventing symptoms of hypocalcemia. This is usually achieved with oral calcium and active vitamin D (calcitriol or alfacalcidol) therapy, but may involve treatment with subcutaneous parathyroid hormone therapy. Treatment is commonly associated with side effects of hypercalciuria, nephrocalcinosis, nephrolithiasis, and renal insufficiency, emphasizing the need for careful monitoring and better future therapies.

5.11. Autoimmune polyendocrine syndromes

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N Engl J Med. 2018 Jun 28;378(26):2543–2544.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=29949487

In brief: This article reviews important developments and major advances in characterizing autoimmunity in patients with autoimmune polyendocrine syndromes, such as the identification of new autoantibody targets associated with distinct diseases and their manifestations. The authors also provide an up to date review of pathophysiological basis, investigations, management and future directions for patients with autoimmune polyendocrine syndromes.

Comment: Autoimmune polyendocrine syndromes are caused by loss of immune tolerance characterized by functional impairment of multiple endocrine glands. These syndromes can be broadly categorized as rare monogenic forms, such as autoimmune polyendocrine syndrome type 1 (APS-1), and the more common polygenic variety, autoimmune polyendocrine syndrome type 2 (APS-2). Autoimmune polyendocrine syndromes are characterized by circulating autoantibodies and lymphocytic infiltration of the affected tissues or organs, eventually causing organ failure. There is marked variability in clinical presentation in affected patients and their families, most likely due to a combination of genetic susceptibility and environmental factors.

This article reviews specific factors that are critical for maintaining immune tolerance, identification of new autoantibody targets associated with distinct diseases and discusses approaches for the appropriate diagnosis and longitudinal follow-up of affected patients. The authors conclude by presenting new directions for future management of Autoimmune polyendocrine syndromes by combining early and refined diagnostics with personalized genomics which would enable immunomodulatory therapy early in the autoimmune process to prevent irreversible organ damage.

5.12. Clinical practice recommendations for the diagnosis and management of X-linked hypophosphataemia

Haffner D, Emma F, Eastwood DM, Duplan MB, Bacchetta J, Schnabel D, Wicart P, Bockenbauer D, Santos F, Levtschenko E, Harvengt P, Kirchhoff M, Di Rocco F, Chaussain C, Brandi ML, Savendahl L, Briot K, Kamenicky P, Rejnmark L, Linglart A
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Nat Rev Nephrol. 2019 May 8. doi: 10.1038/s41581-019-0152-5. [PMID: 31068690].

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=31068690

In brief: In this Evidence-Based Guideline on X-linked hypophosphataemia, the authors identify the criteria for diagnosis of this disease, provide guidance for medical and surgical treatment and explain the challenges of follow-up.

Comment: X-linked hypophosphataemia (XLH) is the most common cause of inherited phosphate wasting and is associated with severe complications such as rickets, lower limb deformities, pain, poor mineralization of the teeth and disproportionate short stature in children as well as hyperparathyroidism, osteomalacia, enthesopathies, osteoarthritis and pseudofractures in adults. The characteristics and severity of XLH vary between patients. Because of its rarity, the diagnosis and specific treatment of XLH are frequently delayed, which has a detrimental effect on patient outcomes.

In this Evidence-Based Guideline, authors recommend that the diagnosis of XLH is based on signs of rickets and/or osteomalacia in association with hypophosphataemia and renal phosphate wasting in the absence of vitamin D or calcium deficiency. The authors suggest that, whenever possible, the diagnosis should be confirmed by molecular genetic analysis or measurement of levels of fibroblast growth factor 23 (FGF23) before treatment. Owing to the multisystemic nature of the disease, patients should be seen regularly by multidisciplinary teams organized by a metabolic bone disease expert. Here, the authors summarize the current evidence and provide recommendations on features of the disease, including new treatment modalities, to improve knowledge and provide guidance for diagnosis and multidisciplinary care.

Basic Science – Growth Plate

5.13. Differential aging of growth plate cartilage underlies differences in bone length and thus helps determine skeletal proportions

Lui JC, Jee YH, Garrison P, Iben JR, Yue S, Ad M, Nguyen Q, Kikani B, Wakabayashi Y, Baron J
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PLoS Biol. 2018 Jul 23;16(7):e2005263.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=30036371

In brief: In this article, Lui et al, make important observations related to the fundamental limits of longitudinal bone growth implicating the growth plate senescence program as a major regulator of bone size.

Comment: A person's right and left arms almost always grow to the same length, but we still don't know why. These authors make key observations related to this fundamental question by comparing developmental changes of growth plates from large and small bones. They find that the same functional, structural, and molecular senescent changes occur in all growth plates, but that these changes occur earlier in growth plates of smaller bones (metacarpals, phalanges) than in growth plates of larger bones (femurs, tibias) and that this differential aging partly explains the differences in final length of the bones. In addition, they identify critical paracrine regulatory pathways, including insulin-like growth factor (Igf), bone morphogenetic protein (Bmp), and Wingless and Int-1 (Wnt) signaling that act in concert to limit growth during development.

During evolution a striking difference in lengths between bones has been achieved and the current study indicates that this is achieved by modulating the progression of the growth plate senescence program. The corollaries of these findings are that the adult size of each bone is controlled by the progression of the growth

plate senescence program, which means that we now have an improved understanding of why my left arm is almost exactly as long as my right arm.

5.14. A radical switch in clonality reveals a stem cell niche in the epiphyseal growth plate

Newton PT, Li L, Zhou B, Schweingruber C, Hovorakova M, Xie M, Sun X, Sandhow L, Artemov AV, Ivashkin E, Suter S, Dyachuk V, El Shahawy M, Gritli-Linde A, Boudierlique T, Petersen J, Mollbrink A, Lundeberg J, Enikolopov G, Qian H, Fried K, Kasper M, Hedlund E, Adameyko I, Sävendahl L, Chagin AS

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Nature. 2019 Mar;567(7747):234–238.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=30814736

In brief: In this article, the authors present evidence that the murine epiphyseal growth plate develops a postnatal stem cell niche with monoclonal properties, that are able to self-renew. They thereby challenge the concept of a continuous depletion of progenitor cells as a limiting factor for bone growth.

Comment: Linear growth depends on a constant generation of chondrocytes at the growth plates. Whereas the process of chondrocyte differentiation and endochondral ossification has been investigated in numerous studies, the precise origin of progenitor cells in the growth plate is not entirely understood. These authors aimed to determine the origin of growth plate chondrocytes and to question the concept of gradually exhausted stem-like chondroprogenitors with limited proliferative potential (1,2).

Using an elegant murine Confetti-reporter system, the authors confirmed that the stem-cell pool in the growth plate's reserve zone was slowly depleted in fetal and neonatal mice. In contrast, the authors observed a sudden acquisition of stem cell renewal properties, leading to a coexistence of a multiclonal fetal and a monoclonal postnatal stem cell niche (Interactive model: <http://chaginlab.com/sim>) suggesting a switch in clonality associated with altered transcriptional activity. Interestingly, the onset of asymmetric stem-cell division was closely associated with the formation of the secondary centre of ossification (SOC), and inhibition of SOC maturation delayed development of monoclonality. Furthermore, the authors show evidence that the mTOR pathway has a central role in the regulation of the mode of clonality.

The hereby described mechanism of cell renewal in the growth plate resembles other tissues in need of continuous self renewal such as hair follicles or bone marrow. While the influence of the mTOR pathway in the regulation of clonality raises hopes for therapeutic options, a plurality of regulating pathways might be involved and remains to be investigated. Nevertheless, the discovery of this novel cellular niche opens doors to novel treatment strategies targeting the regenerative potential of growth plate chondrocytes in conditions such as skeletal dysplasias and osteoarthritis.

References

1. Abad, V. *et al.* The Role of the Resting Zone in Growth Plate Chondrogenesis. *Endocrinology* 143, 1851–1857 (2002).
2. Hunziker, E. B. Mechanism of longitudinal bone growth and its regulation by growth plate chondrocytes. *Microsc. Res. Tech.* 28, 505–519 (1994).

5.15. mir-374-5p, mir-379-5p, and mir-503-5p Regulate Proliferation and Hypertrophic Differentiation of Growth Plate Chondrocytes in Male Rats

Jee YH, Wang J, Yue S, Jennings M, Clokie SJ, Nilsson O, Lui JC, Baron J

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Endocrinology. 2018 Mar 1;159(3):1469–1478.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=29390136

In brief: In this article, a combination of microdissection, microRNA profiling and transfection studies identify 3 microRNAs that are highly expressed in the proliferative zone and appear to be down-stream mediators of PTHrP signaling in the growth plate.

Comment: In the growth plate, PTHrP act as a paracrine factor that maintains chondrocytes in the proliferative pool by inhibiting hypertrophic differentiation and is thus crucial for normal growth plate chondrogenesis and longitudinal bone growth. However, the understanding of the downstream mediators of PTHrP signaling is limited. Recently, cartilage-specific ablation of Dicer result in dwarfism due to decreased proliferation and accelerated differentiation of growth plate chondrocytes.

These authors used a combination of miRNA profiling and transfection studies to identify specific miRNAs that are highly expressed in the proliferative zone and promote proliferation and inhibit expression of hypertrophic chondrocyte marker genes. Three out of four miRNAs were also found to be directly regulated by PTH 1-34, indicating that the identified miRNAs are downstream mediators of PTHrP signaling in proliferative chondrocytes and hence act to maintain chondrocytes in the proliferative pool. This work indicates that PTHrP signaling in the growth plate is, at least in part, mediated through regulation of miRNA.

This study identifies a novel mechanism by which paracrine signals in the growth plate (and other tissues) may be mediated and also point to novel signaling pathways that may be targeted for treatments of growth disorders.

Basic Science – Bone

5.16. Discovery of a periosteal stem cell mediating intramembranous bone formation

Debnath S, Yallowitz AR, McCormick J, Lalani S, Zhang T, Xu R, Li N, Liu Y, Yang YS, Eiseman M, Shim JH, Hameed M, Healey JH, Bostrom MP, Landau DA, Greenblatt MB

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Nature. 2018 Oct;562(7725):133–139.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=30250253

In brief: A newly discovered periosteal stem cell pool with features distinct from other skeletal mesenchymal stem cells (MSCs) is present in murine and human bone and reveals a pivotal function in intramembranous ossification, cortical bone architecture and fracture healing in conditional knockout mouse strains.

Comment: Periosteum is a highly specialized tissue formed by perichondral cells with divergent regenerative capacities: while cells of perichondral lineage can generate chondrocytes and osteoblast for enchondral ossification during fracture repair, very similar cells can be found in craniofacial sutures performing membranous ossification.

This study is the first to identify and characterize a periosteal stem-cell on the surface of mouse and human bones. By establishing Cathepsin K as marker for periosteal stem cells, the authors perform a series of experiments in conditional knock out mice, including single-cell RNA sequencing and transplantation experiments. In contrast to other bone forming MSCs, PSCs were found to mainly give rise to intramembranous bone formation without potential of haematopoietic recruitment. These data explain, for the first time, a cellular basis of different modes of bone development: enchondral and intramembranous ossification. Strikingly, the authors could confirm their murine data in human samples by proving the presence, multipotency and mediation of intramembranous ossification of similar periosteal stem cells.

The distinct and unique properties of PSCs could open new understanding of conditions affecting intramembranous ossification and represent a novel therapeutic target for associated conditions, such as non-union fractures and craniosynostosis.

5.17. Coupling of bone resorption and formation by RANKL reverse signalling

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Nature. 2018 Sep;561(7722):195–200.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=30185903

In brief: This study used various cell-based methods and animal models to investigate the RANK-RANKL signaling pathway in bone homeostasis. The authors show for the first time, that reverse signaling exists from osteoclast to osteoblast and that vesicular RANK, secreted by the osteoclast, relays information to the osteoblast to promote bone formation.

Comment: Bone homeostasis requires coordinated cycles of bone resorption and formation. Signals from osteoclasts to osteoblasts lead to transition from bone resorption to formation. RANKL, a transmembrane protein, has a central role in osteoclastogenesis. Osteoblasts and osteocytes secrete RANKL, which activates the RANK receptor on hematopoietic stem cells, triggering differentiation into osteoclasts. It is less clear how osteoclasts signal to osteoblasts to modify bone formation.

Maturing osteoclasts secrete small extracellular vesicles that contain RANK. Therefore, it is possible that RANKL reverse signaling in osteoblasts is activated by vesicular RANK, secreted from osteoclasts. The authors provide evidence that osteoclasts regulate osteoblasts using the same RANKL–RANK system acting in reverse. They showed that the osteoclastic small extracellular vesicles harbor RANK on their surface. Isolated RANK-bearing vesicles in mouse osteoblast cultures activated the expression of the differentiation-promoting genes *Coll1a1*, *Runx2* and *Osx* in the cells. The presence of RANK-containing vesicles also triggered mineral deposition by osteoblasts. Cellular studies further indicated that stimulation at the early stage promoted osteoblastic mineralization, whereas stimulation at the late stage suppressed osteoblastic mineralization. The timing of vesicular-RANK secretion from osteoclasts and the biphasic properties of RANKL reverse signaling suggest that reverse signaling may contribute to coupling signals.

In light of the bifunctional properties of RANKL, the authors examined the possibility of designing a biological agent to simultaneously activate RANKL reverse signaling in osteoblasts and inhibit RANKL forward signaling to osteoclast precursors. The anti-RANKL antibody denosumab is widely used to treat osteoporosis. By preventing RANKL–RANK forward signaling, denosumab inhibits bone resorption by osteoclasts. However, it transiently also lowers bone formation, because of the tight coupling between osteoclasts and osteoblasts. Here the authors developed a novel anti-RANKL antibody which could potentially uncouple resorption and formation. In a mouse model for post-menopausal osteoporosis, the modified antibody reduced bone resorption but did not suppress bone formation. Based on these findings, we might hopefully in future years expect development of a new osteoporosis drug that combines the benefits of anti-resorptive and bone anabolic therapy.

5.18. Developmental origin, functional maintenance and genetic rescue of osteoclasts

Jacome-Galarza CE, Percin GI, Muller JT, Mass E, Lazarov T, Eitler J, Rauner M, Yadav VK, Crozet L, Bohm M, Loyher PL, Karsenty G, Waskow C, Geissmann F

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Nature. 2019 Apr;568(7753):541–545.

Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=30971820

In brief: Murine knockout studies unravel the developmental origin of osteoclasts in embryonic, erythromyeloid progenitors, acquiring exceptional longevity by constant fusion with monocytes and rejuvenation of cellular nuclei throughout postnatal life.

Comment: Osteoclast function is essential for bone metabolism. Malfunction of bone resorption, as seen in osteopetrosis, causes significant morbidity and often requires invasive treatments including bone marrow transplantation. Whilst monocytic differentiation of postnatal hematopoietic progenitors has been investigated in the last decades, the exact cellular origin of osteoclasts remains unclear (1).

Using multiple conditional murine knockout models, this study aimed to solve the decade-spanning quest for the true origin of osteoclasts. The authors identified a previously unknown embryonic origin of osteoclasts,

undergoing continuous fusion with monocytes from peripheral blood. In these murine models, embryonic osteoclasts directly derived from erythro-myeloid progenitors (EMP) located in the bony anlagen. Furthermore, embryonic osteoclasts were essential for the optimal formation of long bones and membranous ossification, such as teeth eruption and skull growth.

The authors converted the findings of a novel mechanism of long-lived osteoclast with continuous acquisition of new nuclei into a new, cell-based therapeutic approach. They showed that transfusion of monocytic cells could rescue mice with an autosomal-recessive form of osteopetrosis. While textbook chapters on osteoclasts will have to be rewritten based on these novel findings, future studies are needed to prove the applicability of cell-based therapies in osteoclast-driven conditions, such as osteopetrosis.

Reference

1. Xu, F. & Teitelbaum, S. L. Osteoclasts: New Insights. *Bone Res.* 1, 11–26 (2013).

Basic Science – Mineral Metabolism

5.19. Eldecalcitol causes FGF23 resistance for Pi reabsorption and improves rachitic bone phenotypes in the male Hyp mouse

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Endocrinology, Volume 159, Issue 7, July 2018, Pages 2741–2758
Abstract: www.ncbi.nlm.nih.gov/pubmed/?term=29878089

In brief: Eldecalcitol, a long acting active vitamin D3 analogue with lower affinity for vitamin D receptor and resistance to inactivation by vitamin D 24-hydroxylase, causes FGF23 resistance. This leads to complete restoration of renal phosphate transport and NaPi-2a protein levels and improves rachitic bone phenotypes in Hyp mice, a murine model of X-linked hypophosphatemia (XLH).

Comment: FGF23 inhibits phosphate reabsorption in the kidney. Loss-of-function mutations in *PHEX* gene results in excess circulating FGF23, which impairs renal phosphate reabsorption causing hypophosphatemia, and decreased synthesis of the active metabolite of vitamin D, 1,25-dihydroxyvitamin D. Conventional therapy for XLH consists of multiple daily doses of oral phosphate salts and vitamin D metabolites or analogues as replacement therapy. Recently anti-FGF23 antibody therapy, burosumab, was approved to treat children with XLH.

Eldecalcitol [$1\alpha,25$ -dihydroxy- 2β -(3-hydroxypropyloxy) vitamin D3] is an active vitamin D3 analog approved for osteoporosis therapy in Japan. It has a biologically longer life and a lower affinity for the vitamin D receptor and is resistant to vitamin D 24-hydroxylase (*Cyp24a1*; the catabolic enzyme for vitamin D compounds) compared with 1,25-dihydroxyvitamin D. In these contexts, Eldecalcitol has beneficial effects on osteoporotic bone; however, its specific effect on phosphate metabolism and its skeletal effects for hypophosphatemic rickets are unknown.

This study assessed the mechanism of Eldecalcitol on phosphate homeostasis and evaluated the effect of bone growth and mineralization using Hyp mice which, demonstrates clinical features similar to XLH. They found that Eldecalcitol maintains skeletal calcification and adjusts phosphate homeostasis by balancing intestinal phosphate absorption and renal phosphate excretion in WT and Hyp mice. In addition, Eldecalcitol directly facilitated bone development and calcification in hypophosphatemic rickets. However, the mechanism of FGF23 resistance by Eldecalcitol is not known. Eldecalcitol could potentially provide another therapeutic option for patients with XLH.

6. DSD and Gender Dysphoria

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Preface

Our PubMed search for DSD revealed close to 3000 articles and the search for transgender and gender dysphoria about 250 articles for the past 12 months. The topics in DSD were very broad ranging from new and old genes and their mechanism of action to diagnostic themes and new therapeutic options. Additional reports on gender dysphoria with DSD and articles on ethical discussions were published. In transgender, there were many articles on gender dysphoria and autism as well as several reports dealing with the worldwide rise in numbers of youth with gender identity issues.

DSD New Paradigm

6.1. Oestrogen versus androgen in hormone-replacement therapy for complete androgen insensitivity syndrome: a multicentre, randomised, double-dummy, double-blind crossover trial

Birnbaum W, Marshall L, Werner R, Kulle A, Holterhus PM, Rall K, Köhler B, Richter-Unruh A, Hartmann MF, Wudy SA, Auer MK, Lux A, Kropf S, Hiort O

Lancet Diabetes Endocrinol. 2018 Oct;6(10):771–780.

doi: [10.1016/S2213-8587\(18\)30197-9](https://doi.org/10.1016/S2213-8587(18)30197-9).

For the first time a prospective randomized treatment study has been performed for individuals with a DSD. This national multi-center, double-blind, randomized, cross-over trial compared estrogen therapy to androgen replacement in complete androgen insensitivity syndrome (CAIS).

Initially 26 women with CAIS were included, but 10 withdrew or dropped-out, leaving n=16. Participants received either estradiol 1.5 mg/day for 6 months and then testosterone 50 mg/day for 6 months, or the reverse order. A run-in period with estrogen treatment was the same for both groups. The primary outcome was health related quality of life (measured by the questionnaire SF-36). Secondary outcomes were psychological well-being and sexual function.

Estrogen had a positive effect on mental health, and ameliorated psychological distress. Testosterone significantly improved sexual function, especially scores in the desire, arousal, lubrication, and orgasm domains. However, the total score remained in the range of low sexual function during both treatments. The mechanism for this testosterone effect is not clear, but the authors suggest that it could be due to local conversion to estradiol or 3alpha-androstenediol in the central nervous system.

Patients and patient organisations have stated for several years that gonadectomy causes a definite negative change in wellbeing and sexual function. This study answers some of the issues that have been debated, by showing that women with CAIS can benefit from androgen treatment. It is possible that higher doses or combination treatment with both hormones may result in more optimal outcomes in the longer perspective.

6.2. Epigenetic repression of androgen receptor transcription in mutation-negative androgen insensitivity syndrome (AIS type II)

Hornig NC, Rodens P, Dörr H, Hubner NC, Kulle AE, Schweikert HU, Welzel M, Bens S, Hiort O, Werner R, Gonzalves S, Eckstein AK, Cools M, Verrijn-Stuart A, Stunnenberg HG, Siebert R, Ammerpohl O, Holterhus PM

J Clin Endocrinol Metab. 2018 Dec 1;103(12):4617–4627.

doi: 10.1210/jc.2018-00052. PMID: 30124873

This study shows for the first time that epigenetic changes in the androgen receptor gene (*AR*) proximal promoter region may underlie a clinical phenotype of Androgen Insensitivity Syndrome (AIS) in individuals in whom no DNA sequence mutation in *AR* or its promoter were found.

In their previous work, the authors demonstrated reduced *AR* activity in a subset of undervirilised *AR*-mutation negative individuals with clinical AIS. They termed this new clinical entity 'AIS type II'. Here, they quantified *AR* mRNA and *AR* proximal promoter CpG methylation levels in genital skin fibroblasts of individuals with AIS type II and controls. They demonstrate, by a series of inventive and well-built experiments, that over half of AIS type II cases may be caused by epigenetic changes, i.e. increased methylation of specific CpG groups within the proximal *AR* promoter region. Increased methylation at two consecutive sites of this region was associated with decreased *AR* mRNA and protein levels in genital skin fibroblasts of patients as compared to controls. They were able to corroborate their findings by *in vitro* experiments, showing that methylation of a construct containing these CpGs resulted in reduced activation of a reporter gene. Lastly, they identified *RUNXI* as a synergistically acting repressor of *AR* transcription.

Taken together, these data suggest that hypermethylation of the *AR* promoter leads to recruitment of several chromatin modifiers that ultimately induce chromatin compaction and hence inhibit gene transcription.

6.3. Human sex reversal is caused by duplication or deletion of core enhancers upstream of *SOX9*

Croft B, Ohnesorg T, Hewitt J, Bowles J, Quinn A, Tan J, Corbin V, Pelosi E, van den Bergen J, Sreenivasan R, Knarston I, Robevska G, Vu DC, Hutson J, Harley V, Ayers K, Koopman P, Sinclair A

Nat Commun. 2018 Dec 14;9(1):5319.

doi: 10.1038/s41467-018-07784-9. PubMed [citation] PMID: 30552336

Initial steps in the sex determination of the (human) testis depend on *SRY* regulating *SOX9*, but the exact mechanism that controls *SOX9* expression remains unknown. These authors discovered four overlapping copy number variations (CNVs) upstream of *SOX9* as the causes of sex reversal in two 46,XX DSD (with duplications) and two 46,XY DSD patients (with deletions). Prompted by this, they performed studies of these CNVs in cell systems and in mice, and found three essential regulatory elements for normal testis and male sex development, namely eSR-A, eSR-B and eALDI.

This study shows several interesting findings. First, loss of one copy of either of the two identified enhancers of *SOX9* expression (eSR-A or eSR-B) resulted in abnormal testis development and 46,XY sex reversal. By contrast, the addition of one copy upregulated *SOX9*, thereby disturbing the ovarian program and resulted in 46,XX DSD. Second, it describes the exact mechanism how *SFI*, *SRY* and *SOX9* regulate *SOX9* expression. The three newly described enhancers of *SOX9* transcription seem to work both synergistically and specifically to allow for controlled *SOX9* expression and normal testis development. While eSR-A contains *SFI* and *SOX9* consensus binding sites, eSR-B and eALDI contain only *SOX9* and *SRY* binding sites, respectively, but all are responsive to *SFI* and *SOX9*. In addition, the female factor *FOXL2* represses activity of all three elements. Third, these enhancers of *SOX9* are found very far upstream of *SOX9* and are missed in typical genetic sequencing studies for sex reversal. It is hypothesized that they come into contact to work in concert by chromatin looping. Lastly, humans are not mice. *TESCO* (the testis specific enhancer of *SOX9* core element) has been shown to enhance *SOX9* expression in rodents but not in men. But it seems that the equivalent with the same function in humans is eALDI.

In conclusion, alterations in regulatory regions beyond the currently known molecular network controlling gonadal determination may explain genetically unsolved patients with DSD.

6.4. Essential role of BRCA2 in ovarian development and function

Weinberg-Shukron A, Rachmiel M, Renbaum P, Gulsuner S, Walsh T, Lobel O, Dreifuss A, Ben-Moshe A, Zeligson S, Segel R, Shore T, Kalifa R, Goldberg M, King MC, Gerlitz O, Levy-Lahad E, Zangen D

N Engl J Med. 2018 Sep 13;379(11):1042–1049.

doi: [10.1056/NEJMoa1800024](https://doi.org/10.1056/NEJMoa1800024). PMID: 30207912

This case report describes two sisters with 46,XX karyotype and hypergonadotropic hypogonadism – i.e. ovarian dysgenesis. They had normal general development and normal cognition. With estrogen replacement they developed normal sex characteristics and reached their target height. Whole genome sequencing showed that they were compound heterozygous for truncating mutations in the *BRCA2* gene resulting in reduced amounts of *BRCA2* protein.

Functional studies of chromosomal breakage measured in lymphocytes exposed to mitomycin C showed 50 times as many breaks in peripheral lymphocytes from these sisters compared to controls. Hence, the mutation also resulted in impaired DNA repair. This was consistent with the medical history of this family. One of the sisters was a long-term survivor of acute myeloid leukaemia 5 years of age, and one brother had died of leukemia at age 13. The mother was diagnosed with ovarian cancer stage III on ultrasound by an assessment initiated by the genetic study. The *BRCA2* defect as the cause of the ovarian dysgenesis was confirmed via studies in a drosophila model, in which a null mutation in *Dmbrca2*, the fly ortholog of *BRCA2*, caused sterility and gonadal dysgenesis in both sexes.

The findings in this paper add important contributions to our understanding of ovarian development and the causes of ovarian dysgenesis. Autosomal dominant *BRCA2* mutations are a well-established cause of very strong susceptibility to breast and ovarian cancers due to impaired ability to repair DNA double-strand breaks. The current findings show that this mechanism is also essentially involved in ovarian development. In addition, it adds a new clinical perspective since an increased cancer risk may be linked to ovarian dysgenesis in some cases.

6.5. Assembling the jigsaw puzzle: CBX2 isoform 2 and its targets in disorders/differences of sex development

Sproll P, Eid W, Gomes CR, Mendonca BB, Gomes NL, Costa EM, Biason-Lauber A

Mol Genet Genomic Med. 2018 Sep;6(5):785–795.

doi: [10.1002/mgg3.445](https://doi.org/10.1002/mgg3.445). PubMed PMID: 29998616

Chromobox protein homolog 2 (*CBX2*) has two isoforms, *CBX2.1* and the shorter *CBX2.2*. *CBX2.1* was previously shown to be essential for male gonadal development, when a mutation was identified in a girl with 46,XY DSD. *CBX2.1* stimulates male-specific genes (*SFI* and *SOX9*) and inhibits female-specific factors (*FOXL2*). However, the function of *CBX2.2* was unknown.

This study describes two patients with 46,XY DSD, perineal hypospadias, no palpable gonads and compound heterozygous mutations in *CBX2.2*. Possible binding targets of *CBX2.2* were identified using DNA adenine methyltransferase identification (DamID). The functional effects of the mutations were explored using quantitative real-time PCR. The authors found that both *CBX2.2* mutations failed to regulate the expression of *EMX2*, *HOXA13*, and *MAK*, suggesting that failed regulation of these targets might contribute to the pathophysiology.

The authors suggest that the most relevant *CBX2.2* target for sex development seems to be *EMX2*, since deletions have been reported to cause 46,XY DSD ranging from hypospadias to gonadal dysgenesis. In human development, *EMX2* and *CBX2* are simultaneously expressed in the gonadal anlage at 7 weeks of gestation, i.e. prior to *SRY*, suggesting a role in the formation of the early gonad. Other targets of interest were *MAK*, male germ cell associated kinase, involved in cell cycle regulation, and *HOXA13*, for which mutations lead to the hand-foot-genital syndrome, Müllerian duct fusion defects in females and hypospadias in males.

In conclusion, the *CBX2.2* mutations failed to regulate the expression of genes essential for sex development resulting in 46,XY DSD. This study demonstrates the complexity of the regulation of sex determination and development that we are just beginning to understand. *CBX2* adds an important piece to the picture.

6.6. Early-onset complete ovarian failure and lack of puberty in a woman with mutated estrogen receptor β (*ESR2*)

Lang-Muritano M, Sproll P, Wyss S, Kolly A, Hürlimann R, Konrad D, Biason-Lauber A

J Clin Endocrinol Metab. 2018 Oct 1;103(10):3748–3756.

doi: [10.1210/jc.2018-00769](https://doi.org/10.1210/jc.2018-00769). PubMed PMID: 30113650

This case report describes a 16 year old girl with 46,XX karyotype, no pubertal development and streak gonads. The girl was 150 cm tall and had closed epiphyses and osteoporosis. Genetic investigation by whole exome sequencing showed a loss-of-function mutation in the Estrogen Receptor β gene (*ESR2*).

This *ESR2* variant was a loss-of-function heterozygous mutation in a highly conserved residue of the gene. It disrupts estradiol-dependent signaling and has a dominant negative effect. Functional transactivation studies were performed using different cell types of known interest for estrogen function. When stimulated with estradiol, mutant *ESR2* in breast cells or osteoblasts had a much lower basal transactivation potential than the wild type receptor. In ovarian cells, the mutant *ESR2* did show any expression and had a dominant negative effect on the wild-type allele, consistent with its pathological heterozygote effects. The effect was not rescued by increasing estradiol concentrations, suggesting a complete loss of function.

The bone phenotype is difficult to understand. Osteoporosis is expected with estrogen deficiency, however, the epiphyseal closure is unexpected. There are conflicting reports on the localization and function of *ESR1* and *ESR2* in growth plate chondrocytes, and whether predominantly *ESR1* or *ESR2* or both are required. A 2-year follow up during treatment with estrogen, calcium and vitamin D showed some improvement in bone density parameters but the effect was much less notable compared with her changes in breast and uterus size. This suggests a more complex or slower influence of estrogens on bone.

These novel findings suggest that *ESR2* is necessary for human ovarian determination and/or maintenance and that *ESR1* is not sufficient to sustain ovarian function in humans. The results add to the understanding of the function of estrogen in the processes of sex differentiation and development.

Laboratory Medicine in DSD

6.7. Sex differences in reproductive hormones during mini-puberty in infants with normal and disordered sex development

Johannsen TH, Main KM, Ljubicic ML, Jensen TK, Andersen HR, Andersen MS, Petersen JH, Andersson AM, Juul A

J Clin Endocrinol Metab. 2018 Aug 1;103(8):3028–3037.

doi: [10.1210/jc.2018-00482](https://doi.org/10.1210/jc.2018-00482). PubMed ID: 29917083

Minipuberty is a poorly understood developmental event at 1–6 months of age, during which the hypothalamic-pituitary-gonadal axis is transiently activated.

This study assessed serum reproductive hormone concentrations in 1840 healthy boys and girls aged 2 to 5 months. The findings provide sex-specific reference ranges for these hormone concentrations and cut-off values that can be of diagnostic use in DSD. AMH, LH/FSH ratio and testosterone concentrations seemed to be the best classifiers between the sexes in healthy infants during minipuberty. With the exception of the prominent difference in the LH/FSH ratio between the sexes (higher in boys than girls), most of these infancy hormone changes have been previously reported. However, the paper gives a nice summary of all commonly assessed hormones during minipuberty in normal children.

The study also demonstrated the utility of these normative values for the hormonal evaluation of DSD in 27 infants. They showed that LH/FSH ratios correlate well with the sex of rearing in infants with Klinefelter

syndrome, male 45,X/46,XY mosaicism and Turner syndrome; but this is not true for all causes of DSD as shown for CAIS who were found to have high ‘male’ LH/FSH ratios. Importantly, hormone concentrations were measured by highly sensitive analytical methods that are not commonly available in clinical practice, and the provided cut-off values might be specific to these analytical methods. Therefore adoption of these measurements as a diagnostic tool and use of the provided cut-off values needs wider validation.

Gender Dysphoria in DSD

6.8. Psychosexual Aspects, Effects of Prenatal Androgen Exposure, and Gender Change in 46,XY Disorders of Sex Development

Loch Batista R, Inácio M, Prado Arnhold JJ, Gomes NL, Diniz Faria JA, Rodrigues de Moraes D, Frade Costa EM, Domenice S, Bilharinho Mendonça B

J Clin Endocrinol Metab. 2019 Apr 1;104(4):1160–1170.

doi: [10.1210/jc.2018-01866](https://doi.org/10.1210/jc.2018-01866). PubMed PMID: 30388241

This retrospective single center study from Brazil assessed the possible effects of prenatal androgen exposure, degree of virilization of the external genitalia, sex of rearing and diagnosis of DSD on later psychosexual outcomes (including gender identity, gender role and sexual orientation) in 144 individuals older than 18 years with 46,XY. Only prenatal androgen exposure showed significant correlations with all three adult psychosexual outcomes. No such correlation was found for prenatal exposure to estrogens, external genital phenotype at birth, sex of rearing and age at gonadectomy (pre- versus postpubertal) representing sex hormone exposure during puberty.

In this study, individuals were mean age 35 years (range 17–56), most 46,XY DSD individuals were raised female, e.g. 30/32 subjects with *5aRD2* deficiency. This has certainly changed over the last decade and many more 46,XY DSD subjects are now raised male. Thus, some of the observations may be specific to the study group.

Nevertheless, this study supports the following hypotheses: a) the brain is a sexual organ, b) prenatal androgen exposure enhances male-type psychosexual development, but c) undervirilization of the external genitalia does not necessarily correspond to psychosexual development; d) prenatal androgen exposure seems to have a more potent effect than pubertal hormones on psychosexual development, e) estrogens although important for male sexual development of the brain are insufficient to promote the male phenotype, and f) gender issues are more frequently seen in all types of 46,XY DSD. Future longitudinal studies on larger DSD cohorts such as collected in the I-DSD registry will inform similar and more outcomes for better DSD care.

Reference

1. Berenbaum SA, Beltz AM. How Early Hormones Shape Gender Development. *Curr Opin Behav Sci.* 2016 Feb;7:53–60. PMID:26688827.

6.9. Gender dysphoria and XX congenital adrenal hyperplasia: how frequent is it? Is male-sex rearing a good idea?

de Jesus LE, Costa EC, Dekermacher S

J Pediatr Surg. 2019 Feb 22. pii: S0022-3468(19)30117–4.

doi:[10.1016/j.jpedsurg.2019.01.062](https://doi.org/10.1016/j.jpedsurg.2019.01.062). PubMed PMID:30905417

This literature review aimed to analyze the prevalence of gender dysphoria in 46,XX individuals with CAH raised female and male and to identify subgroups with a higher likelihood of gender dysphoria. In total 1770 papers published between 1988 and 2018 were identified, but data from only 28 papers were included. The proportion raised as males varied from 4% to 21% between different studies. In total, gender dysphoria was reported in $n = 73$ individuals with 46,XX raised as males and $n = 36$ individuals with 46,XX raised as females.

Overall, 9% of female-raised 46,XX CAH individuals declared gender dysphoria, but this proportion varied between publications from 6.3% to 27.2%. Three children and 13/27 adults (total 48%) with gender dysphoria lived as a boy or man. Similarly, 10% of male-raised 46,XX CAH individuals declared gender dysphoria.

However, 76% of this group had a late diagnosis, i.e. milder forms of CAH. No specific subgroup with a higher likelihood of gender dysphoria could be identified – partly because of low statistical power. Important information, such as severity of CAH, age at diagnosis, hormonal treatment, surgery, psychological assessment to diagnose gender dysphoria was often missing.

The authors emphasize that it is inappropriate to compare transgender people and patients with DSD. The age at which gender dysphoria is apparent varies widely. Furthermore, many individuals with 46,XX CAH describe themselves as gender fluid and do not seek legal or formal transition to the other gender. Despite limitations in the evidence, this review gives important insights both on what is known so far and what information is missing. The issue is especially important in the light of the ongoing discussion about the timing of genital surgery, and the decision about sex of rearing in severely virilized cases.

Ethical Aspects

6.10. Management of pediatric patients with DSD and ambiguous genitalia: Balancing the child's moral claims to self-determination with parental values and preferences

Diamond DA, Swartz J, Tishelman A, Johnson J, Chan YM
J Pediatr Urol. 2018 Oct;14(5):416.e1–416.e5.
doi: [10.1016/j.jpuro.2018.04.029](https://doi.org/10.1016/j.jpuro.2018.04.029). PubMed ID: 29903608

This paper discusses the management of three DSD cases with difficult sex assignment at a US center. The goal of this report was to explore factors involved in parental decision making and the challenge to reconcile respect for the child's integrity with parental rights to make decisions in what they believe is best for their child. The authors propose a team management approach, which carefully assesses risks and opportunities associated with either gender pathway and discusses with the parents the various treatment options. Pillars of such an approach are provision of full information, psychosocial support and time for parents to consider all the options.

For years now, early genital surgery has been intensely debated. However, so far there has not been convincing evidence that this has effectively resulted in medical practice changes. Although the authors do not take a stand against early genital surgery and the parents ultimately opted for intervention to make the genital appearance more in line with the gender of rearing, the report is convincing in demonstrating that transparency and support in the process of balancing a range of options and without time constraints are highly valued by parents and may be preferred above apparently straightforward and fast solutions. In this respect, the report sets the scene for how contemporary care should be conceived, organized and financed by health care authorities. Such circumstances will provide a more fertile soil for credible management options that aim at empowering children who have a genital difference.

DSD Reviews

6.11. Testis development

Mäkelä JA, Koskeniemi JJ, Virtanen HE, Toppari J
Endocrine Reviews, Volume 40, Issue 4, August 2019, Pages 857–905
doi: [10.1210/er.2018-00140](https://doi.org/10.1210/er.2018-00140). PMID: 30590466

This encyclopedic article reviews the molecular and organizational events that take place during testicular development from the very early start, i.e. the development of the primordial germ cells before gastrulation and of the gonadal ridges at 3–4 weeks post fertilization. This is followed by a step-by step description of progressive testis formation and testicular descent. Lastly, the authors review the genes and mechanisms that when disturbed may give rise to incomplete or atypical gonadal development.

The amount of detail is astonishing, and yet, the authors manage to summarize the data in an agreeably-reading narrative style. Additional focus is placed on highlighting the differences between human and mice data wherever relevant. Many of the figures are conceived as simple and clear drawings, depicting individual cell types or embryonic tissues.

This review is of interest for everyone who is involved in research on gonadal development or who needs detailed and specific information about one or more aspects of testicular development.

6.12. New technologies to uncover the molecular basis of disorders of sex development

Barseghyan H, Délot EC, Vilain E

Mol Cell Endocrinol. 2018 Jun 15;468:60–69.

doi: 10.1016/j.mce.2018.04.003. PubMed PMID: 29655603

Since the description of the first DSD gene, *SRY*, in the early 1990s (OMIM 480000), genetics has become a major player in research and clinical workup of DSD. The advances of today's technologies in genetics and the limitations are summarized in this review, which covers karyotyping, Sanger sequencing, exome sequencing and chromosomal microarrays. In addition, future possibilities with whole genome sequencing and whole genome mapping are predicted to solve more DSD cases.

Although the genetics of DSD has seen a tremendous evolution in the last two decades, with a recent technical shift from a gene-by-gene approach to massively parallel sequencing, the diagnostic yield is currently only about 30–40%. This is due to several factors including technical, interpretative and clinical factors as well as challenges in functional validation of genetic findings. Standardization of phenotyping and genetic workup not only with respect to technical methods, but also with respect to algorithms used for data interpretation is crucial for improving the diagnostic success (1).

Recently, new a pathogenetic insight revealed that the non-coding genome, modifier genes and oligogenic inheritance need to be considered, as also reviewed in (2). Such pathogenetic mechanisms are often difficult to prove with current experimental models. But even these difficulties may be overcome in the near future by using models based on human derived stem cells (3).

References

1. Audi L, Ahmed SF, Krone N, Cools M, McElreavey K, Holterhus PM, Greenfield A, Bashamboo A, Hiort O, Wudy SA, McGowan R; The EU COST Action. GENETICS IN ENDOCRINOLOGY: Approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST Action BM 1303 'DSDnet'. *Eur J Endocrinol.* 2018 Oct 1;179(4):R197–R206.
2. Baetens D, Verdin H, De Baere E, Cools M. Update on the genetics of differences of sex development (DSD). *Best Pract Res Clin Endocrinol Metab.* 2019 Apr 13. pii: S1521-690X(19)30014-4.
3. Ruiz-Babot G, Balyura M, Hadjidemetriou I, Ajodha SJ, Taylor DR, Ghataore L, Taylor NF, Schubert U, Ziegler CG, Storr HL, Druce MR, Gevers EF, Drake WM, Srirangalingam U, Conway GS, King PJ, Metherell LA, Bornstein SR, Guasti L. Modeling Congenital Adrenal Hyperplasia and Testing Interventions for Adrenal Insufficiency Using Donor-Specific Reprogrammed Cells. *Cell Rep.* 2018 Jan 30;22(5):1236–1249.

Food for Thought in Transgender Medicine

6.13. Parent reports of adolescents and young adults perceived to show signs of a rapid onset of gender dysphoria

Littman L

PLoS One. 2018 Aug 16;13(8):e0202330.

doi: 10.1371/journal.pone.0202330. Erratum in: *PLoS One.* 2019 Mar 19;14(3):e0214157.

A sharp and unexplained rise in the incidence of gender dysphoria has been observed worldwide among adolescents, especially natal girls. The causes and outcomes (in terms of persistence-desistence rates) of adolescent-onset gender dysphoria are currently unknown.

This study describes a recently observed phenomenon, ‘rapid-onset gender dysphoria’ (ROGD). It may represent a specific sub-entity of adolescent-onset gender dysphoria that has an atypical presentation. In order to generate hypotheses around the factors contributing to ROGD, the authors recruited participants by advertising through three websites, and asked parents of children with ROGD to complete a custom-made online questionnaire – as such, parents had to notice this to be active. 256 parents completed the survey. The results suggested that ROGD may be triggered by what the authors describe as “social contagion” and “maladaptive coping strategies”.

This study generated a very heated debate, due to the methodological difficulties in its design, the presumed tendentious interpretation of results, and its potential negative impact on the transgender community (1). Nevertheless, this report has an important merit in that it is the first to describe and try to understand a recently observed trend that may be possibly harmful for some vulnerable young individuals. It reminds the pediatric endocrine community to remain cautious that some gender dysphoric adolescents may present with a very complex clinical picture and that long-term outcomes of atypically-presenting and/or rapid onset GD are currently unknown. Even when faced with an increasing pressure to rapidly alleviate the psychological suffering of gender dysphoric youth, careful diagnosis and multidisciplinary follow-up remain crucial in the management of gender dysphoric youth who seek medical transition.

Reference

1. Wadman M. Rapid onset’ of transgender identity ignites storm. *Science*. 2018 Sep 7;361(6406):958–959.

6.14. Genetic link between gender dysphoria and sex hormone signaling

Foreman M, Hare L, York K, Balakrishnan K, Sánchez FJ, Harte F, Erasmus J, Vilain E, Harley VR

J Clin Endocrinol Metab. 2019 Feb 1;104(2):390–396.

doi: [10.1210/jc.2018-01105](https://doi.org/10.1210/jc.2018-01105). PubMed PMID: 30247609.

Polymorphisms in sex hormone genes have been described in gender dysphoric individuals with inconsistent findings. In this case-control study of 380 trans women and 344 control males, functional variants in 12 sex hormone signaling genes were studied. Significant associations were found between gender dysphoria and polymorphisms in *ERα*, *SRD5A2*, *STS* and *SULT2A1*. Some allele combinations were also overrepresented, indicating that oligogenic inheritance may play a role.

Earlier it was thought that gender dysphoria in transgender people results only from psychological conditions, but newer studies suggest that endocrine, neurobiological and genetic factors are involved. The current study supports the hypothesis that genetic variants that alter sex hormone signaling may thereby change the typical sexual differentiation of the developing brain, which may then lead to gender dysphoria in later life. Further evidence for a heritable contribution to gender dysphoria comes from twin studies, in which one third of twin pairs are concordant for gender dysphoria. On the other hand, inconsistencies between alike genetic studies in gender dysphoria have been explained by small sample size of such studies and by the heterogenous character of the transgender population in terms of age at onset of gender dysphoria and of sexual orientation.

Thus, it seems that DSD and transsexualism may share more common grounds in etiology than was earlier thought. But further studies are needed to robustly demonstrate the role of genetics, hormones and other factors that contribute towards gender dysphoria in transgender individuals.

Further recommended reading on same topic in last year:

Fernández R, Guillamon A, Cortés-Cortés J, Gómez-Gil E, Jácome A, Esteva I, Almaraz M, Mora M, Aranda G, Pásaro E. Molecular basis of Gender Dysphoria: androgen and estrogen receptor interaction. *Psychoneuroendocrinology*. 2018 Dec;98:161–167.

6.15. Fertility preservation for a transgender teenager

Nahata L, Campo-Engelstein LT, Tishelman A, Quinn GP, Lantos JD

Pediatrics. 2018 Sep;142(3). pii: e20173142.

doi: [10.1542/peds.2017-3142](https://doi.org/10.1542/peds.2017-3142). PMID:30072573

While awaiting a breakthrough in research on *in vitro* maturation of immature gametes, the best option for transgender youth to secure fertility is to cryopreserve mature sperm, oocytes or gonadal tissue. This often requires postponement or arrest of transitional hormone medication, and this may be one of the main reasons why many trans adolescents do not choose this option. This decision may lead to conflict with their parents, who often wish to keep all options open. For healthcare workers involved in adolescent transgender teams, it is not always clear how to deal with such disagreement.

This article provides guidance for clinicians who are faced with such difficult situations. The format is a reflection of four specialists in the field who balance the arguments and expectations of adolescents against parental wishes, scientific knowledge, and ethical and legal constraints. It is argued that although currently only very few adolescents choose to undergo fertility preserving measures, this should by no means be a reason to limit counseling on this topic. Indeed, transgender adults often have a strong interest in having biological children. Thus, it is crucial for transgender teams and parents to find out how to talk about a topic that is not important now but may become so in the future.

Ultimately, there is a broad consensus that the adolescent, who has been found mature enough to decide on his/her gender, and after having been informed extensively on all options and their consequences, has the final say in this discussion.

New Working Hypotheses in Transgender

6.16. Prevalence of the wish to be of the opposite gender in adolescents and adults with autism spectrum disorder

van der Miesen AIR, Hurley H, Bal AM, de Vries ALC

Arch Sex Behav. 2018 Nov;47(8):2307–2317.

doi: [10.1007/s10508-018-1218-3](https://doi.org/10.1007/s10508-018-1218-3). PubMed ID: 29736809

An increased incidence of autism spectrum disorder (ASD) among adolescents and adults with gender dysphoria has been reported in many studies. This paper describes one of the rare studies taking an opposite approach, by investigating the self-reported wish to be of the opposite gender among over 1300 adolescents and adults with ASD in comparison with healthy controls. Both adolescents and adults with ASD endorsed this item significantly more than controls. In adolescents, more natal girls than natal boys reported that they wanted to be of the opposite gender; in adults, no gender differences could be detected. None of the ASD specific subdomains was associated with a more frequent wish for being the opposite gender.

The study included a very large number of individuals with ASD, and is the first to report an increased wish to be of the opposite gender in adults with ASD. Several hypotheses exist regarding this intriguing association, but for none of them there is currently sufficient evidence. The study has important limitations that are well recognized by the authors. For example, it questions the suitability of the questionnaires and the methodology of self-reporting for a clinical sample of ASD individuals, and highlight differences between demographics of the ASD and control groups. Most importantly, they acknowledge that a positive answer to the question “if you want to be of the opposite gender” does not equal a desire for formal gender change. Nevertheless, given the strength of this association, it is crucial that clinicians working with ASD patients are aware of and open to this issue.

6.17. Gender identities in adolescent population: methodological issues and prevalence across age groups

Kaltiala-Heino R, Lindberg N

Eur Psychiatry. 2019 Jan;55:61–66.

doi: [10.1016/j.eurpsy.2018.09.003](https://doi.org/10.1016/j.eurpsy.2018.09.003). PubMed ID: 30390473

Is the prevalence of gender dysphoria today truly increasing, or do the increasing numbers result because today's world is more open so that these individuals and families no longer hide in the closet? Or are methodological factors playing a role? Fact is that in Europe and North America numbers of adolescents seeking treatment for gender dysphoria have been markedly increasing in the past 10 years.

This large school-based survey in Finland addressed the questions: a) are questionnaires on gender identity in young people prone to dishonest responding, and b) does the prevalence of gender-sex divergence differ between early, middle and late adolescents. The findings clearly show that reporting on gender identity incongruence in youth is very susceptible to unreliable answering, more so in boys than in girls. Nevertheless, overall corrected prevalence of gender identity issues was higher than in previous studies (3% in boys and 4% in girls) and increased in boys from early to late adolescence, while in girls the opposite trend was observed. Also, more other/non-binary than opposite sex identification was reported suggesting a fluid rather than a binary development of gender identity in adolescents.

This study demonstrates the dilemma in clinical practice when taking care of gender dysphoric youth: does the adolescent seeking help show identity explorations as part of normal pubertal development or is he/she manifesting a consolidated gender identity problem? To answer this question, psychological assessment and counseling is compulsory before rushing into medical or even surgical treatments.

Transgender Reviews

6.18. Research Review: Gender identity in youth: treatment paradigms and controversies

Turban JL, Ehrensaft D

J Child Psychol Psychiatry. 2018 Dec;59(12):1228–1243.

doi: [10.1111/jcpp.12833](https://doi.org/10.1111/jcpp.12833). PubMed ID: 29071722

This comprehensive review summarizes the current knowledge on epidemiology, concomitant psychiatric diagnoses, behavioral characteristics and developmental trajectories of gender identity issues. It also reviews determinants of gender identity and expression, and the options and benefits of clinical approaches and treatments.

Reading this review will not only give you a nice overview on past and current understanding in topics of gender identity, it will also illustrate that (too) many questions are unanswered and further studies are needed in several themes. Studies of gender identity are challenging with regard to definitions, cultural themes, therapeutic options etc., and in addition they raise multiple questions on methodological issues, such as data reliability, study size, sample variability or quality and usefulness of currently available instruments (usually questionnaires) – just to mention a few.

With close to 1% of all youth having cross-gender identity questions and needs, it becomes more and more urgent to address these questions as it has been clearly shown that gender minorities are at high risk for depression, anxiety disorders and suicidality, likely due to unmet needs and societal exclusion. While the etiology of atypical gender identity remains unclear, it becomes more and more evident that both biological and psychosocial factors play a role.

6.19. Management of endocrine disease: approach to the management of children and adolescents with gender dysphoria

Martinierie L, Condat A, Bargiacchi A, Bremont-Weill C, de Vries MC, Hannema SE

Eur J Endocrinol. 2018 Oct 12;179(5):R219–R237.

doi: [10.1530/EJE-18-0227](https://doi.org/10.1530/EJE-18-0227). PubMed ID: 30049812

The rapidly increasing number of adolescents with gender dysphoria represents a challenge for psychiatric assessments and the endocrine management of those who are considered to warrant gender change. The diagnostics are more than complicated and the clinical management from the endocrine viewpoint is still a developing field. This review covers both of these issues and gives a broad update of the current situation.

The importance of correct rather than fast diagnosis is emphasized, especially for prepubertal children due to the high rate of desistance (in up to 80% of cases), coupled with our current inability to predict with any certainty those who will persist or desist. Currently, GnRH analog therapy is often started at Tanner stage 2 (B2 or G2), and gender affirming treatment from 16 years or older. The authors warn that, even when started at this stage or later, up to 4% of those who start GnRHa therapy will desist. The ethical dilemma of deciding the age at which the child/adolescent is mature enough to take decisions on own treatment is discussed.

Different effects of treatment options regarding efficacy and some aspects of side effects are described. Cancer and fertility issues are mentioned. The authors advocate regular 3–6 monthly follow-up with measurements of height, weight, sitting height, blood pressure and pubertal development.

In conclusion, firm evidence for many of the recommendations are lacking and there is much need for further research regarding the diagnostics and the right timing for hormonal treatment or surgery, but also concerning the effects and long-term consequences of treatments. The authors call for future studies, and that all teams caring for individuals with gender dysphoria should participate in follow-up studies.

7. Puberty

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Preface

In the puberty field, this year brought new evidence regarding the link between obesity and the onset of puberty. Several high impact basic studies identified new factors involved in the hypothalamic crosstalk between the control of puberty and energy balance.

This chapter also summarizes the discoveries regarding the genetic architecture of hypogonadotropic hypogonadism and central precocious puberty and presents some studies looking at the effects of environmental factors such as physical activity or endocrine disrupting chemicals on pubertal development. New perspectives opened up regarding the treatment of boys with constitutional delay of growth and puberty with aromatase inhibitors.

Basic Science

7.1. PACAP neurons in the ventral premammillary nucleus regulate reproductive function in the female mouse

Ross RA, Leon S, Madara JC, Schafer D, Fergani C, Maguire CA, Verstegen AM, Brengle E, Kong D, Herbison AE, Kaiser UB, Lowell BB, Navarro VM
Elife. 2018 Jun 15;7. pii: e35960.
<https://www.ncbi.nlm.nih.gov/pubmed/29905528>

Using transgenic mice, this team discover a new role for Pituitary adenylate cyclase activating polypeptide (PACAP) in female puberty and reproduction. This neuropeptide relays nutritional state information in the hypothalamus to regulate gonadotropin-releasing hormone release.

Metabolic cues play a critical role in the regulation of pubertal timing and reproduction by modulating the release of kisspeptin and/or (GnRH) at the hypothalamic level (1). While leptin receptors are expressed in a subset of kisspeptin neurons (2), genetic studies have shown that the main site of leptin's action to regulate reproduction is not on kisspeptin neurons directly, but rather on cells in the ventral premammillary nucleus (PMV) (3–6). Because PACAP is expressed in the PMV and appears to play a role in the regulation of GnRH secretion and energy balance, the authors hypothesized that it could be involved in the regulation of reproduction by energy availability. They showed that PACAP-expressing neurons of the PMV make direct contact with kisspeptin neurons in the arcuate and AVPV nuclei and respond to leptin stimulation. Mice with PACAP deleted conditionally from leptin receptor expressing neurons showed delayed onset of puberty and irregular estrus cycles with a blunted LH surge. The data shows that PACAP is responsible for transducing some, but not all, of the metabolic information relayed by leptin to the hypothalamic-pituitary-gonadal axis. In order to investigate the role of PACAP in the PMV directly, they showed that targeted deletion of the PACAP in the PMV in adult females led to increased estrus cycle length and decreased fertility. The magnitude of the LH increase after leptin administration centrally did not differ in control and injected animals, indicating that PACAP from the PMV is necessary for normal reproductive function but not for the relay of leptin signal from this nucleus.

This study sheds a light on the complex crosstalk between energy balance and reproduction, and brings the first evidence of a role for PACAP in the control of puberty, and more specifically in the PMV in ovulatory cycling

and subsequent fertility in females. Kisspeptin neurons in the arcuate nucleus and the AVPV are heterogeneous populations, which can be defined by their response to PACAP.

References

1. Navarro VM, Kaiser UB. 2013. Metabolic influences on neuroendocrine regulation of reproduction. *Current Opinion in Endocrinology & Diabetes and Obesity* 20:335–341.
2. Smith JT, Acochido BV, Clifton DK, Steiner RA. 2006. Kiss-1 neurons are direct targets for leptin in the ob/ob mouse. *Journal of Neuroendocrinology* 18:298–303.
3. Cavalcante JC, Bittencourt JC, Elias CF. 2014. Distribution of the neuronal inputs to the ventral premammillary nucleus of male and female rats. *Brain Research* 1582:77–90.
4. Donato J, Cravo RM, Frazão R, Gautron L, Scott MM, Lachey J, Castro IA, Margatho LO, Lee S, Lee C, Richardson JA, Friedman J, Chua S, Coppari R, Zigman JM, Elmquist JK, Elias CF. 2011. Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. *Journal of Clinical Investigation* 121:355–368.
5. Donato J, Silva RJ, Sita LV, Lee S, Lee C, Lacchini S, Bittencourt JC, Franci CR, Canteras NS, Elias CF. 2009. The ventral premammillary nucleus links fasting-induced changes in leptin levels and coordinated luteinizing hormone secretion. *Journal of Neuroscience* 29:5240–5250.
6. Cravo RM, Frazao R, Perello M, Osborne-Lawrence S, Williams KW, Zigman JM, Vianna C, Elias CF. 2013. Leptin signaling in Kiss1 neurons arises after pubertal development. *PLoS One* 8: e58698.

7.2. SIRT1 mediates obesity- and nutrient-dependent perturbation of pubertal timing by epigenetically controlling Kiss1 expression

Vazquez MJ, Toro CA, Castellano JM, Ruiz-Pino F, Roa J, Beiroa D, Heras V, Velasco I, Dieguez C, Pinilla L, Gaytan F, Nogueiras R, Bosch MA, Rønnekleiv OK, Lomniczi A, Ojeda SR, Tena-Sempere M

Nat Commun. 2018 Oct 10;9(1):4194.

<https://www.ncbi.nlm.nih.gov/pubmed/30305620>

This study identifies Sirtuin 1 (SIRT1), a fuel-sensing deacetylase, as a molecule that restrains female puberty via epigenetic repression of the puberty-activating gene, Kiss1 in rats.

The last few years have brought evidence regarding the epigenetic control of the onset of puberty. It is now clearly established that a switch from epigenetic repression to activation within kisspeptin neurons in the arcuate nucleus is a core mechanism underlying the initiation of female puberty. However, little is known about the pathways conveying epigenetic information from different stimuli, such as nutrition, circadian activity and environmental toxins/endocrine disruptors to the hypothalamic cells controlling the timing of puberty. The KNDy neurons produce Kisspeptin, NKB (neurokinin B) and Dynorphin and drive the changes in GnRH secretion that set-in motion the endocrine manifestations of puberty (1). They serve as nodal portals for nutritional cues to influence reproductive development and are subjected to a repressive epigenetic control, imposed by the Polycomb (PcG) silencing complex that prevents the premature activation of puberty (2).

Sirtuin 1 (SIRT1) is a deacetylase, highly expressed in the brain and acting on histones and other cellular targets. It senses cell energy and modulates life/health span (3, 4). These authors show that SIRT1 is expressed in hypothalamic Kiss1 neurons and suppresses Kiss1 expression, via interaction with the Polycomb silencing complex. As puberty approaches, SIRT1 is evicted from the Kiss1 promoter facilitating a switch in local chromatin configuration from repressive to permissive. These changes are accelerated by early-onset obesity, which induces precocious puberty, and are postponed by undernutrition, which delays puberty. The causal link between these changes and obesity remains to be explored. This study illustrates the potential pathophysiological role that SIRT1 may play in eliciting pubertal perturbations associated with early-onset obesity and undernutrition.

References

1. Lehman, MN, Coolen LM, Goodman RL. 2010. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology* 151: 3479–3489.
2. Lomniczi A, Loche A, Castellano JM, Ronnekleiv OK, Bosch M, Kaidar G, Knoll JG, Wright H, Pfeifer GP, Ojeda SR. 2013 Epigenetic control of female puberty. *Nat. Neurosci.* 16 (3):281–9.

- Nogueiras R, Habegger KM, Chaudhary N, Finan B, Banks AS, Dietrich MO, Horvath TL, Sinclair DA, Pfluger PT, Tschöp MH. 2012 Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. *Physiol. Rev.* 92: 1479–1514.
- Giblin, W, Skinner ME, Lombard DB. 2014. Sirtuins: guardians of mammalian healthspan. *Trends Genet.* 30: 271–286.

7.3. Metabolic regulation of female puberty via hypothalamic AMPK-kisspeptin signaling

Roa J, Barroso A, Ruiz-Pino F, Vázquez MJ, Seoane-Collazo P, Martínez-Sánchez N, García-Galiano D, Ilhan T, Pineda R, León S, Manfredi-Lozano M, Heras V, Poutanen M, Castellano JM, Gaytan F, Diéguez C, Pinilla L, López M, Tena-Sempere M
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<https://www.ncbi.nlm.nih.gov/pubmed/?term=30348767>

The authors show for the first time that central AMP-activated protein kinase (AMPK), the major cellular energy sensor, interplays with Kiss1 to control puberty onset.

AMPK is an indispensable cellular energy sensor (1). Beside its ability to directly sense energy availability of the cell, hypothalamic AMPK is involved in the control of energy balance (1, 2). Fasting and orexigenic hormones activate AMPK, thereby inducing feeding, whereas refeeding and anorectic factors inhibit AMPK, resulting in suppression of food intake (1, 2).

The authors used intra-cerebro-ventricular injections of an AMPK activator as well as virogenetic overexpression of a constitutively active form of AMPK α and observed a profound delay of vaginal opening in female rats. They showed that AMPK is co-expressed in Kisspeptin neurons. While central administration of AMPK activator failed to modify Kiss1 mRNA in anteroventral periventricular nucleus neurons, Kiss1 expression in the arcuate nucleus was significantly blunted by the treatment. In addition, female mice with congenital ablation of AMPK α 1 in Kiss1 neurons were partially protected from the delayed vaginal opening caused by undernutrition.

Thus, AMPK, which operates as a hypothalamic regulator of energy homeostasis, also plays a relevant role in Kiss1 neurons in the arcuate nucleus for the metabolic control of puberty and its inhibition by conditions of energy insufficiency. These data have a considerable translational potential, as early therapies with drugs known to activate AMPK, such as metformin, have been reported to delay menarche in low birth weight girls with precocious pubarche (3) even though the central versus peripheral mechanisms of such effects are still unknown.

References

- López M, Nogueiras R, Tena-Sempere M, Diéguez C. 2016 Hypothalamic AMPK: A canonical regulator of whole-body energy balance. *Nat Rev Endocrinol.* 12:421–432.
- Xue B, Kahn BB 2006 AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. *J Physiol.* 574:73–83.
- Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. 2014 Metformin: From mechanisms of action to therapies. *Cell Metab.* 20:953–966.

Genetics of Puberty

7.4. HS6ST1 insufficiency causes self-limited delayed puberty in contrast with other GnRH deficiency genes

Howard SR, Oleari R, Poliandri A, Chantzara V, Fantin A, Ruiz-Babot G, Metherell LA, Cabrera CP, Barnes MR, Wehkalampi K, Guasti L, Ruhrberg C, Cariboni A, Dunkel L
J Clin Endocrinol Metab. 2018 Sep 1;103(9):3420–3429.
<https://www.ncbi.nlm.nih.gov/pubmed/29931354>

This whole-exome study in 67 probands and 93 relatives from a large cohort of familial delayed puberty identifies a new heterozygous HS6ST1 mutation as a novel cause of delayed puberty.

The underlying pathophysiology of early and delayed puberty remains unexplained in most patients. As illustrated by this article, familial delayed puberty represents an invaluable resource to discover new regulators of the onset of puberty (1).

A whole-exome sequencing study in 67 informative families with self-limited delayed puberty identified 20 rare variants in 12 genes. After filtering for segregation with trait, *HS6ST1* was retained as a candidate gene. One proband and his affected relatives carried a rare and likely damaging *HS6ST1* variant that caused a non-conservative amino acid substitution in the coding sequence. This mutation reduced sulfotransferase activity in vitro. The authors showed that *Hs6st1* mRNA was expressed in peripubertal wild-type mouse hypothalamus and olfactory bulbs. GnRH neuron counts were similar in *Hs6st1*+/- and *Hs6st1*+/+ mice, but vaginal opening was delayed in *Hs6st1*+/- mice despite normal postnatal growth.

Several lines of evidence support a role for heparan sulfate modification in the control of puberty. The *C. elegans* ortholog *hst-6* is known to display genetic interactions with *kal-1*, and anosmin-1 requires heparin sulfate with specific 6-*O*-sulfate modifications to exert its function in vivo (2). *HS6ST1* mutations have been previously identified in patients with idiopathic hypogonadotropic hypogonadism (3, 4) but this study is the first to identify a deleterious mutation as the likely causal factor for self-limited delayed puberty. This finding provides evidence that perturbations in a single allele of a gene regulating the hypothalamic-pituitary-gonadal axis is enough to cause self-limited delayed-puberty, while more deleterious alterations in the same gene, or in combination with additional genes, are required to cause more severe hypogonadotropic hypogonadism phenotypes.

This study helps us to better understand the genetic basis of self-limited delayed-puberty and brings us closer to the possibility of one day establishing a definitive diagnosis in adolescent patients presenting with delayed onset of puberty.

References

1. Howard SR. 2018 Genes underlying delayed puberty. *Mol Cell Endocrinol.* 15:476:119–128.
2. Bülow HE, Berry KL, Topper LH, Peles E, Hobert O 2002 Heparan sulfate proteoglycan-dependent induction of axon branching and axon misrouting by the Kallmann syndrome gene *kal-1*. *Proc Natl Acad Sci USA* 99:6346–6351.
3. Tomberg J, Sykietis GP, Keefe K, Plummer L, Hoang X, Hall JE, Quinton R, Seminara SB, Hughes V, Van Vliet G, Van Uum S, Crowley WF, Habuchi H, Kimata K, Pitteloud N, Bulow HE. 2011 Heparan sulfate 6-*O*-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotropic hypogonadism. *Proc. Natl. Acad. Sci. USA.* 108(28):11524–11529.
4. Pitteloud N, Meysing A, Quinton R, Acierno JS Jr, Dwyer AA, Plummer L, Fliers E, Boepple P, Hayes F, Seminara S, Hughes VA, Ma J, Bouloux P, Mohammadi M, Crowley WF Jr. 2006 Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol.* 254–255:60–69.

7.5. EAP1 regulation of GnRH promoter activity is important for human pubertal timing

Mancini A, Howard SR, Cabrera CP, Barnes MR, David A, Wehkalmi K, Heger S, Lomniczi A, Guasti L, Ojeda SR, Dunkel L

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<https://www.ncbi.nlm.nih.gov/pubmed/?term=30608578>

This whole-exome study from a large cohort of familial self-limited delayed puberty identifies the first EAP1 mutations leading to reduced GnRH transcriptional activity and resulting in a phenotype of self-limited delayed-puberty.

Enhanced at puberty 1 (EAP1) is a nuclear transcription factor, also called interferon regulatory factor 2 binding protein-like (IRF2BPL). Its transcriptional activity facilitates the initiation of female puberty, in a manner that is independent of hypothalamic *Kiss1* expression (1). Its expression has been shown to increase in the hypothalamus of rats and non-human primates at the time of puberty, and EAP1 deficiency leads to delayed puberty and disrupted estrous cyclicity in both rodents (2) and nonhuman primates (3). EAP1 transactivates the GnRH promoter, which facilitates GnRH secretion, and inhibits the preproenkephalin promoter, which represses GnRH secretion. Despite this seemingly important role, no EAP1 mutations had been identified in humans with pubertal disorders. The authors performed whole-exome sequencing in 67 probands and 93 relatives from a

large cohort of familial self-limited delayed puberty. They identified one in-frame deletion and one rare missense variant in *EAP1* in two unrelated families. Using a luciferase reporter assay, *EAP1* mutants showed a reduced ability to trans-activate the GnRH promoter compared to wild-type *EAP1*.

This study confirms the role of *EAP1* in human puberty and illustrates the important role of transcriptional repressors containing Zn finger motifs in the control of GnRH secretion.

References

1. Li C and Li P. 2017 Enhanced at puberty-1 (Eap1) expression critically regulates the onset of puberty independent of hypothalamic Kiss1 expression. *Cell. Physiol. Biochem.* 43: 1402–1412.
2. Heger S, Mastronardi C, Dissen GA, Lomniczi A, Cabrera R, Roth CL, Jung H, Galimi F, Sippell W, Ojeda SR. 2007 Enhanced at puberty 1 (EAP1) is a new transcriptional regulator of the female neuroendocrine reproductive axis. *J. Clin. Invest.* 117: 2145–2154.
3. Dissen GA, Lomniczi A, Heger S, Neff TL, Ojeda SR 2012 Hypothalamic EAP1 (enhanced at puberty 1) is required for menstrual cyclicity in nonhuman primates. *Endocrinology* 153: 350–361.

7.6. Elucidating the genetic architecture of reproductive ageing in the Japanese population

Horikoshi M, Day FR, Akiyama M, Hirata M, Kamatani Y, Matsuda K, Ishigaki K, Kanai M, Wright H, Toro CA, Ojeda SR, Lomniczi A, Kubo M, Ong KK, Perry JRB

Nat Commun. 2018 May. 17;9(1):1977.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=29773799>

This population study reports 26 loci for ages at menarche and menopause in a Japanese population and demonstrates widespread differences in allele frequencies and effect estimates between Japanese and European variants.

Over the past decade, genome-wide association study (GWAS) meta-analyses have explored the genetic architecture of reproductive ageing (1–4), but were limited by their large circumscription to European population. Here, the authors highlight the benefits and challenges of large-scale trans-ethnic approaches to succeed in the detection of key genes and pathways that are poorly represented in European populations.

This article is based on a GWAS for ages at menarche and menopause in 67,029 women of Japanese ancestry from the BioBank Japan Project (5). The authors report multiple novel loci for ages at menarche or menopause and thus identify novel genes and pathways involved in human reproductive ageing, particularly in the Japanese population. In particular, a deleterious variant in *GNRH1*, a known signal of menarche, was identified as a novel locus for menopause timing, suggesting a role for the hypothalamic-pituitary axis in the onset of menopause. This study, together with rodent data, also support a role for receptor-like protein tyrosine phosphatases (PTPRs) in the regulation of puberty timing (6).

The replication at genome-wide significance in the Japanese population of 14 known European signals for menarche or menopause supports a largely shared genetic architecture of reproductive ageing, despite population differences in heritability. However, both effect allele frequencies and effect estimates varied considerably between populations, likely due to a combination of differential genetic drift, selection, recombination and possibly environmental factors. Future studies, both within populations and across populations, should be conducted to comprehensively assess environmental interactions with variants at the level of individual loci and overall heritability. Likewise, additional functional works are required to help further understand the role of all of these genes in the regulation of puberty and menopause.

References

1. Perry JR, *et al.* 2009 Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. *Nat. Genet.* 41: 648–650.
2. Elks CE, *et al.* 2010 Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat. Genet.* 42: 1077–1085.
3. Perry JR, *et al.* 2014 Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche *Nature.* 514: 92–97.
4. Day FR, *et al.* 2017 Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nat. Genet.* 10: 1–19.

5. Nagai A, Hirata M, Kamatani Y, Muto K, Matsuda K, Kiyohara Y, Ninomiya T, Tamakoshi A, Yamagata Z, Mushihiro T, Murakami Y, Yuji K, Furukawa Y, Zembutsu H, Tanaka T, Ohnishi Y, Nakamura Y; BioBank Japan Cooperative Hospital Group & Kubo M. 2017 Overview of the BioBank Japan Project: study design and profile. *J. Epidemiol.* 27: S2–S8.
6. Parent AS, Mungenast AE, Lomniczi A, Sandau US, Peles E, Bosch MA, Rønnekleiv OK & Ojeda SR. 2007 A contactin-receptor-like protein tyrosine phosphatase β complex mediates adhesive communication between astroglial cells and gonadotrophin-releasing hormone neurones. *J. Neuroendocrinol.* 19:847–858

Environmental Factors and Puberty

7.7. Exposure to perfluoroalkyl substances during fetal life and pubertal development in boys and girls from the Danish National Birth Cohort

Ernst A, Brix N, Lauridsen LLB, Olsen J, Parner ET, Liew Z, Olsen LH, Ramlau-Hansen CH

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<https://www.ncbi.nlm.nih.gov/pubmed/?term=30628845>

This population-based cohort study suggests sex-specific associations between prenatal exposure to perfluoroalkyl substances and subsequent altered pubertal development.

Endocrine disrupting chemicals (EDCs) impact populations as much as individuals given their ubiquity in our environment. Developmental exposure to EDCs has been associated with increased risk of genital malformations, hypofertility and testis cancer in human and rodent males, as well as increased risk of breast cancer and alterations of pubertal timing and ovarian function in females (1). Perfluoroalkyl substances (PFASs) are a group of synthetic chemicals used as surfactants or components of surface coatings. They are found in a variety of consumer products, including carpets, textile, coating additives, food paper and packaging, furnishings, waterproof clothing, and cosmetics. These substances are very persistent and contaminate the developing foetus. Several *in vitro* and animal studies indicate that they act as estrogen agonists or androgen antagonists (2–6).

These authors measured several PFAS in maternal plasma from early gestation in 2 cohorts comprising > 1100 mother-child pairs, for whom data on offspring pubertal development were collected biannually from age 11 years until full maturation, using web-based questionnaires. Prenatal exposure to some PFAS was associated with earlier age at onset of puberty in girls. In boys, positive and negative associations with prenatal exposure to different types of PFAS were found. The magnitude (3 to 6 months) and pattern of the associations varied with the child's sex and the nature of the chemical.

This study is one of the first to focus on the effects of prenatal exposure to PFAS on the timing of puberty onset, indicating some alterations of fetal programming by these ubiquitous chemicals.

References

1. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. 2015 EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocr. Rev.* 36(6): E1–E150.
2. Abbott BD. 2009. Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR α), beta (PPAR β), and gamma (PPAR γ) in rodent and human development. *Reprod. Toxicol.* 27: 246–257.
3. Du G, Hu J, Huang H, Qin Y, Han X, Wu D, Qin Y, Han X, Wu D, Song L, Xia Y, Wang X; 2013. Perfluorooctane sulfonate (PFOS) affects hormone receptor activity, steroidogenesis, and expression of endocrine-related genes *in vitro* and *in vivo*. *Environ Toxicol. Chem.* 32(2):353–360.
4. Kjeldsen LS, Bonefeld-Jørgensen EC. 2013. Perfluorinated compounds affect the function of sex hormone receptors. *Environ. Sci. Pollut. Res. Int.* 20: 8031–8044.
5. Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol. Sci.* 99: 366–394.
6. Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Barbee BD, Richards JH, Butenhoff JL, Stevenson LA, Lau C. 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. *Toxicol. Sci.* 74: 369–381.

7.8. Systematic review and meta-analysis of the association between childhood physical activity and age at menarche

Calthorpe L, Brage S, Ong KK

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<https://www.ncbi.nlm.nih.gov/pubmed/?term=30588652>

This meta-analysis reviews the association between physical activity and age at menarche.

The onset of puberty is a multifactorial trait including a wide range of genetic and environmental components. Age at menarche has been associated with several environmental factors such as prenatal exposure, birthweight, childhood nutrition and body mass index, socio-economic circumstances and stress and physical activity (1–3).

This meta-analysis reviews the association between physical activity and age at menarche. The authors included the results obtained in 11 population-based cohorts and 13 athletes versus non-athletes studies. Only 5 cohort studies (one randomized controlled trials and 4 observational cohort studies) reported a significant association between greater pre-menarche physical activity and later menarche timing in the general population. In the randomized controlled trial, an intervention to prevent obesity reduced the likelihood of menarche during the two-year study period. In 12 athletes versus non-athletes studies, menarche occurred on average 1.13 years later in athlete compared to non-athlete girls.

The authors underlined the lack of intervention specificity in the randomized control trial and the existence of possible confounding factors, such as associated changes in diet. Moreover, given the association between energy expenditure and body weight, adjustment for body weight is essential in studies of energy expenditure. Future studies are needed to clarify the magnitude and nature of the effect of childhood physical activity on the timing of menarche in the general population as well as the nature of the physical activity affecting puberty timing.

References

1. Day FR, Thompson DJ, Helgason H, Chasan DI, Finucane H, Sulem P, *et al.* 2017 Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nat. Genet.* 49: 834–41.
2. Kelly Y, Zilanawala A, Sacker A, Hiatt R, Viner R. 2017 Early puberty in 11-year-old girls: Millennium Cohort Study findings. *Arch. Dis. Child.* 102:232–7.
3. Yermachenko A, Dvornyk V. 2014 Nongenetic determinants of age at menarche: a systematic review. *Biomed. Res. Int.* 2014:371583.

7.9. Age at menarche and blood pressure in pregnancy

Petry CJ, Ong KK, Hughes IA, Acerini CL, Dunger DB

Pregnancy Hypertens. 2019 Jan;15:134–140.

<https://www.ncbi.nlm.nih.gov/pubmed/30713829>

This observational cohort study highlights the negative association between age at menarche and blood pressure in pregnancy, and the probable relationship with BMI and insulin resistance.

Age at menarche (AAM) is influenced by genetic and environmental determinants (1). Given that some of the genetic variants associated with AAM are also associated with body mass index (BMI) it is perhaps not surprising that AAM is also linked to some BMI-related pathological conditions (2, 3).

This study analyzed data from 438 mothers from the Cambridge Baby Growth Study. Carlo Acerini who devoted much of his career to this study sadly passed away in May 2019. AAM was recalled using a questionnaire completed by the women. Blood pressure was measured four times during pregnancy (at mean 11.8; 31.4; 37 and 38.8 weeks).

The study showed a negative association between AAM and the four arterial blood pressure measurements, especially with systolic blood pressure. This association was attenuated when adjusted for either pre-pregnancy BMI or insulin resistance, suggesting a potential mechanism involving increased adiposity and insulin resistance. There was no significant association between AAM and hypertensive disorders of pregnancy.

Despite some limitations highlighted by the authors (studied women were not entirely representative of the population, AAM was self-reported, variation in the numbers of participants in the different models), the study suggests that blood pressure in pregnancy is related to AAM and that adiposity and insulin resistance may be

involved in mediating these associations. The findings highlight the role of early AAM in a particular female life course trajectory leading to poor cardiometabolic health.

References

1. Couch FJ, *et al.* 2014 Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. *Nature* 514: 92–97.
2. Luijken J, Van Der Schouw YT, Mensink D, Onland-Moret NC. 2017 Association between age at menarche and cardiovascular disease: A systematic review on risk and potential mechanisms. *Maturitas* 104:96–116.
3. Janghorbani M, Mansourian M, Hosseini E. 2014 Systematic review and meta-analysis of age at menarche and risk of type 2 diabetes. *Acta Diabetol.* 51: 519–528.
4. Petry CJ, Ong KK, Hughes IA, Acerini CI, Dunger DB. 2018 The association between age at menarche and later risk of gestational diabetes is mediated by insulin resistance. *Act. Diabetol.* 55: 853–59.

7.10. Is there a causal relationship between obesity and puberty?

Reinehr T, Roth CL

Lancet Child Adolesc Health. 2019 Jan;3(1):44–54.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=30446301>

This article reviews cross-sectional, longitudinal and intervention studies regarding the bidirectional relationship between obesity and puberty.

An increasing prevalence of obesity amongst children and adolescents is reported globally. Epidemiological cross-sectional and longitudinal studies consistently show that obese girls tend to enter puberty earlier than non-obese girls (1, 2). The situation appears more complex in boys: overweight boys mature earlier, but obese boys mature later (2). In intervention studies, reduction in BMI-SDS reduced the likelihood of puberty onset in the next year in overweight and obese girls, but increased this likelihood in overweight and obese boys (3). Of interest, onset of puberty was associated with an increase of gonadotropins, suggesting a normal hypothalamic-pituitary initiation of puberty.

The authors summarize the hypotheses for the relationship between obesity and puberty timing, and the sex differences. The most promising link is the adipokine leptin and its interaction with the kisspeptin system. Indeed, adipose tissue acts as an endocrine organ secreting adipokines, such as leptin, which stimulates GnRH by activating kisspeptin neurons (4, 5). Interestingly, leptin regulation of puberty is sex-specific: testosterone inhibits leptin secretion from adipocytes (6). This sex difference might explain why the onset of puberty shifts to an earlier age in obese girls, but to a later age in obese boys. Peripheral mechanisms, such as adipose tissue aromatase activity, could also influence the timing of puberty. Finally, puberty is also influenced by insulin resistance associated with obesity: hyperinsulinemia reduces sex hormone binding globulin concentrations and thus increases the bioavailability of sex steroids (7). In addition, prenatal and childhood nutrition, physical activity or endocrine disrupting chemicals are potential mediators linking obesity to puberty timing.

References

1. Kaplowitz PB, Slora EJ, Wasserman RC, Pedlow SE, Herman-Giddens ME. 2001 Earlier onset of puberty in girls: relation to increased body mass index and race. *Pediatrics* 108: 347–53.
2. Wang Y. 2002 Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls. *Pediatrics* 110: 903–10.
3. Reinehr T, Bosse C, Lass N, Rothermel J, Knop C, Roth CL. 2017 Effect of weight loss on puberty onset in overweight children. *J. Pediatr.* 184: 143–150.
4. Casanueva FF, Dieguez C. 1999 Neuroendocrine regulation and actions of leptin. *Front. Neuroendocrinol.* 20: 317–63.
5. Navarro VM, Castellano JM, Garcia-Galiano D, Tena-Sempere M. 2007 Neuroendocrine factors in the initiation of puberty: the emergent role of kisspeptin. *Rev. Endocr. Metab. Disord.* 8: 11–20.
6. Rutters F, Nieuwenhuizen AG, Verhoef SP, Lemmens SG, Vogels N, Westerterp-Platenga MS. 2009 The relationship between leptin, gonadotropic hormones, and body composition during puberty in a Dutch children cohort. *Eur. J. Endocrinol.* 160: 973–78.
7. Sorensen K, Aksglaede L, Munch-Andersen T, Munch-Andersen T, Achmann-Andersen NJ, Petersen JH, Hilsted L, Helge JW, Juul A. Sex hormone-binding globulin levels predict insulin sensitivity, disposition index, and cardiovascular risk during puberty. *Diabetes Care* 2009; 32: 909–14.

7.11. Gonadal function and pubertal development in patients with Silver-Russell syndrome

Goedegebuure WJ, Smeets CCJ, Renes JS, de Rijke YB, Hokken-Koelega ACS

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<https://www.ncbi.nlm.nih.gov/pubmed/30252068>

This longitudinal study of 31 patients (14 males) with Silver-Russell syndrome (SRS) and 123 non-SRS small-for-gestational-age (SGA) patients (65 males) shows that Sertoli cell dysfunction is more common in SRS males, with 11p15 loss of methylation (LOM), but gonadal function seems to be unaffected in SRS females.

Literature describes that SRS males have an increased risk of genital abnormalities (1, 2) while SRS in females is associated with Mayer–Rokitansky–Küster–Hauser syndrome (3). However, data on gonadal function and fertility in patients with SRS are lacking.

These authors describe a longitudinal study, which analyzed gonadal function and pubertal progression in SRS patients from childhood until early adulthood. These data were compared to those of patients born SGA without SRS. Onset and progression of puberty were similar in SRS and non-SRS SGA children. However, Sertoli cell dysfunction was more common in SRS males than non-SRS SGA males: >25% of SRS males had a low post-pubertal inhibin B level <5th percentile on normal references; two SRS males also had a high FSH level >95th percentile. Gonadal function did not seem to be impaired in females with SRS.

This is the first study looking at gonadal function in patients with SRS. More research is warranted to investigate the etiology of Sertoli cell dysfunction in SRS males, especially those with a loss of methylation in 11p15, who appear to have an increased risk of gonadal dysfunction.

References

1. Price SM, Stanhope R, Garrett C, Preece MA, Trembath RC. 1999 The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. *J Med Genet.* 36:837–842.
2. Bruce S, Hannula-Jouppi K, Peltonen J, Kere J, Lipsanen-Nyman M. 2009 Clinically distinct epigenetic subgroups in Silver-Russell syndrome: the degree of H19 hypomethylation associates with phenotype severity and genital and skeletal anomalies. *J Clin Endocrinol Metab.* 94:579–587.
3. Abraham MB, Carpenter K, Baynam GS, Mackay DJ, Price G, Choong CS. 2014 Report and review of described associations of Mayer–Rokitansky–Küster–Hauser syndrome and Silver–Russell syndrome. *J Paediatr Child Health.* 51:555–560.
4. Bellver-Pradas J, Cervera-Sanchez J, Boldo-Roda A, Martin-Cortes A, Ferreres-Gomez L, Serra-Serra V, Romeu-Sarrio A. 2001 Silver-Russell syndrome associated to Mayer–Rokitansky–Küster–Hauser syndrome, diabetes and hirsutism. *Arch Gynecol Obstet.* 265: 155–157.

7.12. Prevalence of cranial MRI findings in girls with central precocious puberty: a systematic review and meta-analysis

Cantas-Orsdemir S, Garb JL, Allen HF

J Pediatr Endocrinol Metab. 2018 Jul 26;31(7):701–710.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=29902155>

This meta-analysis assesses the prevalence of intracranial lesions in girls with central precocious puberty and hence evaluates the benefit of routine MRIs in girls with puberty onset at age 6–8 years.

Central precocious puberty (CPP) is defined as the development of secondary sexual characteristics before age 8 years in girls and 9 years in boys (1) and is due to premature activation of the hypothalamic-pituitary gonadal axis. It can be idiopathic, genetic or associated with central nervous system (CNS) abnormalities (3). Girls with CPP have a lower incidence of CNS abnormalities than boys, but this rate varies between studies (3–7). Moreover, the incidence of CNS abnormalities seems to increase with younger ages at puberty onset (1), although data are inconsistent (8). In light of ongoing discussions regarding the decreasing age of normal puberty (9, 10), the utility of brain MRI in girls with CPP is still a matter of debate.

This paper reports the first systematic review and meta-analysis to provide an overall estimate of the prevalence of CNS lesions in girls with CPP, with a particular focus on CPP in girls aged 6–8 years old. The authors identified 15 studies from six electronic databases from 1990 through December 2015, describing a total of 1853 girls with CPP aged <8 years. Across all studies, the overall incidence of positive MRI was 7% (excluding lesions with questionable relationship with CPP). From the five studies that stratified data by age, the pooled prevalence of positive MRI was 25% in girls <6 years vs. 3% in girls aged 6–8 years. A similar trend was observed in CPP girls aged <7 vs. 7–8 years old. The most common CNS lesion was hypothalamic hamartoma, which in most cases does not require surgical intervention. Across all CPP girls, the incidence of CNS tumours was 1.6%.

Thus, MRI should be performed in girls with CPP before 6 years old (1, 11, 12). However, there is no clear benefit of routine MRI in girls with CPP older than 6 years without any neurological concern; such decisions require a consideration of the likelihood of detecting an intracranial lesion requiring intervention.

References

1. Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR. 2009 Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics* 123: e752–62.
2. Brito VN, Latronico AC, Arnhold IJ, Mendonça BB. 2008 Update on the etiology, diagnosis and therapeutic management of sexual precocity. *Arq Bras Endocrinol Metab.* 52: 18–31.
3. Chalumeau M, Chemaitilly W, Trivin C, Adan L, Breart G & Brauner R. 2002 Central precocious puberty in girls: an evidence-based diagnosis tree to predict central nervous system abnormalities. *Pediatrics.* 109:61–7.
4. Pedicelli S, Alessio P, Scirè G, Cappa M, Cianfarani S. 2014 Routine screening by brain magnetic resonance imaging is not indicated in every girl with onset of puberty between the ages of 6 and 8 years. *J Clin Endocrinol Metab.*; 99: 4455–61.
5. De Sanctis V, Corrias A, Rizzo V, Bertelloni S, Urso L, Galluzzi F, Pasquino AM, Pozzan G, Guarneri MP, Cisternino M, De Luca F, Gargantini L, Pilotta A, Sposito M, Tonini G. 2000 Etiology of central precocious puberty in males: the results of the Italian Study Group for Physiopathology of Puberty. *J Pediatr Endocrinol Metab*; 13:687–93.
6. Choi KH, Chung SJ, Kang MJ, Yoon JY, Lee JE, Shin CH, Yang SW. 2013 Boys with precocious or early puberty: incidence of pathological brain magnetic resonance imaging findings and factors related to newly developed brain lesions”. *Ann Pediatr Endocrinol Metab.* 18:183–90.
7. Ng SM, Kumar Y, Cody D, Smith CD, Didi M. 2003 Cranial MRI scans are indicated in all girls with central precocious puberty. *Arch Dis Child.* 88:414–8.
8. Mogensen SS, Akglaede L, Mouritsen A, Sørensen K, Main KM, Gideon P, Juul A. 2012 Pathological and incidental findings on brain MRI in a single-center study of 229 consecutive girls with early or precocious puberty. *PLoS One* 7:e29829.
9. Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM. 1997 Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics* 99:505–12.
10. Kaplowitz PB, Oberfield SE. 1999 Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. *Pediatrics* 104(Pt 1):936–41.
11. Kaplowitz PB. 2016 Do 6–8 year old girls with central precocious puberty need routine brain imaging? *Int J Pediatr Endocrinol.* 2016:19.
12. Chalumeau M, Hadjiathanasiou CG, Ng SM, Cassio A, Mul D, Cisternino M, Partsch CJ, Theodoridis C, Didi M, Cacciari E, Oostdijk W, Borghesi A, Sippell WG, Bréart G, Brauner R. 2003 Selecting girls with precocious puberty for brain imaging: validation of European evidence-based diagnosis rule. *J Pediatr.* 143:445–50

7.13. High prevalence of syndromic disorders in patients with non-isolated central precocious puberty

Wannes S, Elmaleh-Bergès M, Simon D, Zénaty D, Martinerie L, Storey C, Gelwane G, Paulsen A, Ecosse E, De Roux N, Carel JC, Léger J

Eur J Endocrinol. 2018 Dec 1;179(6):373–380.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=30324796>

This observational cohort study including children followed for central precocious puberty (CPP) in a single academic centre in Paris, France, identifies a large proportion of patients with complex disorders without structural hypothalamic lesions on MRI.

Patients with CPP undergo an etiological diagnostic workup, which typically includes neuroimaging in order to identify the possible underlying condition (see comment 7.12). Based on their clinical experience, the authors hypothesized a high prevalence of associated disorders among patients with non-isolated CPP. They identified 63 children (42 girls and 21 boys) with non-isolated CPP, representing 16% of their whole clinic population with CPP. In 45% of those patients, hypothalamic lesions were visible on MRI, including hamartomas, optic gliomas, malformations with inter-hypothalamic adhesions or associated with syndromic midline abnormalities or arachnoid cysts. The remaining 55% had no structural hypothalamic lesions, but also had narcolepsy, RASopathy, encephalopathy, autism spectrum disorder with or without chromosomal abnormality, or other complex syndromic disorder. Average age at puberty onset was 4.95 years in patients with hypothalamic lesions and 7.3 years in patients without. Future studies should explore the pathophysiological mechanisms underlying CPP in these disorders.

This study suggests that hypothalamic disturbances not visible on MRI may be associated with several complex disorders in patients with non-isolated and potentially non-idiopathic CPP.

Reference

1. Carel JC, Leger J. 2008 Clinical practice. Precocious puberty. *New England Journal of Medicine* 358: 2366–2377.

7.14. Pituitary deficiency and precocious puberty after childhood severe traumatic brain injury: a long-term follow-up prospective study

Dassa Y, Crosnier H, Chevignard M, Viaud M, Personnier C, Flechtner I, Meyer P, Puget S, Boddaert N, Breton S, Polak M

Eur J Endocrinol. 2019 May 1;180(5):281–290.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=30884465>

This longitudinal prospective study of 61 children with a 5-10 years follow-up post severe traumatic brain injury evaluates the prevalence of pituitary deficiency and precocious puberty.

In children, retrospective and prospective studies report variable rates of hypothyroidism and growth hormone deficiency (GHD) after severe traumatic brain injury (TBI) (1–5), but little is known about the long-term history of pituitary function post-TBI. Here, 61 children were followed up for 5–10 years post traumatic brain injury. At 1-year post-TBI 17 children had GHD, and of these GHD was confirmed 3–4 years post-TBI in 5/17 children. Four children developed CPP, on average 5.7 years post-TBI. Having a pituitary dysfunction at 1-year post-TBI was significantly associated with pituitary dysfunction or CPP at 5+ years post-TBI.

This study confirms that severe TBI in childhood can lead to permanent pituitary dysfunction and shows that GHD and CPP may appear many years after the traumatic injury. The authors recommend systematic hormonal assessment at 1-year post severe TBI and prolonged monitoring of growth and puberty.

References

1. Einaudi S, Matarazzo P, Peretta P, Grossetti R, Giodano F, Altare F, Bondone C, Andreo M, Ivani G, Genitori L, De Sanctis C. 2006 Hypothalamo-hypopyseal dysfunction after traumatic brain injury in children and adolescents: a preliminary retrospective and prospective study. *Journal of Pediatric Endocrinology and Metabolism* 19: 691–703.
2. Niederland T, Makovi H, Gal V, Andreka B, Abraham CS & Kovacs J. 2007 Abnormalities of pituitary function after traumatic brain injury in children. *Journal of Neurotrauma* 24: 119–127.
3. Poomtavorn P, Maixner W, Zacharin M. 2008 function in paediatric survivors of severe traumatic brain injury. *Archives of Disease in Childhood* 93: 133–137.
4. Auble BA, Bollepalli S, Makoroff K, Weis T, Khoury J, Colliers T & Rose SR. 2014 Hypopituitarism in pediatric survivors of inflicted traumatic brain injury. *Journal of Neurotrauma* 31: 321–326.
5. Sancho P. 2017 Pituitary dysfunction after traumatic brain injury: are there definitive data in children? *Archives of Disease in Childhood* 102: 572–577.

7.15. Letrozole versus testosterone for promotion of endogenous puberty in boys with constitutional delay of growth and puberty: a randomised controlled phase 3 trial

Varimo T, Huopio H, Kariola L, Tenhola S, Voutilainen R, Toppari J, Toiviainen-Salo S, Hämäläinen E, Pulkkinen MA, Lääperi M, Tarkkanen A, Vaaralahti K, Miettinen PJ, Hero M, Raivio T

Lancet Child Adolesc Health. 2019 Feb;3(2):109–120

<https://www.ncbi.nlm.nih.gov/pubmed/?term=30612946>

This randomised, controlled, open-label trial at four paediatric centres in Finland evaluates aromatase inhibition with letrozole to induce puberty in boys with constitutional delay of growth and puberty.

Treatment of delayed puberty aims to promote pubertal development and skeletal growth, while avoiding early epiphyseal maturation and gonadal injury. The aromatase inhibitor letrozole inhibits the conversion of androstenedione to estrone, and testosterone to estradiol. Hence, letrozole lowers estrogen concentrations and delays epiphyseal maturation in boys (1–3). By decreasing central feedback inhibition, letrozole also activates gonadotropin secretion and thus promotes testicular growth and testosterone secretion (1, 2).

Boys aged at least 14 years with constitutional delay of growth and puberty, who wanted medical intervention and exhibited the first signs of puberty, were randomly assigned to receive oral letrozole 2.5 mg once daily for 6 months (n=15) or standard treatment: 6 × 4-weekly intramuscular injections of low-dose (~1 mg/kg) testosterone (n=14). At 12-months, both letrozole and testosterone treatments induced similar changes in Tanner stage. Additionally, letrozole was more efficacious than testosterone in promoting testicular growth (+7.2 mls vs. +2.2 mls). Letrozole-induced gonadotropin secretion and high concentrations of intra-testicular testosterone might affect development of seminiferous epithelium. However, circulating concentrations of inhibin B remained stable during epiphyseal maturation.

This study suggests letrozole as an alternative treatment to testosterone for boys with constitutional delay of growth and puberty. Furthermore, it may have advantages to testosterone for testicular growth and potentially also for adult height, by suppressing the rate of bone maturation, although available data on this are yet inconclusive.

References

1. Wickman S, Dunkel L. 2001 Inhibition of P450 aromatase enhances gonadotropin secretion in early and midpubertal boys: evidence for a pituitary site of action of endogenous E. *J Clin Endocrinol Metab.* 86: 4887–94.
2. Hero M, Norjavaara E, Dunkel L. 2005 Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. *J Clin Endocrinol Metab.* 90: 6396–402.
3. Hero M, Wickman S, Dunkel L. 2006 Treatment with the aromatase inhibitor letrozole during adolescence increases near-final height in boys with constitutional delay of puberty. *Clin Endocrinol.* 64: 510–13.

7.16. Toward more targeted and cost-effective gonadotropin-releasing hormone analog treatment in girls with central precocious puberty

Kaplowitz PB, Backeljauw PF, Allen DB

Horm Res Paediatr. 2018;90(1):1–7.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=30048994>

The authors discuss why GnRH analog therapies should not be used in all girls with central precocious puberty (CPP), but only in those cases where the predicted benefits outweigh the risks and high cost of the treatment.

Epidemiological studies in Europe and USA show declining average age at onset of puberty (1). Consequently, an increasing proportion of girls are categorized as ‘precocious’, according to the widely accepted definition of CPP. GnRH analog treatment is used to suppress the hypothalamic-pituitary-gonadal axis in girls with CPP, in order to: a) slow the rate of skeletal maturation and thus increase predicted adult height (2); b) reduce the distress of early physical changes and menarche (3). The authors argue that this treatment is very long (2–4 years) and expensive (\$20,000–30,000 per year). They analyze the existing data on the benefits of CPP treatment: 1) do

GnRH analogs increase average height in children with CPP? 2) do girls with CPP have more psychological problems than prepubertal girls at the same age?

Studies show that the greatest height gain from GnRH analog treatment occurs in girls with onset of puberty <6 years old (4–7). The decision to initiate therapy in girls with onset at 6–8 years old should be individualized (8), especially in most of these girls who have slowly progressive CPP and will achieve target range adult heights without treatment (9).

It remains unclear whether psychosocial stress should be considered a predictable consequence of early puberty supporting a decision to start GnRHa treatment (10), and if so, whether treatment relieves such stress (11).

Thus, the decision regarding GnRHa therapies should attempt to balance benefits, risks and costs for each child individually and should be decided after an informed discussion with the family.

References

1. Sorensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A. 2012 Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. *Horm Res Paediatr.* 77: 137–45.
2. Comite F, Cassorla F, Barnes KM, Hensch KD, Dwyer A, Skerda MC, Loriaux DL, Cutler GB Jr, Pescovitz OH. 1986 Luteinizing hormone releasing hormone analogue therapy for central precocious puberty. Long-term effect on somatic growth, bone maturation, and predicted height. *JAMA* 255: 2613–6.
3. Sonis WA, Coite F, Bleu J, Pescovitz OH, Rahn CW, Hensch KD, Cutler GB Jr, Loriaux DL, Klein RP. 1985 Behavior problem and social competence in girls with true precocious puberty. *J Pediatr.* 106: 156–60.
4. Paul D, Conte FA, Grumbach MM, Kaplan SL. 1995 Long-term effect of gonadotropin-releasing hormone agonist therapy on final and near-final height in 26 children with true precocious puberty treated at a median age of less than 5 years. *J Clin Endocrinol Metab.* 80(2): 546–51.
5. Cassio A, Cacciari E, Balsamo A, Bal M, Tassinari D. 1999 Randomised trial of LHRH analogue treatment on final height in girls with onset of puberty aged 7.5–8.5 years. *Arch Dis Child.* 81(4): 329–32.
6. Pasquino AM, Pucarelli I, Accardo F, Demiraj V, Segni M, Di Nardo R. 2008 Long-term observation of 87 girls with idiopathic central precocious puberty treated with gonadotropin-releasing analogs: impact on adult height, body mass index, bone mineral content, and reproductive function. *J Clin Endocrinol Metab.* 93: 190–5.
7. Berek A. 2017 A critical appraisal of the effect of gonadotropin-releasing hormone analog treatment on adult height of girls with central precocious puberty. *J Clin Endocrinol Metab.* Suppl 2:33–48.
8. Carel JC, Eugster EA, Rogol A, Chizzoni L, Palmert MR, Antoniazzi F, Berenbaum S, Bourguignon JP, Chrousos GP, Coste J, Deal S, de Vries L, Foster C, Heger S, Holland J, Jahnukainen K, Juul A, Kaplowitz P, Lahlou N, Lee MM, Lee P, Merke DP, Neely EK, Oostdijk W, Phillip M, Rosenfield RL, Shulman D, Styne D, Tauber M, Wit JM. ESPE-LWPES GnRH Analogs Consensus Conference Group. 2009 Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics.* 123(4):e752–62.
9. Massart F, Federico G, Harrell JC, Saggese G. 2009 Growth outcome during GnRH agonist treatments for slowly progressive central precocious puberty. *Neuroendocrinology.* 90: 307–14.
10. Schoelwer MJ, Donahue KL, Didrick P, Eugster EA. 2017 One-year follow-up of girls with precocious puberty and their mothers: do psychological assessments change over time or with treatment? *Horm Res Paediatr.* 88: 347–53.
11. Wojnusz S, Callens N, Sütterlin S, Andersson S, De Schepper J, Gies I, Vanbesien J, De Waele K, Van Aken S, Craen M, Vögele C, Cools M, Haraldsen IR. 2017 Cognitive, emotional, and psychosocial functioning of girls treated with pharmacological puberty block-age for idiopathic central precocious puberty. *Front Psychol.* 7: 1053

8. Adrenals

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Preface

For this year's chapter on 'Adrenals', we have searched the PubMed for articles on 'adrenal' or 'steroidogenesis' published in English between June 1, 2018 and May 31, 2019. Our search yielded more than 5,000 citations. We have examined all citations individually and selected the following collection of basic research and clinical articles. Whenever possible, we have avoided topics that have been discussed in the Yearbook 2018, unless progress in the field has been incremental. Emerging themes for this year's chapter include: i) Endogenous glucocorticoids control host resistance to viral infection through the tissue-specific regulation of PD-1 expression on NK cells; ii) Steroidogenic differentiation and PKA signaling are programmed by histone methyltransferase EZH2 in the adrenal cortex; iii) Associations between adrenarcheal hormones, amygdala functional connectivity and anxiety symptoms in children; iv) An Endocrine Society Clinical Practice Guideline for the diagnosis and management of Congenital Adrenal Hyperplasia Due to Steroid 21-hydroxylase deficiency; and v) Epigenetic alterations associated with early prenatal dexamethasone treatment.

Mechanism of the Year: Endogenous glucocorticoids suppress immunopathology and promote host resistance to viral infection without compromising protective immunity

8.1. Endogenous glucocorticoids control host resistance to viral infection through the tissue-specific regulation of PD-1 expression on NK cells

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Nat Immunol. 2018; 19(9): 954–962.
<https://www.ncbi.nlm.nih.gov/pubmed/30127438>

The immune system protects the host organism against infectious diseases, however, pathogen elimination frequently entails collateral tissue damage and inflammation, which decrease host fitness (1). Neuroendocrine-immune interactions play an important role in these regulatory processes (2, 3). Endogenous glucocorticoids (cortisol in humans and corticosterone in rodents) regulate many physiological and developmental processes through their ubiquitously expressed glucocorticoid receptor (GR). The HPA neuro-endocrine pathway restores homeostasis by limiting and resolving inflammation in many conditions (2). The HPA axis is activated during infection with viruses, such as influenza 10 and cytomegalovirus (CMV) (4). Murine CMV (MCMV) is a beta

herpesvirus used as a model for systemic human CMV infection, which can cause severe disease, especially in immunocompromised patients. Mice rendered glucocorticoid-deficient by adrenalectomy are more susceptible to MCMV-induced death, due to the deleterious effects of cytokine-induced over-inflammation, and resistance is restored by corticosterone replacement (5). In all these conditions, the underlying mechanisms of glucocorticoids remain unclear, because GRs are expressed by many hematopoietic and non-hematopoietic cells and can impact on multiple signaling pathways. During acute infection, the cytokine interferon- γ (IFN- γ), produced by innate lymphoid cells (ILCs) is essential for antiviral defense. These IFN- γ -producing ILCs include spleen and liver natural killer (NK) cells and liver ILC1s, which all express the GR (6).

In this study, the authors investigated the role of the GR in ILC subsets relevant to MCMV infection. They used mice with a conditional deletion of the GR gene in ILCs expressing NK cells, ILC1s and a subset of ILC3s. They found that endogenous glucocorticoids produced rapidly after infection induced the selective and tissue-specific expression of the immune checkpoint PD-1 on the surface of spleen NK cells. Concomitantly, PD-1 ligands were upregulated in several immune cell subsets. PD-1 signalling was required for host survival to infection and acted by limiting the production of IFN- γ by NK in the spleen, which prevented immunopathology. This neuroendocrine-immune axis controlled immune tolerance but did not impair viral clearance.

The glucocorticoid-dependent induction of PD-1 expression on NK cells in MCMV infection is a previously unrecognized mechanism by which the HPA axis suppresses immunopathology and promotes autoimmune disease resistance without compromising protective immunity. Therefore, these findings demonstrate a major role for the HPA axis in promoting host resistance to an infectious disease through regulation of the PD-1 inhibitory pathway in an ILC subset. A deeper understanding of the physiological and pathological conditions in which this glucocorticoid-PD-1 pathway exerts some beneficial or detrimental effects could provide a rational basis for the development of new therapeutic strategies.

References

1. Medzhitov R, Schneider DS, Soares MP. Disease tolerance as a defense strategy. *Science*. 2012; 335:936–941.
2. Webster JL, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. *Annu Rev Immunol*. 2002; 20:125–163.
3. Irwin MR, Cole SW. Reciprocal regulation of the neural and innate immune systems. *Nat Rev Immunol*. 2011; 11: 625–632.
4. Ruzek MC, Miller AH, Opal SM, Pearce BD, Biron CA. Characterization of early cytokine responses and an interleukin (IL)-6-dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection. *J Exp Med*. 1997; 185:1185–1192.
5. Ruzek MC, Pearce BD, Miller AH, Biron CA. Endogenous glucocorticoids protect against cytokine-mediated lethality during viral infection. *J Immunol*. 1999; 162:3527–3533.
6. Quatrini L, *et al*. Host resistance to endotoxic shock requires the neuroendocrine regulation of group 1 innate lymphoid cells. *J Exp Med*. 2017; 214(12): 3531–3541.

New Mechanisms

8.2. Isolation and characterization of adrenocortical progenitors involved in the adaptation to stress

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Humans are constantly challenged by multiple stressors, to which the body's response and adaptation are essential. The adrenal gland plays a major role in the response to both physiological challenges and stress. Maintenance of the adrenal is partly accomplished by proliferation and differentiation of adult progenitors and stem cells in the adrenal cortex and medulla. In previous studies, the authors demonstrated a direct effect of adrenocortical growth factors and androgens on proliferation and differentiation of adult adrenomedullary

chromaffin cells (7) and chromaffin progenitor cells (8). They also showed that Nestin⁺ stem-like cells in the adrenal medulla play an important role under stress, predominantly differentiating into chromaffin cells (9).

In the present study, they characterized a distinct population of Nestin⁺ progenitors in the murine adrenal cortex and analyzed its role in stress. Under basal conditions, these progenitors very slowly migrate centripetally through the different zones of the adrenal cortex to the cortical–medullary boundary. However, under stress, the progenitors migrate faster and differentiate into steroidogenic cells. In addition, they isolated the Nestin⁺ cells from the adrenal cortex, characterized them *in vitro* and showed that they display progenitor characteristics and are able to generate functional cells producing steroid hormones. These findings demonstrate the coordinated action of stress-inducible stem cells to ensure tissue remodeling and cellular and functional adaptation to stress.

References

7. Sicard F, *et al.* Age-dependent regulation of chromaffin cell proliferation by growth factors, dehydroepiandrosterone (DHEA), and DHEA sulfate. *Proc Natl Acad Sci USA* 2007; 104:2007–2012.
8. Chung KF, Qin N, Androutsellis-Theotokis A, Bornstein SR, Ehrhart-Bornstein M. Effects of dehydroepiandrosterone on proliferation and differentiation of chromaffin progenitor cells. *Mol Cell Endocrinol* 2011; 336:141–148.
9. Rubin de Celis MF, *et al.* Multipotent glia-like stem cells mediate stress adaptation. *Stem Cells* 2015; 33:2037–2051.

8.3. Steroidogenic differentiation and PKA signaling are programmed by histone methyltransferase EZH2 in the adrenal cortex

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The adrenal cortex plays a central role in regulating body homeostasis through the production of glucocorticoids and mineralocorticoids that control many important physiologic functions, such as metabolism, inflammation and arterial blood pressure. The production of these hormones, known as steroidogenesis, is achieved by differentiated steroidogenic cells that arise from undifferentiated progenitors and are constantly renewed throughout life (10). Epigenetic factors play an essential role in programming and maintaining cell-fate decisions during embryonic development and in adult tissue homeostasis (11).

In this study, the authors investigated the role of histone methyltransferase EZH2, a key epigenetic factor, in adrenocortical development and homeostasis by targeted inactivation of EZH2 in adrenal cortex steroidogenic cells. Their data show that EZH2 ablation is associated with deficient zona fasciculata differentiation, resulting in primary glucocorticoid insufficiency, as well as disruption of the unidirectional renewal and differentiation process. Altogether, these data demonstrate an all-encompassing role of EZH2 in programming steroidogenic cells for optimal response to differentiation signals and in preventing their dedifferentiation to a progenitor-like state.

References

10. Yates R, *et al.* Adrenocortical development, maintenance, and disease. *Curr Top Dev Biol* 2013; 106:239–312.
11. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature* 2011; 469:343–349.

8.4. Associations between adrenarcheal hormones, amygdala functional connectivity and anxiety symptoms in children

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Psychoneuroendocrinology. 2018; 97: 156–163.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=30036793>

Anxiety disorders are common in childhood and adolescence and the transition from childhood to adolescence seems to be a vulnerable period for the development of anxiety symptoms. This article elucidates the impact of

androgens (DHEA, DHEAS and testosterone) on functional connectivity of the amygdala while processing fearful facial expressions and the relation to anxiety symptoms. 83 children (40 boys, 43 girls; mean age 9.5 years) underwent a functional MRI scan of the brain while looking at fearful and calm facial expressions. They also completed the Spence Children's Anxiety Scale (SCAS). The concentrations of androgens were also determined in saliva in the morning. Amygdala connectivity was analyzed.

Androgen concentrations (DHEA; DHEAS; T) did not directly correlate with SCAS scores. However, in boys, both DHEA and T were positively associated with left amygdala connectivity with the visual cortex. In addition, T was related to more positive left amygdala connectivity with the anterior cingulate cortex (ACC). In girls, DHEAS concentrations showed a negative correlation with the right amygdala connectivity to the fusiform face area (FFA) and to the insula. In the mediation analysis in boys, the authors showed that higher DHEAS concentrations were indirectly related to greater SCAS social anxiety and obsessive-compulsive (OC) symptoms through more positive left amygdala-visual cortex connectivity. In girls, increased DHEAS was indirectly associated with less anxiety, through less positive amygdala-fusiform gyrus connectivity. In both sexes, DHEAS concentrations were indirectly related to less SCAS OC symptoms through weaker positive amygdala-cerebellum connectivity.

These findings highlight important sex differences in the relation between adrenarcheal hormones, functional connectivity of the amygdala and anxiety during childhood. They also suggest that amygdala connectivity may be an important neural mechanism in the link between adrenarcheal hormones and anxiety symptoms in children.

Important for Clinical Practice

8.5. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline

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<https://www.ncbi.nlm.nih.gov/pubmed/30272171>

Since the publication of the 2010 Endocrine Society clinical practice guideline for Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency, there have been several advances in the diagnosis and management of the condition. Neonatal diagnosis methods have been refined to use gestational age in addition to birth weight for cut-point interpretation or to employ liquid chromatography–tandem mass spectrometry (LC-MS/MS) as a secondary screening test. The standard for confirming a diagnosis of CAH continues to be serum 17-hydroxyprogesterone (17OHP) concentrations, most often following cosyntropin stimulation. The advent of commercially available serum 21-deoxycortisol measurements may simplify identification of CAH carriers. New human and animal data convey further concerns regarding prenatal dexamethasone treatment. No international registry has yet been established for the long-term outcomes of individuals treated prenatally with dexamethasone. Although non-invasive prenatal diagnosis of fetal sex is now commonly performed, prenatal CAH genotyping has been reported only in a proof-of-concept study and is not routinely available.

This updated clinical practice guideline includes more detailed protocols for adults, especially pregnant women. It suggests more moderate use of stress dosing during minor illness or minor surgery in patients with CAH. Over time, the approach to genital reconstructive surgery has changed, incorporating more shared decision-making among parents, patients, surgeons, endocrinologists, mental health providers, and support groups. A systematic review and meta-analysis of published literature on surgery for females with CAH through early 2017 could not identify enough scientifically rigorous studies delineating a favorable benefit-to-risk ratio for either early or late elective genital reconstructive surgery for females with CAH. CAH should not be equated with other, rarer 46,XX or XY disorders of sex development in formulating treatment guidelines and policies. The main goal is consistently directed at preserving functional anatomy and fertility. In another new meta-analysis, investigators

found no direct well-controlled evidence of cardiovascular or metabolic morbidity and mortality associated with CAH. Thus, it is recommended that individuals with CAH should be monitored according to conventional guidelines for monitoring CAH-affected children, adolescents, and adults. Regular follow-up of adult patients with CAH in specialist centers is an important goal, and there is a need for improved mental health monitoring. Finally, in this guideline, the authors discuss potential new therapies and future ways to improve quality of life for individuals with CAH.

8.6. Circadian rhythm of glucocorticoid administration entrains clock genes in immune cells: A DREAM trial ancillary study

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Conventional glucocorticoid (GC) therapy in adrenal insufficiency (AI) does not fully mimic the endogenous cortisol circadian rhythm, and this may adversely affect long-term health. In the recent DREAM trial (Dual Release Hydrocortisone vs. Conventional Glucocorticoid Replacement in Hypocortisolism) patients with AI showed an atypical inflammation, with more classic monocytes and impaired innate immune responses. Treatment with once-daily modified-release hydrocortisone improved circulating immune cell profiles and reduced infections compared to standard GC replacement therapy.

The current study aimed to investigate whether patients with AI enrolled in the DREAM trial had an altered expression of circadian genes in PBMCs compared to control subjects. Furthermore, they investigated whether the pro-inflammatory state seen on conventional GC treatment was associated with dysregulation of such genes, whether the more physiological treatment restored clock gene expression, and whether restoration of clock gene expression correlated with clinical outcome.

Gene expression profiling of 84 circadian clock genes was performed in: A) 29 patients with AI on standard multiple daily hydrocortisone (HC) treatment; B) 26 patients with AI on modified release HC treatment; C) 16 control subjects. PBMCs were freshly isolated in the morning at baseline and 12 weeks after randomization (A, B, C). At baseline, 19 genes were differentially expressed in patients with AI compared to controls, for example downregulation of CLOCK and ARNTL, whereas PER3 and TIMELESS were upregulated. Most genes in the CREB pathway cluster (CAMK2D, CREB1, CREB3, MAPK1, PRKAR1A, PRKAR12A, and PRKCB) were under-expressed in patients with AI. The relative expression of several genes correlated with the metabolic and immune phenotype. At week 12, patients on once-daily modified release HC exhibited an increase in expression levels of ARNTL, ARNTL2, CLOCK and RORA and reduced the previously overexpressed PER3 and TIMELESS levels. Of the 19 differentially expressed genes, 16 were also modulated in lymphocytes sorted from the entire subset of pooled PBMCs. There was also a correlation between changes in expression of several clock genes (CAMK2D, CSNK1A1, GUSB, ONP3, PER3, PRF1, SP1, TIMELESS, WEE1) and changes in clinical outcome, such as glycated hemoglobin, blood pressure, and levels of circulating soluble CD16, ADAM17, pro-inflammatory monocytes and frequency of infections. This suggests that reprogramming of circadian gene expression/resynchronisation using once-daily modified release HC is linked to improvements in clinical outcome in patients with AI.

8.7. Complement component 4 variations may influence psychopathology risk in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Patients with Congenital adrenal hyperplasia (CAH) suffer from multiple comorbidities, including mental illness. Studies that investigated the prevalence of psychiatric disorders in patients with CAH showed that any psychiatric diagnosis were more common in CAH subjects compared with population controls. In particular, the

risk of alcohol misuse was increased in females with CAH compared with controls, and appeared most common among the girls and women with the most severe null genotype. The risk of stress and adjustment disorders was doubled compared with female population controls. Furthermore, suicidality, other psychiatric disorders (excluding suicidality), and alcohol misuse were increased in males with CAH compared with controls. There was no increased risk for any neurodevelopmental disorder.

CYP21A2, the gene mutated in, lies within the major histocompatibility complex within a region (RCCX) that is error prone during meiosis. Neighbouring *CYP21A2* are genes that encode the complement component 4 isotypes, C4A and C4B, which are implicated in a variety of diseases, both immune related and psychiatric.

This study aimed to evaluate C4A and C4B copy number variation in patients with CAH in relation to psychiatric morbidity and autoimmunity. The authors determined comorbidity, copy numbers of C4A and C4B in patients with CAH and carrier relatives, and evaluated serum C4 concentrations. Only 30% of subjects had the expected two copies in each of the two C4 genes. High C4A copy number was associated with increased risk of an externalizing psychiatric condition. C4B copy number determined total C4 copy number and serum C4 concentrations, negatively correlated with carriership of a 30-kb deletion, and positively correlated with carriership of the p.V281L variant. No association was found between C4 copy number and autoimmune disease. This study provides evidence that RCCX genotype as it relates to C4 may be an additional risk factor for psychiatric comorbidity in patients with CAH.

8.8. The Natural History of Adrenal Insufficiency in X-Linked Adrenoleukodystrophy: An International Collaboration

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<https://www.ncbi.nlm.nih.gov/pubmed/?term=30252065>

Primary adrenal insufficiency (AI) is a major clinical manifestation in boys and men with X-linked adrenoleukodystrophy (ALD), a progressive neurodegenerative inborn error of metabolism readily diagnosed by detecting elevated plasma very-long-chain fatty acids (VLCFAs), in particular the ratios of C26:0/C22:0 and C24:0/C22:0 (12). Other clinical manifestations include slowly progressive spinal cord disease, rapidly progressive inflammatory demyelinating cerebral disease and primary hypogonadism (12). The aim of this retrospective international multicentre study was to describe the natural history of AI in boys and men with ALD, as well as to assess associations between the risk for developing AI, spinal cord disease, or cerebral disease and plasma C26:0/C22:0 and C24:0/C22:0 ratios.

The findings showed that the lifetime prevalence of AI in ALD is ~80%. The cumulative probability of AI was highest in the first decade of life (46.8%), remained prominent until 40 years of age (an additional 28.6%), and decreased substantially thereafter (an additional 5.6%). Plasma C26:0/C22:0 and C24:0/C22:0 ratios, although diagnostic for ALD, are not associated with the (age-dependent) risk of developing AI, spinal cord disease, or cerebral disease. Abnormal 08:00h plasma ACTH and cortisol concentrations preceded endocrine symptoms in many patients (43/92, 46.7%), warranting regular assessments of the adrenal function in asymptomatic ALD males.

Over time, long-term prospective follow-up of babies diagnosed through newborn screening will elucidate the true natural history of AI in ALD. Meanwhile, the development of treatment strategies toward reduction of VLCFA accumulation or even restoration of the genetic defect continues, and in the future these might be able to prevent onset of AI all together.

Reference

12. Kemp S, Huffnagel IC, Linthorst GE, Wanders RJ, Engelen M. Adrenoleukodystrophy - neuroendocrine pathogenesis and redefinition of natural history. *Nat Rev Endocrinol.* 2016;12(10): 606–615.

8.9. A Randomized Trial of Epinephrine in Out-of-Hospital Cardiac Arrest

Perkins GD, Ji C, Deakin CD, Quinn T, Nolan JP, Scopinari C, Regan S, Long J, Slowther A, Pocock H, Black JJM, Moore F, Fothergill RT, Rees N, O'Shea L, Docherty M, Gunson I, Han K, Charlton K, Finn J, Petrou S, Stallard N, Gates S, Lall R, PARAMEDIC2 Collaborators

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In attempting to reduce the rate of death and disability associated with cardiac arrest worldwide, emergency medical workers have few effective treatments other than early initiation of cardiopulmonary resuscitation (CPR) and prompt defibrillation. Epinephrine (adrenaline) has potentially beneficial effects in cardiac arrest through the constriction of arterioles mediated by α -adrenergic receptors. Potentially harmful effects on the heart are mediated through β -adrenergic stimulation, which causes dysrhythmias and increased myocardial oxygen demand and increases the risk of recurrent cardiac arrest. In addition, α -adrenergic stimulation causes platelet activation, which promotes thrombosis and impairs the microvascular blood flow in the cerebral cortex, which in turn increases the severity of cerebral ischemia during CPR and after a return of spontaneous circulation.

The International Liaison Committee on Resuscitation, a consortium of 7 major organizations involved in the field of resuscitation, initiated the PARAMEDIC2 (Prehospital Assessment of the Role of Adrenaline: Measuring the Effectiveness of Drug Administration in Cardiac Arrest) trial in 8016 UK patients to determine whether epinephrine (1.0 mg every 3–5 minutes) is beneficial or harmful as a treatment for out-of-hospital cardiac arrest. In this trial, epinephrine during resuscitation for out-of-hospital cardiac arrest significantly improved survival at 30 days compared to placebo. Patients in the epinephrine group had a higher rate of return of spontaneous circulation, a higher frequency of transport to hospital, and a higher rate of treatment in the ICU. However, more patients in the epinephrine group survived with severe neurologic disability, and there was no effect of epinephrine on the rate of survival with a favorable neurologic outcome.

These findings show that the use of epinephrine for out-of-hospital cardiac arrest improves survival, however, at the risk of increased severe neurological disability, thereby making the decision of using it in the context of out-of-hospital cardiac arrest a very difficult one.

8.10. Treatment of Primary Aldosteronism with mTORC1 Inhibitors

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<https://www.ncbi.nlm.nih.gov/pubmed/?term=31087053>

Primary aldosteronism (PA) is one of the most common causes of secondary hypertension, affecting ~6% of the adult general hypertensive population. Patients with PA are at increased risk of cerebrovascular and cardiovascular morbidity, renal abnormalities and metabolic syndrome. The activity of mTORC1 (mammalian target of rapamycin complex 1) is increased in the adrenal glands of patients with PA.

The current study analyzed the expression of mTORC1 components in aldosterone-producing adenomas (APA) in mice and in patients with PA, and investigated whether mTORC1 inhibition affects aldosterone levels, blood pressure and renin levels. Six pairs of APA and matched control adrenal cortex samples were subjected to deep quantitative mass spectrometry analysis. The expression of mTORC1 components was unchanged in APA and thus does not explain the increased mTORC1 activity observed in APAs. Systemic inhibition of mTORC1 using rapamycin in mice decreased plasma aldosterone levels but the changes were not significant and were independent of Angiotensin II. Whether the effect is direct on aldosterone synthesis or indirect via paracrine factors regulating aldosterone metabolism remains to be investigated.

To assess the systemic mTORC1 effect in human PA, 14 patients with PA were treated with everolimus for two weeks. Ten patients reported some type of adverse event, 1 patient developed pancreatitis (serious adverse

event), and 4 patients were defined as treatment responders ($\geq 30\%$ reduction in aldosterone levels), although the aldosterone levels were returned to baseline after two weeks of wash-out period. Other steroid hormone levels did not differ between the treatment and non-treatment phase. Furthermore, 24-h steroid metabolome profiles of the patients responding to everolimus treatment suggested that everolimus affects aldosterone synthesis specifically since both aldosterone levels and its major metabolite tetrahydroaldosterone were reduced, but no change was observed in the levels of other steroids with mineralocorticoid action. Patients with PA treated with everolimus showed significant reductions in blood pressure and lowered the renin suppression. The effect was independent of heart rate, urine volume, and ADH.

The above results in mice and patients with PA suggest that increased mTORC1 activity in APA most likely depends on downstream signaling changes rather than overexpression of its components. mTORC1 inhibition is a potential therapy for hypertension in PA, however, further studies are needed to identify potential responders, and to define the most appropriate dose and safety and efficacy profiles.

New Genes

8.11. Susceptibility to corticosteroid-induced adrenal suppression: a genome-wide association study

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Inhaled corticosteroids (ICS) are widely used by patients with asthma or chronic obstructive pulmonary disease (COPD). Although ICS are generally well tolerated and have fewer systemic adverse effects than oral corticosteroids, some patients develop systemic adverse effects. Adrenal suppression is a clinically important adverse effect, particularly in children with asthma, but its detection is challenging because presentation can range from asymptomatic biochemical changes to non-specific lethargy to florid adrenal crisis and death. The aim of the Pharmacogenetics of Adrenal Suppression with Inhaled Steroids (PASS) study was to undertake the first pharmacogenomic assessment of corticosteroid-induced adrenal suppression among children with asthma using ICS.

The authors found common variants in the platelet-derived growth factor D (PDGFD) gene associated with adrenal suppression. These findings were validated in separate groups of children with asthma (the PASS study) and adults with COPD (the Pharmacogenomics of Adrenal Suppression in COPD [PASIC] study) - the rs591118 variant showed genome-wide significance in both groups. The validation in the adult cohort is especially remarkable since these patients had a different chronic disease, had multiple comorbidities, and were on multiple medications, further reinforcing this novel finding. Heterogeneity between studies was higher for COPD (44%) than for asthma (0%), which could reflect greater heterogeneity in COPD.

These data support the idea of a genetic basis for inter-individual variation in susceptibility to corticosteroid-induced adrenal suppression. The findings offer the potential to develop translational pathways to prevent corticosteroid-induced adrenal suppression, thereby improving the benefit–risk ratio of this important therapy.

8.12. Targeted next-generation sequencing detects rare genetic events in pheochromocytoma and paraganglioma

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<https://www.ncbi.nlm.nih.gov/pubmed/?term=30877234>

Parangliomas and pheochromocytomas (PPGL) are rare neuroendocrine tumours that can arise either from the adrenal medulla (pheochromocytomas, PCC) or from extra-adrenal paraganglia (parangliomas, PGL). PPGLs are considered to be the most heritable of human tumours with at least 35% having inherited forms of the disease (13). Driver mutations can also be identified at somatic level, and overall, germline or somatic mutations in one of the 18 known genes involved in PPGL pathogenesis are present in ~60% of tumours (14). These PPGL genes include VHL, NF1, RET, SDHA, SDHB, SDHC, SDHD, SDHAF2, TMEM127, FH, MAX, EPAS1, HRAS, EGLN1, ATRX, MET, MDH2 and SLC25A11, demonstrating a high degree of genetic heterogeneity in the aetiology of these tumours. Published guidelines recommend that genetic testing should be considered in all patients with PPGL.

This study aimed to validate a unique customized ‘MASTR Plus SDHv2’ PPGL gene panel using next generation sequencing (NGS) of germline and tumour DNAs, including DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissues in order to rapidly identify driver mutations in patients and tumours for guiding follow-up, targeted therapies in case of metastatic PPGL and familial genetic counselling.

The NGS custom-designed targeted panel significantly improved the diagnostic accuracy for mutation detection in PPGL compared with former genetic approaches. In total, the NGS panel assessed in both retrospective and prospective cohorts, encompassing 768 patients’ germline and/or tumour DNA, showed higher accuracy for PPGL genetic testing than conventional methods with a significant increase of the mutation detection rate (78% vs. 65%). Furthermore, the NGS panel brought an additional value in diagnosing co-occurring variants and mosaicism, both genetic mechanisms not easily identified so far. These data are essential for guiding genetic counselling, which indicates or exempts (in the presence or the absence of a germline mutation) specific management and follow-up, as well as predictive genetic testing in the relatives or to reassure (in front of truly somatic mutations) patients about the risk of relapse.

References

13. Toledo RA, Burnichon N, Cascon A, Benn DE, Bayley JP, Welander J, Tops CM, Firth H, Dwight T, Ercolino T, Mannelli M, Opocher G, Clifton-Bligh R, Gimm O, Maher ER, Robledo M, Gimenez-Roqueplo AP, Dahia PLM. Consensus Statement on next generation-sequencing-based diagnostic testing of hereditary pheochromocytomas and paragangliomas. *Nat Rev Endocrinol* 2017; 13:233–47.
14. Favier J, Amar L, Gimenez-Roqueplo AP. Paranglioma and pheochromocytoma: from genetics to personalized medicine. *Nat Rev Endocrinol* 2015; 11:101–11.

New Hope

8.13. GnRH antagonist treatment of malignant adrenocortical tumors

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Incidence of adrenocortical carcinomas shows a bimodal distribution, being more common in children < 10 years and in adults aged 40–50 years. Their prognosis is poor, with only 10–25% 5-year survival. Ectopic expression of LHCGR and GNRHR has been reported in ACTH-independent adrenal hyperplasia and in aldosterone-producing adrenal carcinomas, suggesting the possibility of GnRH analogues as a potential new treatment strategy.

This study investigated the expression of several GPCRs in human adrenocortical carcinomas (h-ACC) and mouse adrenal tumors, and further analyzed the molecular mechanisms of GnRH antagonist action on adrenocortical tumor (ACT) cells *in vitro* and *in vivo*. Thirteen formalin fixed h-ACC samples were assessed with: A) immunohistochemistry; B) *in situ* hybridization and qPCR to identify the expression of GPCRs.

The authors found that ACC expressed the genes *GNRHR*, *LHCGR* but not *FSHR*. Cell cultures of *Cα1*, *Y-1* and *H295* cell lines were treated with Cetrorelix acetate (CTX; a GnRH antagonist) and in all cells the cell viability and proliferation were decreased. Furthermore, GNRHR knock-down experiments in *H295* cells confirmed that these CTX actions were mediated through GNRHR. They then used *inhα/Tag* mice to study the effects of CTX treatment on ACT *in vivo*. cDNA microarray analyses were run to identify the plausible biological processes and

pathways affected by CTX in ACTs. Expressed genes were clustered with the PANTHER classification system and the most interesting processes were growth, biological adhesion, immune system, development and response to stimulus. Among dysregulated pathways, the authors identified p53, apoptosis signaling, EGFR signaling, FGF signaling, G-protein signaling pathways, angiogenesis and Wnt signaling pathways. CTX treatment of *inh α /Tag* mice showed that in the ACTs the expression of tumour biomarkers such as Gata4, Lhcgr, Cyclin A1 (*Ccna1*) was down-regulated, whereas expression of *Sgcd* and *Mmp24* was up-regulated together with other genes related to cell growth suppression and tumour suppression. Taken together, the *in vivo* and *in vitro* data showed that GnRH antagonist treatment acts directly on ACC tumors to cause their regression.

8.14. A report on state-wide implementation of newborn screening for X-linked adrenoleukodystrophy

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X-linked adrenoleukodystrophy (X-ALD) is the most common peroxisomal disorder, with an estimated incidence in the USA of \sim 1:17,000 all births (male and female) and 1:21,000 male births. X-ALD is caused by mutations in the X chromosome gene *ABCD1*, which encodes the peroxisomal membrane protein, ATP-binding cassette sub-family D member one, also known as adrenoleukodystrophy protein, or ALDP. ALDP transports very long chain fatty acids (VLCFA) from the cytosol into the peroxisome where the VLCFA are metabolized by beta-oxidation. Pathogenic mutations in *ABCD1* cause absent or abnormally functioning ALDP, resulting in an accumulation of VLCFA in plasma and tissues, including the brain, spinal cord, and adrenal cortex.

The current paper describes a retrospective review of Minnesota Department of Health C26:0-LPC newborn screening results and follow-up outcomes from the first year of screening. This is one of the first reports describing outcomes of population screening of X-ALD on a newborn screening platform. It showed that over the first year of screening in Minnesota, 14 infants screened positive for X-ALD, and all were subsequently confirmed positive.

These results suggest that C26:0-LPC detection by LC-MS/MS is an effective and specific population-based screening assay for X-ALD, with a high positive predictive value with confirmation of all screen positive results. As five females were also detected, this newborn screening assay may also be a reliable test for detecting female heterozygotes and more sensitive than serum VLCFA levels. The benefits of adding X-ALD to newborn screening programs and the potential lives saved are predicted to be significant. However, there are unique challenges with adding this condition to newborn screening, including detection of variants of uncertain significance, inability to predict phenotypic severity in confirmed cases, and downstream diagnoses of numerous family members based on the X-linked inheritance pattern.

New Concerns

8.15. Epigenetic alterations associated with early prenatal dexamethasone treatment

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Prenatal treatment with dexamethasone (DEX) has been used to avoid virilization in girls with Congenital Adrenal Hyperplasia (CAH). However, it has potential short- and long-term risks and has been associated with

cognitive impairments. Here, the authors investigate whether epigenetic modification of DNA during early developmental stages may be a key mediating mechanism by which prenatal DEX treatment could result in poor outcomes in the offspring by comparing genome-wide DNA methylation, from peripheral CD4+ T-cells, between prenatal DEX-treated individuals without CAH and population controls.

In total, 9672 differentially methylated probes (DMPs) were associated with DEX treatment and 7393 DMPs were associated with an interaction between DEX and sex. Associated DMPs were enriched in intergenic regions located near epigenetic markers for active enhancers. Functional enrichment of DMPs was seen in immune functioning, inflammation, and also nonimmune-related biological pathways. DEX-associated DMPs enriched near single nucleotide polymorphisms (SNPs) associated with inflammatory bowel disease, and DEX*sex interaction DMPs were enriched near SNPs associated with asthma. DMPs were also identified in genes involved in the regulation and maintenance of methylation and steroidogenesis. Methylation in the *BDNF*, *FKBP5*, and *NR3C1* genes were associated with performance on several Wechsler Adult Intelligence Scale–Fourth Edition subscales.

This study highlights the importance of studying the potential risks of the prenatal DEX treatment. Furthermore, the study also indicates a possible effect on the treated individuals immune-functioning and susceptibility to future disease, which should be addressed in future studies evaluating the prenatal treatment.

New Paradigms

8.16. Autoantibodies reactive to adrenocorticotrophic hormone can alter cortisol secretion in both aggressive and nonaggressive humans

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It is accepted that aggressive behavior can be viewed as a strategy by humans and animals to cope with stress, implying that neurobiological mechanisms involved in stress responses should underlie both physiological and pathological aggression (15, 16). The hypothalamic–pituitary–adrenal (HPA) axis is a key system in the stress response, linking the brain to cortisol secretion via pituitary release of the ACTH. Both deficient and increased activation of the HPA axis have been associated with aggressive behavior. The molecular mechanisms underlying altered activation of the HPA axis that may predispose to aggressive behavior, including proactive violent aggression typical of murder, are currently unknown.

The current study tested the hypothesis that altered activation of the HPA axis in aggressive humans may involve ACTH-reactive immunoglobulins (Igs). Indeed, humans naturally and ubiquitously display IgG and other classes of Igs nonspecifically reactive with ACTH and other peptide hormones, supporting their constitutive contribution to peptidergic signaling (17, 18). Increased plasma levels of ACTH-reactive IgG have been found in male prisoners and adolescents with conduct disorder (18). However, it is unknown whether ACTH-reactive IgG may influence ACTH-induced cortisol secretion and whether such an influence can be different in aggressive subjects.

To address these questions, the authors analyzed plasma levels and affinity kinetics of ACTH-reactive IgG in prisoners who had committed violent acts of aggression, including murder, and compared the results with those from healthy nonaggressive controls [prisoners in whom violence was not a major feature and bodybuilders who were on active treatment with performance-enhancing substances (PES) and who previously had been characterized by increased physical aggressiveness but not hostility and anger]. They then studied the functional relevance of the observed differences in IgG affinity and epitope binding for ACTH with regard to IgG's ability to modulate ACTH-induced cortisol secretion *in vitro*. They also studied aggressive behavior in mice after peripheral injections of ACTH and IgG from aggressive and control subjects. Furthermore, to determine the presence of other autoantibodies potentially interfering with the stress axis in aggressive subjects, they

performed an immunohistochemical analysis of IgG binding to the rat brain and pituitary as well as guinea pig adrenal cortex sections.

They showed that ACTH-reactive IgGs can regulate ACTH-induced cortisol secretion in the adrenal gland, and they exhibit a clear-cut difference in ACTH epitope binding in violent aggressors vs. controls. Additionally, IgG from a subset of aggressive subjects selectively bind to hypothalamic vasopressin neurons. Thus, using several *in vitro* and *in vivo* approaches, the study reveals a molecular mechanism involved in the variability of stress response relevant to the neurobiology of aggression and possibly other stress-related conditions.

References

15. de Boer SF, Olivier B, Veening J, Koolhaas JM. The neurobiology of offensive aggression: Revealing a modular view. *Physiol Behav* 2015; 146:111–127.
16. Nelson RJ, Trainor BC. Neural mechanisms of aggression. *Nat Rev Neurosci* 2007; 8: 536–546.
17. Fetissov SO, *et al.* Autoantibodies against neuropeptides are associated with psychological traits in eating disorders. *Proc Natl Acad Sci USA* 2005; 102:14865–14870.
18. Fetissov SO, *et al.* Aggressive behavior linked to corticotropin-reactive autoantibodies. *Biol Psychiatry* 2006; 60:799–802.

Reviews

8.17. Autoimmune Addison's disease - An update on pathogenesis

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Ann Endocrinol (Paris). 2018; 79(3): 157–163.

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This review summarizes the current knowledge regarding the genetic susceptibility to autoimmune Addison's disease (AAD) and the genes outside the MHC complex associated with AAD. Fourteen genes (CTLA-4, PD-L1, PTPN22, NALP1, STAT4, CIITA, BACH2, FCRL3, GPR174, GATA3, NFATC1, CLEC16A, CYP27B1, and VDR) have been reported to be associated with AAD and the majority are involved in the function of the T cell.

The theories regarding the pathophysiology of AAD are also discussed, as well as the plausible involvement of the 21-hydroxylase antibodies in the process of adrenal destruction, as a major T cell autoantigen. Two epitopes targeted by CD8 + T cells have been described, the 21OH342-361 and 21OH431-438 regions, and one epitope (21OH342-350) has been described as a HLA-A2-restricted epitope. Clones of the latter produced IFN γ upon stimulation with the cognate peptide and were able to lyse target cells expressing 21OH through production of granzyme B. A central question to the pathogenesis of AAD is what drives the infiltration of mononuclear cells into the adrenal cortex. Patients with AAD have increased serum levels of interferon induced chemokines CXCL9 and CXCL10, which potentially could recruit self-reactive lymphocytes to the adrenal cortex.

The authors further discuss the role of viruses in promoting adrenal autoimmunity, as well as the importance of type I interferons and checkpoint inhibitors (antibodies) targeting CTLA-4 and PD-1. Patients treated for malignancies with these types of drugs have developed AAD and other endocrinopathies. Finally, the potential role of immunomodulatory (rituximab) and regenerative therapies in AAD are discussed.

8.18. Developmental programming of the HPA axis and related behaviours: epigenetic mechanisms

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Maternal exposure to glucocorticoid therapy, or stress and anxiety during pregnancy, have been linked to alterations in the exposed offspring in their functioning of the hypothalamic-pituitary-adrenal (HPA) axis and stress-related behaviours. Moreover, it appears that these effects are maintained across multiple generations.

There is considerable heterogeneity in the literature regarding the observed effects in the offspring. The programmed phenotype seems to be dependent on very specific factors, such as timing and dose of exposure. It is also evident that sex of the offspring and age at which assessment of outcome is undertaken are important. Mechanistic studies in animals and similar associations in humans are linking early exposures to adversity with changes in gene regulatory mechanisms, including modifications of DNA methylation and altered levels of miRNA.

These findings suggest that epigenetic mechanisms represent a fundamental link between early life adversity and developmental programming of diseases later in life. Epigenetic studies come, however, with a set of challenges that need to be addressed in order to help interpret results, and how phenotypes are transmitted between generations still needs to be explained. The new knowledge gained from studies that are addressing these issues will be very helpful in identifying individuals who are at risk of developing poor outcomes and for whom early intervention is most efficacious. These interventions can be identified and refined further by advancing our understanding of the underlying targets and factors that drive these outcomes. With time, poor outcomes associated with early life adversity can possibly be prevented, ameliorated or reversed.

Food for Thought

8.19. Placental H3K27me3 establishes female resilience to prenatal insults

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Sex differences in human disease have been extensively described for some of the most prevalent health conditions affecting societies today, from hypertension to diabetes, arthritis, and cancers. However, the mechanism for different disease vulnerability between sexes is not known.

Here the authors investigate whether female prenatal resilience is driven by sex differences in placental transcriptional control by the X-linked gene, O-linked N-acetylglucosamine transferase (*OGT*) and its regulation of the histone repressive mark, H3K27me3. The study included both human placental tissue and a mouse model of prenatal stress to elucidate the underlying mechanisms. The study provides evidence that one mechanism whereby *OGT* contributes to variation in vulnerability to prenatal stress between sexes is by establishing sex-specific trophoblast gene expression patterns and via regulation of the canonically repressive epigenetic modification, H3K27me3. Moreover, the authors show that high levels of H3K27me3 in the female placenta create resilience to the altered hypothalamic programming associated with prenatal stress exposure. The results highlight the importance of the placenta as a mediator between the maternal milieu and fetal development. Understanding these mechanisms may confer knowledge on the programming of the brain and on how male-biased neurodevelopmental diseases develop.

8.20. Associations of prenatal depressive symptoms with DNA methylation of HPA axis-related genes and diurnal cortisol profiles in primary school-aged children

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De Psychopathol. 2019; 31(2): 419–431.

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Environmental stimuli, especially in the pre- and postnatal periods, can have long-lasting effects on offspring development and health. Prenatal exposure to maternal depression, anxiety or stress may alter functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Here, epigenetic modifications of DNA in genes related to the HPA

axis are investigated as a mechanism underlying the association between prenatal depression and altered child HPA activity.

In a longitudinal study, the authors investigated DNA methylation changes in children related to prenatal depressive symptoms, as well as associations with the child's basal HPA activity. Children exposed to prenatal depressive symptoms in their mothers showed lower bedtime cortisol and a steeper diurnal slope. Regarding total cortisol release, prenatal exposure was related to lower cortisol release in boys, and higher cortisol release in girls. Furthermore, prenatal depressive symptoms were associated with altered methylation in the glucocorticoid receptor gene (*NR3C1*), the mineralocorticoid receptor gene (*NR3C2*), and the serotonin receptor gene (*SLC6A4*), with some sex-specific effects. In boys, prenatal depressive symptoms predicted bedtime cortisol mediated by *NR3C2* methylation.

This study provides evidence that alterations in DNA methylation, here found especially in the *NR3C2* (the mineralocorticoid receptor gene), is an underlying mediating mechanism between prenatal exposure to maternal depression and offspring outcome.

9. Oncology and Chronic Disease

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Long Term Outcomes of Childhood Cancer: A Changing Burden

9.1. The changing burden of long-term health outcomes in survivors of childhood acute lymphoblastic leukaemia: a retrospective analysis of the St Jude lifetime cohort study

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The St Jude Lifetime (SJLIFE) Cohort is a retrospective cohort of childhood cancer survivors (CCS), who are followed prospectively in order to evaluate long-term health outcomes. This study evaluated chronic health conditions in 980 childhood lymphoblastic leukaemia survivors of the SJLIFE cohort, compared to 272 matched community controls. Survivors were diagnosed with paediatric acute lymphoblastic leukaemia between Aug 28th 1963 and July 19th 2003 at St Jude Children's Research Hospital. Enrolled patients were aged ≥ 18 years old at recruitment and had at least 10 years of follow-up after diagnosis. The large sample size and the accurate data collection are the main strengths of the study that allow a reliable analysis of the changes in the prevalence of late outcomes and the identification of changing patterns in late outcomes, concurrently with evolving treatment modalities.

Different disorders of attention and cognitive and executive performance have become increasingly more common in patients than in controls in more recent years. Conversely, a decrease in health conditions that are immediately life-threatening, as stroke and seizures, was observed, concurrently with an increased incidence of milder conditions as chronic peripheral neuropathy. The incidence of pituitary defects showed a decreasing trend with time, but growth hormone deficiency and hypogonadism were remained more common in survivors than in controls, even in the most recent years. Impaired glucose metabolism became more frequent with time, and a trend towards obesity was registered in both survivors and controls. Compared to previous years, survivors treated in recent years showed a higher prevalence of musculoskeletal disorders, probably related to a reduced bone mineral density. This finding correlated with an impairment of mobility and aerobic function. Attention and executive function improved as treatment protocols restricted chemo-radiotherapy. Among survivors who did not receive radiotherapy, attention did not differ from population normative data or controls, except for a less focused attention.

Treatment protocols of childhood acute lymphoblastic leukaemia have significantly changed during the last three decades, with a progressive improvement of overall survival. The cumulative burden of severe health conditions involving multiple organ systems has decreased, after the elimination of cranial-spinal and cranial radiotherapy from treatment protocols. The pattern of toxic effects has remarkably changed over time, with a reduction of early life-threatening conditions. Late morbidities, as chronic musculoskeletal and endocrine disorders, predominate in patients treated more recently. Maintaining health status and quality of life still requires careful medical surveillance, counselling, and lifestyle changes.

9.2. The late effects of radiation therapy on skeletal muscle morphology and progenitor cell content are influenced by diet-induced obesity and exercise training in male mice

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In recent years, improved knowledge of the metabolic risks in childhood cancer survivors (CCS) has increasingly focused research on modifiable factors and preventive strategies (1–2). It is well known that therapeutic irradiation can cause detrimental changes in body composition, but it is still unknown whether the specific effects of radiation on skeletal muscle are influenced by physiological factors, such as obesity and exercise training.

This study evaluated, on a mouse model, the late effects of diet-induced obesity and exercise training on irradiated skeletal muscle morphology and cellular dynamics. Forty mice were divided into control and high-fat-diet groups with or without exercise training; all mice were then exposed to total body irradiation. After continuing for another 4 weeks in their intervention group, tissue collection was performed. Diet-induced obesity resulted in increased muscle fibrosis, while obesity and exercise training both increased muscle adiposity. Exercise training enhanced myofibre cross-sectional area and the number of satellite cells (myogenic stem cells that contribute to muscle growth and/or repair, and are depleted in irradiated skeletal muscle) committed to the myogenic lineage, independently of diet. High-fat-diet group demonstrated an increase in the expression of the inflammatory marker p-NFκB, regardless of exercise training.

This study suggests that radiation exposure during muscle development induces long-term impairment of skeletal muscle health, which may contribute to reduced quality of life in irradiated CCS. The findings need to be confirmed, as muscle cell dynamics were analyzed at a single post-radiation time point, which does not allow to document pre-radiation differences and the long-term evolution of muscle damage.

References

1. Srivastava R, Batra A, Dhawan D, Bakhshi S. Association of energy intake and expenditure with obesity: A cross-sectional study of 150 pediatric patients following treatment for leukemia. *Pediatr Hematol Oncol.* 2017; 34: 29–35.
2. Slater ME, Ross JA, Kelly AS, Dengel DR, Hodges JS, Sinaiko AR, Moran A, Lee J, Perkins JL, Chow LS, Baker KS, Steinberger J. Physical activity and cardiovascular risk factors in childhood cancer survivors. *Pediatr Blood Cancer* 2015; 62: 305–310.

9.3. Insulin and glucose homeostasis in childhood cancer survivors treated with abdominal radiation: A pilot study

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Observational studies in large cohorts of cancer survivors have reported that cancer survivors exposed to abdominal radiation have an increased risk of both insulin-dependent and non-insulin-dependent diabetes mellitus, with a prolonged latency between radiation exposure and diabetes onset. The irradiation of the pancreatic tail, where insulin-producing beta-cells are concentrated, has been implicated as the main risk factor.

This cross-sectional study analysed a relatively small group (40 subjects) of childhood cancer survivors treated with abdominal radiotherapy (aRT) at age ≤ 21 years. In this study, 70% ($n = 28$) of the enrolled patients were survivors of neuroblastoma treated with aRT; 26/28 patients had stage 4 disease, receiving aggressive multimodal treatment. The enrolled patients underwent oral glucose tolerance test (OGTT) and assessment of diabetes-related autoantibodies [autoantibodies against insulin (IAA), glutamic acid decarboxylase (GAD-65), and islet antigen-2 (IA-2)]. Impaired glucose tolerance was found more prevalent in patients previously treated

with aRT, independently of obesity and in the absence of pancreatic autoimmunity. Two patients showed isolated GAD65 positivity, associated with normal glucose tolerance. Three of the four individuals with impaired fasting glucose showed also insulin resistance, as measured by HOMA-IR. Four additional subjects with normal glucose tolerance were insulin resistant. No participant had absolute insulinopenia.

The correlation between aRT and diabetes has been already described, with discordant data about the dose–response relationship. A few previous studies had analyzed specific markers of pancreatic autoimmunity leading to beta-cell damage and type 1 diabetes in childhood cancer survivors. Overall, these auto-antibodies were not found in the analyzed subjects. (1–3), confirming that pancreatic autoimmunity is not implicated in the pathogenesis of diabetes mellitus in cancer survivors. Despite the established association between radiation dose to the pancreatic tail and diabetes risk, this study shows that autoimmunity and absolute insulinopenia, as might be expected after direct radiation-induced damage to the insulin producing beta-cells, does not seem to play a role in the pathophysiology of glucose metabolism derangement. Abdominal therapeutic irradiation has been associated with specific body composition changes (fat redistribution with central and visceral fat accumulation) and the various components of the metabolic syndrome, which were not systematically assessed in this study. Further research into alternative pathways leading to diabetes after aRT is needed.

References

1. Mohn A, Di Marzio D, De Berardinis A, Di Marzio A, Capanna R, Fioritoni G, Chiarelli F. Long-term follow-up of children treated for acute lymphoblastic leukemia and the recovery of beta-cell function. *Haematologica* 2006; 9: 1424–1425.
2. Davies JH, Evans BA, Jenney ME, Gregory JW. In vitro effects of combination chemotherapy on osteoblasts: Implications for osteopenia in childhood malignancy. *Bone* 2002; 31: 319–326.
3. D’Annunzio G, Bonetti F, Locatelli F, Pistorio A, Lorini R. Insulin resistance in children and adolescents after bone marrow transplant for haematological malignancies. *Haematologica* 2006; 91(12 Suppl).

9.4. Diabetes risk in childhood cancer survivors: A population-based study

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This retrospective population-based study focused on diabetes prevalence in childhood cancer survivors (CCSs) using administrative health care databases of the Canadian National Health System. The authors compared 10,438 CCSs (mean age 10.7 ± 6.8 years; mean follow-up time 11.2 ± 6.9 years) with 52,190 age- and sex-matched controls. CCSs had a 55% higher rate of developing diabetes than matched controls. Diabetes was more common in CCSs with cancer diagnosis between 6 and 10 years of age, and in patients treated for leukaemia or lymphoma. CCSs developed diabetes at an earlier age than matched controls. This difference was more evident in males and was confirmed after exclusion of confounding factors, such as income, rurality, age at cancer diagnosis and year of cancer diagnosis.

This large study highlights the importance of regular diabetes screening during long-term follow up of CCSs (1–2). The study has some limitations: concurrent factors potentially affecting the risk of diabetes, as specific cancer treatments, obesity, lifestyle, relapses and/or associated comorbidities were not analyzed. Further studies are needed in order to clinically characterize CCSs who are at higher risk of diabetes and need regular and lifelong metabolic screening.

References

1. Meacham LR, Sklar CA, Li S, Liu Q, Gimpel N, Yasui Y, Whitton JA, Stovall M, Robison LL, Oeffinger KC. Diabetes mellitus in long-term survivors of childhood cancer. Increased risk associated with radiation therapy: a report for the childhood cancer survivor study. *Arch Intern Med*. 2009 Aug 10; 169: 1381–1388.
2. Holmqvist AS, Olsen JH, Andersen KK, de Fine Licht S, Hjørth L, Garwicz S, Moëll C, Anderson H, Wesenberg F, Tryggvadottir L, Malila N, Boice JD Jr, Hasle H, Winther JF; ALiCCS study group. Adult life after childhood cancer in Scandinavia: diabetes mellitus following treatment for cancer in childhood. *Eur J Cancer*. 2014 Apr; 50: 1169–1175.

9.5. Total body irradiation for hematopoietic stem cell transplantation during early childhood is associated with the risk for diabetes mellitus

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Hematopoietic stem cell transplantation (HSCT) is a curative treatment for life-threatening malignancies. However, late HSCT adverse effects cause substantial morbidity among long-term survivors. Metabolic complications, such as diabetes mellitus and hyperlipidemia, are the major late effects of pediatric HSCT, but the pathogenesis of these complications is unclear. Different mechanisms leading to insulin resistance have been implicated, including TBI related muscular damage leading to secondary muscular insulin resistance and a chronic systemic inflammatory state.

This retrospective study evaluated general clinical conditions and metabolic complications of a small group of HSCT survivors. Among 22 participants, 4 developed diabetes mellitus and 9 hyperlipidemia. All patients with diabetes mellitus also developed hyperlipidemia. No patient with diabetes mellitus was obese, but all showed substantial insulin resistance. Ten participants had received total body irradiation (TBI), including the four participants with diabetes mellitus and 5/9 participants with hyperlipidemia, revealing that TBI may be an independent risk factor for diabetes. The age at TBI of patients with diabetes was significantly lower than those without diabetes; all patients with diabetes had received TBI before 6 years of age.

These results suggest that weight control is not enough to prevent metabolic complications and body weight is not predictive of metabolic complications. Therefore, close monitoring of metabolic biomarkers, such as fasting blood glucose, HbA1c, immunoreactive insulin, seems to be essential. This is the first study to report TBI during early childhood as a significant risk factor for diabetes mellitus. The authors recommend a conditioning regimen without TBI for patients younger than 6 years of age, whenever possible, and close monitoring of metabolic status in patients who underwent TBI before the age of 6.

TBI has been widely employed as a conditioning regimen of HSCT, especially for high risk malignancies. TBI regimens have been described as significantly superior to Busulfan regimens without TBI, with disease-free survival being 57% for TBI and 20% for Busulfan (2). These results suggest that innovative therapies are required to avoid TBI, including novel technologies like T-cell therapy (3). Such treatments could represent a future option to avoid TBI, and we expect that these novel therapies will be greatly beneficial for young children with cancer, not just for what concerns their anti-tumor effects, but also in reducing severe late effects of TBI.

References

1. Kavanagh K, Dendinger MD, Davis AT, Register TC, DeBo R, Dugan G, Cline JM. Type 2 Diabetes is a Delayed Late Effect of Whole-Body Irradiation in Nonhuman Primates. *Radiat Res*. 2015; 183: 398–406.
2. Bunin N, Aplenc R, Kamani N, Shaw K, Cnaan A, Simms S. Randomized trial of busulfan vs total body irradiation containing conditioning regimens for children with acute lymphoblastic leukemia: a Pediatric Blood and Marrow Transplant Consortium study. *Bone Marrow Transplant*. 2003; 32: 543–548.
3. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. *EMBO Mol Med*. 2017; 9: 1183–1197.

9.6. Late cardiovascular morbidity and mortality following pediatric allogeneic hematopoietic cell transplantation

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Early cardiovascular disease is relatively common among childhood cancer survivors (CCS). A high risk of accelerated atherosclerosis and a 8.2-fold higher cardiac mortality rate have been reported (1). Arterial stiffness, an independent risk factor for cardiovascular morbidity and mortality in adults, seems to increase in young adult

CCS (2). In hematopoietic cell transplantation (HCT) survivors, the risk of cardiovascular death is 2–4 fold higher than the general population, and cardiovascular adverse events occur earlier than average (3).

This multicenter, retrospective study recorded cardiovascular outcomes in 661 long-term survivors of paediatric allogeneic HCT for hematologic malignancies. A low cumulative incidence of these outcomes was found: coronary artery disease 0.2%, cerebrovascular accident 0.6%, cardiomyopathy 3%, cardiac-related death 0.5%. Patients who received anthracycline chemotherapy and cranial or chest radiation showed higher risks. Overweight/obesity was present in 52% of patients at last follow up; diabetes requiring medications in 7% and dyslipidemia in 18% of survivors.

The low incidence of adverse events is comforting, but should be interpreted in the light of the patients' young age (median age 18.5 years at last follow-up) and the relatively short follow up duration (median 8 years, range 2–19). Lipid measurements were missing in a large proportion of patients, suggesting the lack of a regular monitoring and uniformity in clinical practice between centers. The study population included patients aged 21 years or less at the time of transplant who survived, relapse-free at least 2 years following the first allogeneic HCT for hematologic malignancy between 1 January 1995 and 31 December 2008. Standards of care radically changed during the 13-year study window, with a progressive increase in the attention on specific metabolic risks of HCT patients.

The main strength of the study is certainly its size that makes it the largest study currently available on cardio-metabolic outcomes following HCT in childhood. Its limitations include the retrospective design, the lack of uniformity in standards of care during the study window, the potential underestimation of the true incidence of diabetes and/or hypertension (having considered only patients who required drugs), and the use of BMI as the only index of overweight/obesity, which does not provide information on body composition alterations in these patients.

References

1. Mertens AC1, Yasui Y, Neglia JP, Potter JD, Nesbit ME Jr, Ruccione K, Smithson WA, Robison LL. Late mortality experience in five-year survivors of childhood and adolescent cancer: the Childhood Cancer Survivor Study. *J Clin Oncol.* 2001; 19: 3163–3172.
2. Krystal JI, Reppucci M, Mayr T, Fish JD, Sethna C. Arterial stiffness in childhood cancer survivors. *Pediatr Blood Cancer.* 2015; 62: 1832–1837.
3. Armenian SH, Chow EJ. Cardiovascular disease in survivors of hematopoietic cell transplantation. *Cancer.* 2014; 120: 469–479.

9.7. No evidence of overweight in long-term survivors of childhood cancer after glucocorticoid treatment

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Glucocorticoids still represent one of the pillars in the treatment of paediatric hematologic malignancies, but they cause excessive weight gain through several mechanisms. However, it is unclear whether these glucocorticoids have a long-lasting effect on body weight, because studies have focused mainly on the acute effects during or shortly after treatment (1–2).

This large Swiss study examined the relationship between cumulative glucocorticoid dose and overweight in childhood cancer survivors (CCS) long after the completion of treatment (median time from diagnosis 17 years). CCS treated with glucocorticoids only had the same prevalence of overweight and obesity compared to that of siblings and the general population (24%), but higher prevalences were evident in patients who had undergone cranial radiotherapy (37%) or cranial radiotherapy plus glucocorticoid treatment (49%). No correlation was found between cumulative corticosteroid dose and overweight. The strengths of the study are the large sample size (1936 CCS, comparison groups: 546 siblings, 9591 subjects from a random sample of the general Swiss

population) and the separate analysis of patients with the three types of cancer most often treated with glucocorticoids (acute lymphoblastic leukaemia, non-Hodgkin and Hodgkin lymphoma).

These results reassuringly show that glucocorticoids used for the treatment of childhood cancer are not associated with long-term risk of overweight. However, the study has some limitations. They relied on self-reported height and weight, and BMI was the only parameter used to evaluate overweight. It is well known that BMI fails to measure the complex changes of body composition induced by cancer treatment, with lean mass impairment, fat tissue redistribution and increased abdominal adiposity. Another limitation is that glucocorticoid cumulative dose was estimated only indirectly by the type of protocol used.

References

1. Chow EJ, Pihoker C, Hunt K, Wilkinson K, Friedman DL. Obesity and hypertension among children after treatment for acute lymphoblastic leukemia. *Cancer*. 2007; 110: 2313–2320.
2. Arpe ML, Rørvig S, Kok K, Mølgaard C, Frandsen TL. The association between glucocorticoid therapy and BMI z-score changes in children with acute lymphoblastic leukemia. *Support Care Cancer*. 2015; 23: 3573–3580.

Fertility Issues in Chronic Diseases: New Insights

9.8. Long-term effects of childhood cancer treatment on hormonal and ultrasound markers of ovarian reserve

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Childhood cancer treatment may compromise ovarian function in female childhood cancer survivors (CCSs), leading to delayed or arrested puberty, infertility, subfertility and adverse pregnancy outcomes. This Dutch nationwide retrospective cohort study collected measurements performed between 2008 and 2014. In total, 1749 female 5-year CCSs, diagnosed before age 18 years between 1963 and 2002 and 1201 controls were invited to participate. Ovarian reserve was assessed by anti-Müllerian hormone (AMH), follicle stimulating hormone (FSH), inhibin B levels, and antral follicle counts (AFC). In total, 564 CCSs and 390 controls participated in the clinical part of the study.

The proportion of CCSs with abnormal ovarian reserve markers was remarkably low, even after treatment with alkylating agents (6.5–13.0%). Above age 35 years, a higher proportion of CCSs showed abnormalities of ovarian reserve markers compared to controls (AMH: 26% vs. 4%; AFC: 20% vs. 3%; inhibin B: 42% vs. 16%); while AMH and FSH levels showed differences also below age 35. Combined treatment with alkylating agents and gonadotoxic radiotherapy resulted in the lowest AMH, AFC, and inhibin B levels, and the highest FSH levels in all age groups. Clear dose–effect relationships were seen for procarbazine and abdominal/pelvic radiotherapy. Treatment with busulfan, melphalan, chlorambucil or lomustine also increased the risk of reduced ovarian reserve. Abdominal/pelvic RT affected all ovarian reserve markers at almost any dose, a finding consistent with previous studies and a clear dose–effect relationship was established for AFC and inhibin B.

This study surprisingly shows that the majority of CCSs do not show signs of a reduced ovarian reserve. Moreover, the results are important in order to design future childhood oncology protocols in which the curative effect of the treatment is balanced with the risk of gonadotoxicity. Specific subgroups of CCSs (in particular those treated with alkylating agents and radiotherapy) appear to have a higher risk of early gonadal failure. These CCSs should be counselled adequately and new patients receiving such treatments should be referred early to a reproductive specialist for parenthood and fertility preservation counselling.

9.9. Co-transplantation of mesenchymal stem cells improves spermatogonial stem cell transplantation efficiency in mice

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Spermatogonial stem cell transplantation (SSCT) could become a fertility restoration tool for childhood cancer survivors. However, the colonization efficiency of transplanted spermatogonial stem cells (SSCs) in animal models is about 12%, and the effectiveness of this procedure needs to be improved before clinical implementation become feasible. Co-transplantation of mesenchymal stem cells (MSCs) increased colonization efficiency of SSCs, by restoring after gonadotoxic treatment the SSC specific tissue environment that provides a niche for the germ cells.

This is an experimental study on a mouse model of spermatogenesis damage. The model is similar to the damage induced by chemotherapies in cancer survivors because both spermatogonial stem cells and their surrounding mesenchymal niche were affected. The model was developed and used to transplant SSCs, MSCs, a combination of SSCs and MSCs, or a combination of SSCs and TGFβ1-treated MSCs. A significant increase in testis size and testis-to-body ratio and resumption of spermatogenesis was found in all transplanted groups compared to controls. The best results were observed in mouse undergoing spermatogonial stem cells transplantation (SSCT) and TGFβ1-treated mesenchymal stem cells + SSCT (MSi-SSCT). The proportion of tubules containing spermatogenesis (TFI) was higher in the MSi-SSCT group, in which germline-specific gene expression was also confirmed. TGFβ1-treatment was also related to a higher localization of MSCs in the testis and less evident localization in different organs.

These findings suggest that TGFβ1-treated mesenchymal stem cell may exert an anti-inflammatory effect resulting in a better engraftment of the transplanted germinal cells. More pre-clinical studies are needed to clarify the involved mechanisms and the reproductive safety has to be proven, but MSi-SSCT could represent an effective tool to improve colonization efficiency of transplanted spermatogonial stem cells.

9.10. Cryopreservation of ovarian tissue may be considered in young girls with galactosemia

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Galactosemia is an autosomal recessive genetic disorder of the galactose metabolism, caused by impaired activity of galactose-1-phosphate uridylyltransferase (GALT). Complete deficiency or severely reduced activity of GALT affects approximately 1:30,000 to 1:50,000 persons. Girls and women with classic galactosemia have reduced ovarian reserve with elevated serum levels of follicle-stimulating hormone (FSH) and reduced concentrations of anti-Müllerian hormone (AMH) compared to age-matched controls (1–2). It has been estimated that more than 80% of girls with classic galactosemia experience premature ovarian insufficiency in early adulthood. The pathogenesis of the observed accelerated follicle loss is unknown.

In this retrospective study, follicle density was estimated in ovarian cortical tissues from six pre-pubertal girls with galactosemia below the age of 12 years, and from 31 girls below the age of 18 years who had one ovary removed for fertility preservation prior to gonadotoxic treatment. Expression of AMH and other glycoproteins important for follicle development was analyzed with immunohistochemistry. Girls with galactosemia below the age of 5 years presented with morphological normal follicles and follicle densities within the 95% confidence interval of controls. No follicles were detected in the ovary from an 11.7-year-old girl with galactosemia. The expression of specific glycoproteins was not significantly different in girls with galactosemia and in controls.

These findings suggest that young girls with galactosemia maintain vital follicles during the first years of life, and cryopreservation of ovarian tissue in early childhood may represent a therapeutic option for future fertility in this patient group.

References

1. Sanders RD, Spencer JB, Epstein MP, Pollak SV, Vardhana PA, Lustbader JW, Fridovich-Keil JL. Biomarkers of ovarian function in girls and women with classic galactosemia. *Fertil Steril*. 2009; 92: 344–351.
2. Spencer JB, Badik JR, Ryan EL, Gleason TJ, Broadaway KA, Epstein MP, Fridovich-Keil JL. Modifiers of ovarian function in girls and women with classic galactosemia. *J Clin Endocrinol Metab*. 2013; 98: E1257–1265.

9.11. Ovarian reserve in young juvenile idiopathic arthritis patients

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In recent years, attention has been increasing on the ovarian reserve in juvenile rheumatic diseases (1–4). Premature ovarian failure is described in 3.5–7.5% of juvenile idiopathic arthritis (JIA) patients, mainly in older subjects (mean age 35 years) and in patients treated with chlorambucil for amyloidosis (5–6). This cross-sectional study analyzed ovarian function during the early follicular phase in 28 post-pubertal JIA patients (mean age 22.6 years) and 28 healthy age-matched controls. A higher median menarche age, a lower median AMH levels with higher LH and estradiol levels were observed in JIA patients, without any correlation with current age, disease duration, number of active/limited joints, inflammation markers, cumulative glucocorticoid and methotrexate doses. These results suggest a precocious impairment of ovarian reserve, not associated with hypothalamic-pituitary-gonadal axis dysfunction. This impairment appears to be related to disease activity, rather than to its treatment, and an early and progressive ovarian damage due to autoimmune mechanisms is hypothesized.

This chronic inflammatory disease occurs mainly in females during their early reproductive age, making the issue of ovarian reserve and future fertility particularly relevant and probably still underestimated. A strength of this study is the rigorous selection criteria of JIA patients and controls (<40 years, without gynecologic diseases and hypothalamic-pituitary-ovary axis dysfunction), however the small sample size is a limitation. The confirmation of impaired ovarian reserve and its impact in future fertility in JIA patients is an important aspect to be evaluated in larger, prospective studies.

References

1. De Araujo DB, Yamakami LY, Aikawa NE, Bonfá E, Viana VS, Pasoto SG, Pereira RM, Serafini PC, Borba EF, Silva CA. Ovarian reserve in adult patients with childhood-onset lupus: a possible deleterious effect of methotrexate? *Scand J Rheumatol*. 2014; 43: 503–511.
2. Aikawa NEI, Sallum AM, Leal MM, Bonfá E, Pereira RM, Silva CA. Menstrual and hormonal alterations in juvenile dermatomyositis. *Clin Exp Rheumatol*. 2010; 28: 571–575.
3. Aikawa NE, Sallum AM, Pereira RM, Suzuki L, Viana VS, Bonfá E, *et al*. Subclinical impairment of ovarian reserve in juvenile systemic lupus erythematosus after cyclophosphamide therapy. *Clin Exp Rheumatol*. 2012; 30: 445–449.
4. Henes M, Froeschlin J, Taran FA, Brucker S, Rall KK, Xenitidis T, Igney-Oertel A, Lawrenz B, Henes JC. Ovarian reserve alterations in premenopausal women with chronic inflammatory rheumatic diseases: impact of rheumatoid arthritis, Behçet's disease and spondyloarthritis on anti-Müllerian hormone levels. *Rheumatology (Oxford)* 2015; 54: 1709–1712.
5. David J, Vouyiouka O, Ansell BM, Hall A, Woo P. Amyloidosis in juvenile chronic arthritis: a morbidity and mortality study. *Clin Exp Rheumatol*. 1993; 11: 85–90.
6. Packham JC, Hall MA. Premature ovarian failure in women with juvenile idiopathic arthritis (JIA). *Clin Exp Rheumatol*. 2003; 21: 347–350.

9.12. Diagnosis of GH deficiency as a late effect of radiotherapy in survivors of childhood cancers

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GH deficiency (GHD) is common among children treated for cancer, especially among childhood cancer survivors (CCS) with tumors/surgery in the hypothalamic-pituitary (HP) region, CCS exposed to HP radiation or CCS exposed to cranial (CIR), craniospinal (CSI), or total body irradiation (TBI). However, most of existing data on GHD diagnostic work-up derive from people who were not CCSs, despite suspected differences related to the specific nature of the radiation-induced HP axis damage. Therefore, an Endocrine Society taskforce was charged to develop guidelines on the management of growth disorders in CCSs. This is a preliminary report produced by the taskforce, aiming to evaluate the existing data on GHD screening in CCS by the use of IGF-1 or IGFBP-3 measurements, compared with GH provocative tests and diagnosing GHD by using different GH dynamic tests.

Fifteen studies were analyzed [8 studies examined IGF-1 and 7 studies analyzed IGFBP-3]; 477 patients were included. Overall, both IGF-1 and IGFBP-3 showed poor diagnostic accuracy. The studies were remarkably heterogeneous; when calculations were possible, the sensitivity and specificity of IGF-1 varied from 47% to 66% and 77% to 100%, respectively. IGFBP-3 had a lower sensitivity (20%). IGF-1 and IGFBP-3 were strongly correlated and the simultaneous use of both tests did not improve the diagnostic accuracy. Provocative tests remained the most accurate tools to identify GHD in CCS, despite remarkable variability in the testing protocols and the absence of standardized testing strategies. The insulin tolerance test (ITT) seems to be the most accepted reference test, when used alone or in combination with arginine. The ability of GHRH stimulation, with or without arginine, to diagnose GHD was equivocal across different studies; in one study GHRH with arginine stimulation had 66% sensitivity and 88% specificity compared to ITT. Insufficient data were available to assess the accuracy of serial GH testing (nocturnal or over 24 hours).

Evaluating the GH axis in CCSs allows an opportunity to treat these patients with available and effective replacement therapy. The controversy regarding the benefit-to-risk ratios of such therapies highlights the importance of appropriate patient selection and accuracy of GHD testing. According to this systematic review, dynamic tests are still the cornerstone of GHD diagnosis. Additional research is needed to establish the best provocative test for CCS. In the meantime, reliance on the ITT (as the gold standard) seems to be appropriate, even if this test is not feasible at many institutions. The use of GHRH with arginine stimulation test should be limited in CCS. The pituitary gland is located outside the blood-brain barrier and intravenous GHRH infusion may act as a maximal direct stimulus on the somatotrophic cells. In CCS patients, this test can elicit a high and falsely normal GH response, because it does not investigate the true spontaneous function of the HP axis damaged by primary tumour, surgery and/or therapeutic irradiation. CCSs probably need a lower testing threshold because of their established cranial pathologies and/or HP radiation. In the presence of low height velocity associated or not to concurrent HP defects, a blunted GH response to any of the provocative tests should be considered sufficient to establish GHD diagnosis, due to the high pre-test diagnostic suspicion.

On the other hand, a direct bone damage with skeletal growth impairment independently of GHD, due to treatment with agents such as TBI, imatinib, and cis-retinoic acid, may contribute to poor linear growth, short adult stature and/or altered body proportions.

9.13. The predictive value of insulin-like growth factor 1 in irradiation-dependent growth hormone deficiency in childhood cancer survivors

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This single-centre, retrospective study analyzes the screening role of low IGF-1 levels in the diagnosis of growth hormone deficiency (GHD) in a cohort of 158 childhood cancer survivors (CCS) and in a selected sub-cohort of 117 CCS who received radiation for tumours not directly involving the hypothalamic-pituitary (HP) area (RT-NHP group).

The aim of the study was to assess the value of IGF-1 levels in predicting GH status, both at childhood GHD diagnosis and at final height retesting. IGF-1 levels < -2 SDS showed a low sensitivity for GHD (31.9%); test sensitivity was higher (45.6%) in patients with severe GHD, defined as GH peak < 3 mcg/l on stimulation tests. A significant negative correlation was found between pituitary radiation dose and GH peak on stimulation testing. IGF-1 levels showed no correlation with the number of hormonal defects in patients with multiple pituitary deficiencies. Conversely, when patients with childhood GHD in the RT-NHP group were retested after final height achievement, an IGF-1 level < -2 SDS predicted adulthood GHD in 100% of cases.

Previous reports on GHD in irradiated patients (1–6) seem to confirm that IGF-1 does not represent a suitable tool for screening purposes in irradiated patients. An overall poor sensitivity of IGF-1 levels < -2 SDS is reported in radiation-induced GHD. Sensitivity is higher in patients with severe GHD, while normal plasma IGF-1 concentrations despite a diagnosis of GHD are frequently found after low radiation doses to the HP area. According to the recommendation jointly provided by the European Endocrine Society and the Pediatric Endocrine Society there is a potential diagnostic reliability of low IGF-1 levels only in CCS with severe GHD (7).

The strengths of this interesting, well-designed study are its large, homogeneous cohort and its value in clinical practice. The limitations are instead related to its retrospective nature and to the lack of a control group of non-oncological GHD patients, which could provide more information about the role of IGF-1 in irradiation-dependent GHD.

References

1. Ham JN, Ginsberg JP, Hendell CD, Moshang T Jr. Growth hormone releasing hormone plus arginine stimulation testing in young adults treated in childhood with cranio-spinal radiation therapy. *Clin Endocrinol (Oxf)*. 2005; 62: 628–632.
2. Tillmann V, Shalet SM, Price DA, Wales JK, Pennells L, Soden J, Gill MS, Whatmore AJ, Clayton PE. Serum insulin-like growth factor-I, IGF binding protein-3 and IGFBP-3 protease activity after cranial irradiation. *Horm Res*. 1998; 50: 71–77.
3. Achermann JC, Hindmarsh PC, Brook CG. The relationship between the growth hormone and insulin-like growth factor axis in long-term survivors of childhood brain tumours. *Clin Endocrinol (Oxf)*. 1998; 49: 639–645.
4. Blum WF, Ranke MB, Kietzmann K, Gauggel E, Zeisel HJ, Bierich JR. A specific radioimmunoassay for the growth hormone (GH)-dependent somatomedin-binding protein: its use for diagnosis of GH deficiency. *J Clin Endocrinol Metab*. 1990; 70: 1292–1298.
5. Rosenfeld RG, Wilson DM, Lee PD, Hintz RL. Insulin-like growth factors I and II in evaluation of growth retardation. *J Pediatr*. 1986; 109: 428–433.
6. Sfeir JG, Kittah NEN, Tamhane SU, Jasim S, Chemaitilly W, Cohen LE, Murad MH. Diagnosis of GH Deficiency as a Late Effect of Radiotherapy in Survivors of Childhood Cancers. *J Clin Endocrinol Metab*. 2018; 103: 2785–2793.
7. Sklar CA, Antal Z, Chemaitilly W, Cohen LE, Follin C, Meacham LR, Murad MH. Hypothalamic-Pituitary and Growth Disorders in Survivors of Childhood Cancer: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2018; 103: 2761–2784.

9.14. GH therapy in childhood cancer survivors: a systematic review and meta-analysis

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This systematic review and meta-analysis was conducted by a US Endocrine Society Task Force in order to inform new guidelines on the management of childhood cancer survivors (CCS). The aim of this review was to evaluate the effects of GH therapy on final height, risk of diabetes mellitus, lipid abnormalities, metabolic syndrome, quality of life, secondary tumors, and disease recurrence among CCS with tumors/surgery in the hypothalamic-pituitary (HP) region and CCS subjected to cranial (CIR), or craniospinal (CSI), or total body

(TBI) irradiation. Twenty-nine observational studies were included. Sixteen studies compared CCSs treated or untreated with GH. Thirteen studies compared CCSs treated with GH against matched healthy controls, or controls with idiopathic GHD or idiopathic short stature.

In CCS, GH therapy was associated with no increase in tumour relapse or secondary neoplasia occurrence, and with a height gain of +0.61 SDS [95% CI, 0.08 to 1.13]. The risks of diabetes, abnormal lipid profile, metabolic syndrome, and poor quality of life were not increased. One study showed that the risk of a second neoplasia in CCS treated with GH was lower after an extended follow-up (1). This risk became non-significant after adjusting for sex, age at primary diagnosis, CIR dose/time, and treatment type in a later report on the same cohort (2). The dose of GH and treatment modalities did not differ between patients with and without recurrence, and there was no association between risk of recurrence with the cumulative duration of GH therapy or the time elapsed since treatment start.

There have been no definitive studies on how long to wait after the completion of cancer therapy to start GH therapy. US Pediatric Endocrine Society guidelines suggest to wait for 12 months (3). The strengths of this review relate to the comprehensive literature search. However, the available evidence is all observational, with related limitations. Most studies assessing GH therapy had relatively short follow-up duration, which limits the assessment of long-term risks of recurrence and secondary neoplasia. Additional studies with a longer follow up are needed to better define these risks.

References

1. Sklar CA, Mertens AC, Mitby P, Occhiogrosso G, Qin J, Heller G, Yasui Y, Robison LL. Risk of disease recurrence and second neoplasms in survivors of childhood cancer treated with growth hormone: a report from the Childhood Cancer Survivor Study. *J Clin Endocrinol Metab.* 2002; 87: 3136–3141.
2. Patterson BC, Chen Y, Sklar CA, Neglia J, Yasui Y, Mertens A, Armstrong GT, Meadows A, Stovall M, Robison LL, Meacham LR. Growth hormone exposure as a risk factor for the development of subsequent neoplasms of the central nervous system: a report from the childhood cancer survivor study. *J Clin Endocrinol Metab.* 2014; 99: 2030–2037.
3. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, Rossi WC, Feudtner C, Murad MH; Drug and Therapeutics Committee and Ethics Committee of the Pediatric Endocrine Society. Guidelines for growth hormone and insulin-like growth factor-I treatment in children and adolescents: growth hormone deficiency, idiopathic short stature, and primary insulin-like growth factor-I deficiency. *Horm Res Paediatr* 2016; 86: 361–397.

Biologic Agents and Growth in Chronic Inflammatory Diseases

9.15. Growth during Tocilizumab therapy for Polyarticular-course juvenile idiopathic arthritis: 2-year data from a phase III clinical trial

Bharucha KN, Brunner HI, Calvo Penadés I, Nikishina I, Rubio-Pérez N, Oliveira S, Kobusinska K, Schmeling H, Sztajn bok F, Weller-Heinemann F, Zholobova E, Zulian F, Allen R, Chaitow J, Frane J, Wells C, Ruperto N, De Benedetti F Paediatric Rheumatology International Trials Organisation and the Pediatric Rheumatology Collaborative Study Group. *J Rheumatol.* 2018; 45(8): 1173
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In recent years, biologic agents have clearly been shown to be effective in maintaining remission and improving linear growth in children with inflammatory bowel disease and other chronic inflammatory diseases (1–3). This prospective cohort study analyzed growth in 187 patients (143 females, mean age 11 ± 4 years; including 123 with Tanner stage ≤ 3 at baseline) with polyarticular-course juvenile idiopathic arthritis treated with the biologic drug tocilizumab, an anti-Interleukin-6 (IL-6) receptor antibody. Tocilizumab was associated with improved growth profiles from baseline to the end of the second year of therapy: height SDS increased in 72% of patients, with mean height gain +0.40 SDS.

The study did not include essential auxological elements, such as target height and bone age evaluation. Patients with chronic inflammatory diseases often show pubertal delay and blunted pubertal growth spurt; for these reasons Tanner stage assessment was planned at “selected visits”, but the pubertal progression was neither

analysed nor commented on. A further limitation is the lack of laboratory measurements, such as IGF-1 and bone turnover markers. Finally, a significant proportion of patients (46%) were concurrently treated with steroids, but the impact of steroid therapy dose and duration on growth was not considered.

IL-6 appears to be the major proinflammatory cytokine involved in growth retardation in chronic inflammatory diseases. Previously, a 50% to 70% reduction in growth rate was observed in transgenic mice expressing high levels of circulating IL-6, associated with low IGF-1 levels and partially reversed by the administration of an anti-IL-6 receptor antibody (4). The pathophysiological link between IL-6 and IGF-1 is clearly interesting, but a more complete evaluation of the factors interfering with linear growth and pubertal spurt is needed.

The therapeutic switch from chronic glucocorticoid therapy to the use of biologic agents has profoundly changed the clinical scenario of chronic inflammatory diseases, even if a few data suggest a vanishing effect with time. A role of biological agents in improving growth of patients with chronic inflammatory diseases has been suggested by previous studies (1–3), and this effect has been directly correlated with early response to therapy and sustained remission. Nevertheless, new longitudinal studies should include an accurate assessment of height velocity, puberty, essential laboratory data, and confounding effects of steroid treatment.

References

1. Marino A, Stagi S, Simonini G, Carli N, Caparello MC, Giani T, Pagnini I, De Masi S, Cimaz R. Growth and body mass index in a cohort of patients with juvenile idiopathic arthritis: effects of second line treatments. *Clin Exp Rheumatol*. 2018; 36: 929–933.
2. Church PC, Guan J, Walters TD, Frost K, Assa A, Muise AM, Griffiths AM. Infliximab maintains durable response and facilitates catch-up growth in luminal pediatric Crohn's disease. *Inflamm Bowel Dis*. 2014; 20: 1177–1186.
3. Walters TD, Faubion WA, Griffiths AM, Baldassano RN, Escher J, Rummel FM, Hyams JS, Lazar A, Eichner S, Huang B, Li Y, Thakkar RB. Growth Improvement with Adalimumab Treatment in Children with Moderately to Severely Active Crohn's Disease. *Inflamm Bowel Dis*. 2017; 23: 967–975.
4. De Benedetti F, Alonzi T, Moretta A, Lazzaro D, Costa P, Poli V, Martini A, Ciliberto G, Fattori E. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. *J Clin Invest* 1997; 99: 643–650.

10. Type 1 Diabetes Mellitus

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10.1. Transition to adult diabetes care in Germany - high risk for acute complications and declining metabolic control during the transition phase

Kapellen TM, Mütter S, Schwandt A, Grulich-Henn J, Schenk B, Schwab KO, Marg W, Holl RW, DPV initiative and the Competence Network Diabetes Mellitus funded by the German Federal Ministry of Education and Research Hospital for Children and Adolescents, University of Leipzig, Germany
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Transition of patients with chronic diseases from pediatric to adult care has become recognized as an important part of health care provision and its multifaceted challenges are increasingly being studied. This transition period is associated with deterioration of metabolic control and general health in young adults with type 1 diabetes (T1D) (1). The aim of this multicenter study, based on routine care records in the German/Austrian DPV database, was to compare metabolic control, the number of acute complications and the prevalence of microvascular complications in adolescents and young adults with diabetes before and after transfer to adult care.

The study included 1283 young adults with available records of the last pediatric treatment year and the first year after transition to adult care. HbA1c levels increased from 8.95% (74 mmol/mol) before to 9.20% (77 mmol/mol) in the first year after transition. In addition, rates of diabetic ketoacidosis with hospitalization (0.100 to 0.191 per annum, $P < 0.0001$) and severe hypoglycemia (0.23 to 0.46 per annum, $P = 0.013$) doubled during transition. Probably most importantly, microvascular complications increased dramatically between the last visit in pediatric diabetes care and the first visit in adult care. The rise of microvascular complications was unrelated to the duration of transition (short or long) nor to type of transition modality.

It is concluded that transition from pediatric to adult T1D care carries a high risk for impaired metabolic control and microvascular comorbidities. Structured transition programs with case management are likely to improve the transition process and outcomes. The establishment of transition clinics and close cooperation between specialists in pediatric and adult medicine is urgently needed. It is interesting to note that such improvements and changes in diabetes care are demanded by patients and would ensure better uptake of health care services after transfer (1).

Reference

1. Busse FP, Hiermann P, Galler A, Stumvoll M, Wiessner T, Kiess W, Kapellen TM. Evaluation of patients' opinion and metabolic control after transfer of young adults with type 1 diabetes from a pediatric diabetes clinic to adult care. *Horm Res*. 2007;67:132–8.

10.2. Closed-loop insulin delivery in suboptimally controlled type 1 diabetes: a multicentre, 12-week randomised trial

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The appearance of new technologies and treatment modalities are changing the fate of patients with diabetes. While until recently the global epidemic of type 1 diabetes (T1D) has been largely neglected (1), much data are being published on the impact of new technologies on metabolic control, quality of life and microvascular

complications. As it is still difficult for patients with T1D to achieve good metabolic control and reduce disease burden in daily life, indeed new insight into the usefulness of new devices is urgently needed.

This large multinational randomized controlled trial compared the effectiveness of day-and-night hybrid closed-loop insulin delivery systems versus sensor-augmented pump therapy over 12 weeks of free-living in patients with poorly controlled T1D. Patients aged 6 years or older with HbA1c 7.5–10.0% on insulin pump therapy were recruited from four hospital outpatient clinics in the UK and two centers in the USA. The primary outcome was the proportion of time that glucose concentration was within the target range of 3.9–10.0 mmol/l at 12 weeks post randomization. 114 patients were screened, and 86 eligible patients were randomly assigned to receive hybrid closed-loop therapy ($n=46$) or sensor-augmented pump therapy ($n=40$; control group).

Duration of target range glucose concentrations was significantly higher in the closed-loop group (65%, s.d. 8) compared with the control group (54%, s.d. 9; mean difference 10.8%, 95% CI 8.2–13.5; $P<0.0001$). Importantly, in the closed-loop group HbA1c reduced from 8.3% (s.d. 0.6) at screening to 8.0% (s.d. 0.6) after the 4-week run-in, to 7.4% (s.d. 0.6) after the 12-week intervention period. In the control group, HbA1c was 8.2% (s.d. 0.5) at screening, 7.8% (s.d. 0.6) after run-in, and 7.7% (s.d. 0.5) after intervention; reductions in HbA1c were significantly greater in the closed-loop group compared with the control group (mean difference in change 0.36%, 95% CI 0.19–0.53; $P<0.0001$). However, it is interesting to note, that metabolic control also improved in the control group! Compared to controls, the closed-loop intervention also reduced time spent in hypoglycaemia (glucose <3.9 mmol/l: mean difference in change -0.83% , -1.40 to -0.16 ; $P=0.0013$) and in hyperglycaemic (glucose >10.0 mmol/l: -10.3% , -13.2 to -7.5 ; $P<0.0001$). No difference between groups was seen in: variability in sensor-measured glucose (mean difference in coefficient of variation -0.4% ; $P=0.50$); total daily insulin dose (mean difference 0.031 U/kg per day; $P=0.09$) or body weight (mean difference 0.68 kg; $P=0.19$). No severe hypoglycaemia event occurred in either group. One diabetic ketoacidosis event occurred in the closed-loop group, due to infusion set failure, which shows the potential risk of any new technology namely technical error. Two participants in each group had significant hyperglycaemia, and there were 13 other adverse events in the closed-loop group and three in the control group.

The authors conclude that hybrid closed-loop insulin delivery improves glucose control while reducing the risk of hypoglycaemia across a wide age range in patients with suboptimally controlled T1D. However, it might be hypothesized that intensifying diabetes education and increasing attention and care for the patient alone can improve metabolic outcomes and reduce acute complications. It is technologies applied wisely and with care that will improve life for patients with T1D.

Reference

1. Kiess W, Gorski T, Penke M, Klamt S, Kapellen TM. Diabetes mellitus in children and adolescents - a global epidemic which has become neglected. *J Pediatr Endocrinol Metab.* 2015;28:247–50.

10.3. Psychosocial benefits of insulin pump therapy in children with diabetes type 1 and their families: The pumpkin multicenter randomized controlled trial

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It has been debated whether or not new technologies, in particular the combination of insulin pumps and continuous glucose testing, will improve patient satisfaction and quality of life rather than only metabolic control and risk of microvascular complications. Indeed, the use of continuous subcutaneous insulin infusion (CSII) has been increasing among pediatric patients with type 1 diabetes (T1D), particularly in high income countries. Metabolic benefits cannot alone explain this increasing popularity. Therefore, it has been hypothesized that the main benefits of CSII from the patient's perspective may be related to subjective psychosocial outcomes (patient-reported outcomes [PRO]).

In this multicenter open randomized controlled trial, patients with T1D aged 6–16 years and treated with multiple daily injections (MDI) were randomized either to CSII starting immediately or 6 months later after continuing on multiple daily injections. The primary outcomes were patient-reported diabetes-specific

health-related quality of life (DHRQOL) and diabetes burden of the main caregiver (parents or grandparents). Secondary outcomes were family stress, fear of hypoglycemia, and overall satisfaction with treatment. HbA1c levels were assessed additionally.

211 patients were randomized over 4 years, and eventually data from 186 caregivers and 170 patients were included in the intention-to-treat analyses. Children aged 8–11 years in the immediate CSII group reported improved DHRQOL at follow-up compared to MDI (median difference [MD] 9.5, 95% CI: 3.6–16.7, $P=0.004$). However, there were no group differences in the adolescent age-group 12–16 years (MD 2.7; 95% CI –3.2–9.5; $P=0.35$). Main caregivers of the CSII group reported a small decline in overall diabetes burden at follow-up compared to the MDI group (MD 0; 95% CI –1–0; $P=0.029$). Secondary PROs were in favor of CSII.

The authors favour the use of insulin pumps and concluded that CSII can offer substantial psychosocial benefits. However, the data should be seen with great caution since patient satisfaction is related to age, and psychosocial factors are very likely to influence quality of life and also the impact of new technologies on quality of life in a direct way.

10.4. Excess mortality and cardiovascular disease in young adults with type 1 diabetes in relation to age at onset: a nationwide, register-based cohort study

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Despite great improvements in diabetes care, people with type 1 diabetes (T1D) remain at increased risk of mortality and morbidity, in particular from cardiovascular disease (CVD). How exactly diabetes leads to CVD and which factors contribute to the increased risk profile is still largely unknown. Molecular mechanisms are being studied extensively (1). However, simple factors such as age are not considered in current guidelines. This study of the Swedish National Diabetes Register examined how age at diagnosis of T1D influences excess mortality and CVD risk compared to matched controls from the general population.

All patients from Sweden with at least one registration between Jan 1, 1998, and Dec 31, 2012 were included. The authors used Cox regression, with adjustment for diabetes duration, to estimate the excess risks of disease stratified by age at T1D onset (0–10, 11–15, 16–20, 21–25, and 26–30 years). A huge cohort of 27,195 patients with T1D were compared to an even larger number (135,178) of matched controls. 959 patients with T1D and 1501 controls died during follow-up (median duration 10 years).

Patients who developed T1D at a young age (0–10 years) had much higher risks for all-cause mortality (hazard ratio: 4.11; 95% CI 3.24–5.22); CVD mortality (7.38; 3.65–14.94); non-CVD mortality (3.96; 3.06–5.11); CVD events (11.44; 7.95–16.44); coronary heart disease (30.50; 19.98–46.57); acute myocardial infarction (30.95; 17.59–54.45); stroke (6.45; 4.04–10.31); and heart failure (12.90; 7.39–22.51). Corresponding risks for patients who developed T1D in the oldest age group (26–30 years) were 2.83 (95% CI 2.38–3.37) for all-cause mortality, 3.64 (2.34–5.66) for CVD mortality, 2.78 (2.29–3.38) for non-CVD mortality, 3.85 (3.05–4.87) for CVD disease events, 6.08 (4.71–7.84) for coronary heart disease, 5.77 (4.08–8.16) for acute myocardial infarction, 3.22 (2.35–4.42) for stroke, and 5.07 (3.55–7.22) for heart failure. Hence, the excess risks differed by up to 5-times across the diagnosis age groups. The highest overall incidence rate, noted for all-cause mortality, was 1.9 (95% CI 1.71–2.11) per 100 000 person-years for patients with T1D. Sadly, onset of T1D before 10 years of age resulted in a loss of 17.7 life-years (95% CI 14.5–20.4) for women and 14.2 life-years (12.1–18.2) for men.

In summary, young age at onset of T1D is indeed one of the most important determinants of survival, as well as all CVD outcomes. In addition, risk of CVD disease is also gender related, with higher excess risks in women. Even greater care for cardioprotection, such as optimal treatment of hyperlipidemia and hypertension, are warranted in patients with early-onset T1D. Children with T1D are indeed a vulnerable group and need our greatest attention.

Reference

1. Nurten E, Vogel M, Michael Kapellen T, Richter S, Garten A, Penke M, Schuster S, Körner A, Kiess W, Kratzsch J. Omentin-1 and NAMPT serum concentrations are higher and CK-18 levels are lower in children and adolescents with type 1 diabetes when compared to healthy age, sex and BMI matched controls. *J Pediatr Endocrinol Metab.* 2018;31:959–969.

10.5. Repaglinide versus insulin for newly diagnosed diabetes in patients with cystic fibrosis: a multicentre, open-label, randomised trial

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Cystic fibrosis (CF)-related diabetes (CFRD) impacts significantly on mortality and quality of life. Impaired glucose metabolism and CFRD are associated with poor weight and height gain and impaired lung function in children and adolescents (1). In that study, height and weight were lower in CF patients with diabetes than those without, not only at diagnosis of CFRD, but also years before that. After CFRD diagnosis, height and weight declined even further in CFRD patients. In contrast, no reduction of BMI was observed. All analyzed lung function parameters showed a marked decline in CFRD patients starting 1 year prior to CFRD diagnosis (1).

Hence, life expectancy of patients with cystic fibrosis largely depends upon complications and comorbidities and importantly upon the treatment of accompanying conditions such as CFRD. Currently, the recommended treatment for CFRD is insulin, but some patients receive oral antidiabetic drugs to ease the treatment burden. Clinical trials to assess the efficacy and safety of oral antidiabetic drugs in patients with CFRD are needed.

This multicentre, open-label, industry-sponsored randomised controlled trial in 49 centres in Austria, France, Germany, and Italy included patients with CF aged 10+ years with newly diagnosed CFRD. Patients were randomised to receive either insulin injections or oral repaglinide. The primary outcome was mean change in HbA1c from baseline to 24 months of treatment. Instead of intention-to-treat analyses, patients who stopped treatment early because of lack of efficacy were excluded. Out of 34 patients in the repaglinide group and 41 in the insulin group, 30 and 37, respectively, were included in the analyses.

At 24 months, HbA1c was no different between groups (mean change from baseline 0.2% [s.d. 0.7%], 1.7 mmol/mol [8.1] with repaglinide vs -0.2% [1.3%], -2.7 mmol/mol, [14.5] with insulin; mean difference -0.4% , (95% CI -1.1 to 0.2 [-4.4 mmol/mol, -11.5 to 2.7], $P=0.15$). As expected for patients with CF, the most frequent adverse events were pulmonary events (43/107 [40%] with repaglinide and 60/133 [45%] with insulin), and the most frequent serious adverse events were pulmonary events leading to hospital admission (5/10 [50%] and 7/13 [54%], respectively).

In summary, oral repaglinide was as efficacious and safe as insulin in patients with CFRD. Whether repaglinide is equally effective in increasing BMI and life expectancy in such patients will need further study. Since patients with CF have to adhere to multiple treatments, avoiding the need for injections seems worthwhile.

Reference

1. Terliesner N, Vogel M, Steighardt A, Gausche R, Henn C, Hentschel J, Kapellen T, Klamt S, Gebhardt J, Kiess W, Prenzel F. Cystic-fibrosis related-diabetes is preceded by and associated with growth failure and deteriorating lung function. *J Pediatr Endocrinol Metab.* 2017 Aug 28;30(8):815–821.

10.6. Clinical trial of fluid infusion rates for pediatric diabetic ketoacidosis

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Neurological complications of diabetic ketoacidosis (DKA) are still causes of significant mortality and morbidity in type 1 diabetes (T1D). Children are still being reported with acute cerebral infarction (CI) and extra pontine myelinolysis (EPM) at onset of T1D (1). Frequently, their clinical management had not been optimal and putative risk factors for such complications had been neglected. Not only cerebral edema (CE), but also other severe neurological complications such as CI should be suspected when neurological deterioration occurs during DKA, and these may still cause brain injuries ranging from mild to severe (1).

This large randomized, controlled trial, involving 13 US centers, tested the rate of fluid administration and sodium chloride content of intravenous fluids in relation to neurologic outcomes in children with DKA. Children were randomly assigned to one of four treatment groups in a 2-by-2 factorial design (0.9% or 0.45% sodium chloride content and rapid or slow rate of administration). The primary outcome was a decline in mental status as proven by two consecutive Glasgow Coma Scale (GCS) scores of < 14. Secondary outcomes included clinically apparent brain injury during treatment for diabetic ketoacidosis, short-term memory during treatment for diabetic ketoacidosis, and memory and IQ two to six months after recovery from diabetic ketoacidosis. 1389 episodes of DKA were reported in 1255 children. GCS score declined to < 14 in 3.5% (48 episodes), and clinically apparent brain injury occurred in 0.9% (12 episodes).

No significant difference between treatment groups was observed in: episodes of GCS score < 14, the magnitude of decline in GCS score, duration of time spent with GCS < 14; tests of short-term memory; or incidence of clinically apparent brain injury during treatment for DKA. Memory and IQ scores measured after recovery from DKA did not differ between groups. Serious adverse events other than altered mental status were rare and occurred with similar frequency in all groups.

In summary, neither the rate of administration nor the sodium chloride content of intravenous fluids influenced neurologic outcomes in children with DKA in tertiary care centers when treatment was otherwise conducted according to recommended guidelines.

In retrospect, one might agree that sodium chloride or fluid infusion rate alone were unlikely to be the single and most relevant risk factor for the development of neurological complications in DKA. Other risk factors might be of more relevance, such as initial conscious level, bicarbonate use, rate of blood glucose lowering, and lack of intensive care unit monitoring. It is still recommended that not only an exceeded rehydration therapy but also a rapidly reduced serum osmolality due to an unbalanced rapid blood sugar decrease and serum sodium increase should be avoided in order to prevent neurological complications. A structured and well-defined rehydration strategy in the first 6–12 hours of therapy following an in-house standard operation procedure protocol remains crucial for recovery and can reduce neurological complications of patients with DKA.

Reference

1. Petzold S, Kapellen T, Siekmeyer M, Hirsch W, Bartelt H, Siekmeyer W, Kiess W. Acute cerebral infarction and extra pontine myelinolysis in children with new onset type 1 diabetes mellitus. *Pediatr Diabetes*. 2011;12:513–7.

10.7. Worse metabolic control and dynamics of weight status in adolescent girls point to eating disorders in the first years after manifestation of type 1 diabetes mellitus: findings from the diabetes patienten verlaufsdokumentation registry

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Psychosocial issues and psychiatric disease are prevalent in young patients with type 1 diabetes (T1D). Most importantly, eating disorders, anxiety-related behavioral problems as well as depression have all been shown to occur (1). In a nationwide population-based survey, this group had previously reported on relationships between eating disorder and depressive symptoms and HbA1c levels (1). Screening positive for an eating disorder was associated with more severe depressive symptoms among women. However, neither eating disorder symptoms

nor severity of depressive symptoms were associated with HbA1c among women, while HbA1c increased with the severity of depressive symptoms among men (β 0.14, $P=0.006$). Because of the high prevalence of eating disorder and depressive symptoms, their interrelationship, and their associations with metabolic control, regular mental health screening is recommended for young adults with T1D (1).

This study from the DPV group assessed indications of eating disorders in 31,556 T1D girls aged >6 months and <23 years, including 155 (0.49%) girls with anorexia nervosa, 85 (0.27%) with bulimia nervosa, 45 (0.14%) with binge eating disorder, and 229 (0.73%) with eating disorders not otherwise specified. Patient characteristics, weight changes, numbers of patients with severe hypoglycemia and diabetic ketoacidosis (DKA), changes in HbA1c, use of pumps, and prevalence of celiac disease and autoimmune thyroiditis were compared between girls with and without eating disorders using multiple logistic regression analyses.

Eating disorders were significantly associated with late pubertal age, non-usage of pumps, no migration background, higher HbA1c, and higher frequencies of DKA and severe hypoglycemia, but not to celiac disease. Importantly, the differences in HbA1c levels, prevalence of DKA and severe hypoglycemia were already detectable in the first years after onset of T1DM. A decrease in BMI-SDS increased the risk for comorbid anorexia nervosa (7.1-fold [95% CI 3.6–14.3] compared with stable BMI-SDS, and 6.9-fold [95%CI 3.4–14.1] compared with increase of BMI-SDS.

In conclusion, girls with T1D should be monitored for the development of eating disorders starting immediately after T1D onset: poor metabolic control and higher rates of DKA and severe hypoglycemia in the first years after T1D onset are alarming signs. Weight loss after initiation of insulin treatment is specific for anorexia nervosa. Systematic screening for eating disorders and also depression especially at a late pubertal age in girls with T1D is recommended.

Reference

1. Bächle C, Lange K, Stahl-Pehe A, Castillo K, Scheuing N, Holl RW, Giani G, Rosenbauer. Symptoms of Eating Disorders and Depression in Emerging (1) J. Adults with Early-Onset, Long-Duration Type 1 Diabetes and Their Association with Metabolic Control. *PLoS One*. 2015;10:e0131027. doi: 10.1371/journal.pone.0131027.

10.8. One potato, two potato,... assessing carbohydrate counting accuracy in adolescents with type 1 diabetes

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Diabetes education includes nutritional education and the provision of practical guidelines as to the interrelation between insulin requirements and carbohydrate intake. Carbohydrate (CHO) counting has therefore been a recommended daily practice to help patients to manage blood glucose levels in type 1 diabetes (T1D). Evidence suggests that CHO estimates should be within 10–15 g of the actual meal for optimal postprandial blood glucose control.

This study assessed the accuracy of CHO counting in adolescents with T1D. Adolescents (aged 12–18 years) with T1D who self-identified as regular CHO counters were recruited from the SickKids Diabetes Clinic, Toronto. Adolescents completed the PedsCarbQuiz (PCQ) and estimated CHO content of test trays (three meals and three snack trays) that were randomly assigned. Analyses were conducted to identify factors associated with accuracy of counting and CHO counting knowledge (PCQ score).

140 adolescents (78 females, mean age 14.7, s.d. = 1.8) participated. The average PCQ score was $81 \pm 10\%$. 42% of adolescents accurately estimated meal trays (i.e. within 10 g of the actual CHO content), 44% estimated CHO inaccurately (within 10–20 g), while 14% were significantly inaccurate counters (> 20 g variation). PCQ scores were higher in teens who CHO counted accurately than in those with significant inaccuracy, and a longer duration of diabetes correlated with a lower PCQ score.

Fewer than half of teens in this study were accurate CHO counters. It is disturbing to see that longer diabetes duration actually related to less accurate CHO counting. This points to a potential weaning off the educational efforts. It is assumed that in other socioeconomic and cultural environments adherence to CHO counting might even be lower. These results indicate the high need for regular clinical accuracy checks and reeducation.

10.9. Continuous glucose monitoring and glycemic control among youth with type 1 diabetes: International comparison from the T1D Exchange and DPV Initiative

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New technologies such as continuous glucose monitoring, insulin pumps and closed-loop systems are likely to change diabetes care and hopefully will reduce the burden of disease management, decrease diabetes associated comorbidities, and increase life expectancy.

To assess the change in rates of pediatric real-time or intermittent scanning continuous glucose monitoring (CGM) use over the past 5 years, and how it impacts glycemic control, data from two different national registries were compared: the US-based type 1 diabetes Exchange Registry (T1DX) and the German/Austrian DPV (Prospective Diabetes Follow-Up Registry). Registry participants aged < 18 years with T1D duration \geq 1 year comprised 29,007 individuals in 2011 and 29,150 in 2016. Demographic data, CGM use and HbA1c were obtained from routine medical records.

As expected, CGM use increased from 2011 to 2016 in both registries across all age groups, regardless of gender, ethnic minority status or insulin delivery method. The increase in CGM use was most pronounced in the youngest patients, and usage rates remained lowest for adolescent patients in 2016. For both registries in 2016, mean HbA1c was lower among CGM users than non-users regardless of insulin delivery method, and CGM users were more likely to achieve glycemic target of HbA1c < 7.5% (56% vs 43% for DPV and 30% vs 15% for T1DX, $P < 0.001$). T1DX participants had a higher mean HbA1c compared with DPV despite whether they were CGM users or non-users; however, the difference was less pronounced in CGM users.

The authors conclude that pediatric CGM use increased in both USA and Germany and this was associated with lower mean HbA1c regardless of insulin delivery modality. However, it is interesting to note that despite the effects of GCM there was also a registry effect as patients in the US-based registry did less well than patients in the German registry. Hence, cultural, socioeconomic and physician-related factors are also important for obtaining good metabolic control in T1D.

10.10. Sex as a determinant of type 1 diabetes at diagnosis

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Despite good metabolic control some patients with type 1 diabetes (T1D) may develop comorbidities early while others despite high HbA1c levels remain free of complications for long durations. Risk factors for T1D complications are both of intrinsic (e.g. genetic) and extrinsic (e.g. environmental) origin. Amongst the intrinsic risk factors for progressive disease, ethnicity, gender and age at T1D onset are debated.

This study tested the hypothesis that girls have a more aggressive disease process than boys at the very beginning of T1D. Sociodemographic and clinical characteristics, autoantibody expression, and the genetic risk as assessed by the presence of HLA DR-DQ haplotypes were analyzed in relation to sex of the patients in a large cohort of 4993 children and adolescents diagnosed with T1D between January 2003 and December 2016 in Finland. Interestingly, the cohort comprised a prominent male preponderance (56.6%) and boys were significantly older than girls at T1D onset (mean 8.3 vs 7.7 years, $P < 0.001$).

Age-adjusted analyses showed a worse metabolic decompensation in girls than boys at T1D diagnosis. Boys tested more often positive for anti-insulin autoantibodies, anti-islet antigen-2 autoantibodies, and anti-zinc transporter 8 autoantibodies, whereas girls had a higher frequency of anti-glutamic acid decarboxylase autoantibodies (GADA) and higher GADA and islet cell antibody titers. No difference in the genetic risk profile between girls and boys was detected.

These data show that metabolic derangement was more severe in girls already at diagnosis of T1D. Importantly, this difference was independent of age. The immunologic aggressiveness of the disease is more variable as the predominance of different autoantibodies varies between the sexes with a higher frequency of GADA in girls, while the 3 other autoantibodies were more common in boys. This study very clearly points to genetic factors that are responsible for the progression of T1D and strengthen the view that sex is an important factor that contributes to the aggressiveness of autoimmune disease.

10.11. Changes in diabetes medication regimens and glycemic control in adolescents and young adults with youth-onset type 2 diabetes: The SEARCH for diabetes in youth study

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There is a small but significant debate as to whether or not type 1 (T1D) and type 2 diabetes (T2D) are the same disease with albeit different course. It might therefore be prudent to include into a chapter on T1D a publication on T2D. This study aimed to describe recent medication patterns and changes in medication patterns and glycemic control in adolescents and young adults with incident T2D. Data from the SEARCH for Diabetes in Youth Study, were included in cross-sectional analyses of treatments for adolescents and young adults with incident T2D in two time periods: 2002–2005 and 2008/2012. In addition, a longitudinal analysis of medications and glycemic control for a subset of patients with baseline and follow-up visits was carried out.

In the cross-sectional analysis, of 646 patients classified as having incident T2D, a majority in each year period received metformin (64.9% vs 70.4%) and/or insulin (38.1% vs 38.4%), while few used sulfonylureas (5.6% vs 3.6%) with non-significant changes over time. There was a significant reduction in thiazolidinedione use over time (5.0% vs 2.0%, $P < 0.05$). In the longitudinal analysis, 322 participants were followed for 7 years, on average. Baseline metformin users had a lower HbA1C (6.4% [46.7 mmol/mol]) compared to insulin users (8.4% [68.2 mmol/mol], $P < 0.001$) or insulin plus any oral diabetes medication (ODM) (7.7% [60.4 mmol/mol], $P < 0.001$). Among baseline metformin users ($n = 138$), 29.7% reported using metformin at follow-up, with the remainder adding (19.6%) or switching to insulin (8.0%), ODM (15.9%), or lifestyle only (26.8%). Of those receiving insulin (\pm ODM) ($n = 129$), 76% reported insulin use at follow-up. Overall, only 35% were at A1C goal ($< 7.0\%$, 53 mmol/mol) at follow-up.

Youth-onset T2D is still largely being treated with metformin and/or insulin. The majority did not achieve the American Diabetes Association (ADA)-recommended A1C goal 7 years after diagnosis. Most importantly, adolescents classified as having T2D but requiring insulin might have been misdiagnosed and later on might actually turn out to have T1D and obesity. It is important to note that classification of diabetes into T1D or T2D is difficult, might even be misleading and in some cases might very well prohibit early and appropriate use of insulin as first line treatment. It is disturbing that many of the adolescents upon follow-up did not meet treatment targets as recommended by ADA. This fact might be attributable in part to the large number from poor family backgrounds with low education and low incomes.

10.12. Efficacy of growth hormone treatment in children with type 1 diabetes mellitus and growth hormone deficiency – An analysis of KIGS data

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This study aimed to analyze growth hormone (GH) doses and first-year growth response in prepubertal patients with the combination of type 1 diabetes (T1D) and growth hormone deficiency (GHD).

A total of 69 patients with T1D and GHD treated with GH have been enrolled in KIGS (Pfizer International Growth Database). Of these, 24 prepubertal patients had developed T1DM before GHD and were included in

this analysis. For many years the presence of T1D in patients who were to be treated with GH seemed to have been regarded as a contraindication for GH use. In addition, GH therapy has falsely been associated with the development of T1D in children with GHD. This study analyzed in a large cohort from a registry of one pharmaceutical company the impact of diabetes on GH treatment. In the registry, of 30,570 patients with GHD without T1DM, 15,024 were prepubertal and served as controls.

Patients with T1DM and GHD had similar characteristics to the GHD-alone group. Neither age (10.2 ± 3.13 vs 8.42 ± 3.46 years, $P=0.14$), height SDS corrected for midparental height SDS at start of treatment (-1.62 ± 1.38 vs -1.61 ± 1.51 , $P=0.80$), nor GH dose (0.24 ± 0.08 mg/kg/wk vs 0.20 ± 0.04 mg/kg/wk, $P=0.09$) were different between those with and without T1D. First-year catch-up growth was comparable between the two patient groups (first treatment year height velocity 7.54 ± 3.11 cm/year compared with 8.35 ± 2.54 cm/year in control patients, $P=0.38$). Height SDS of children with both T1DM and GHD improved from -2.62 ± 1.04 to -1.88 ± 1.11 over the first year of GH treatment.

In conclusion, the short-term response to GH therapy appears similar in patients with T1D who then developed GHD and in those with GHD alone. Thus, T1D does not compromise the GH response in children with GHD. The study also clearly shows that GH treatment was safe in both subgroups of patients. T1D is not a contraindication for GH treatment in those children who need it.

10.13. Longitudinal assessment of hippocampus structure in children with type 1 diabetes

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According to these authors, earlier studies had shown that children with type 1 diabetes mellitus (T1D) experience mild cognitive alterations compared to healthy age-matched controls. This might develop during the course of the disease and relate to both hypo- and hyperglycemia. Another explanation points to psychosocial factors or common underlying yet unknown alterations of the central nervous system. The neural basis of these cognitive differences is unclear, but may be due in part to the effects of dysglycemia on developing neurons.

This study examined longitudinal changes in hippocampus volume using structural magnetic resonance imaging in 142 young children with T1D and 65 age-matched controls (4–10 years old) at two time points, 18 months apart.

Longitudinal hippocampus growth did not differ between children with T1D and healthy controls, however, among T1D children slower hippocampus growth was associated with both greater exposure to hyperglycemia (higher HbA1c) and greater glycemic variability. These observations indicate that the current practice of tolerating some hyperglycemia to minimize the risk of hypoglycemia in young children with T1D may be sub-optimal for the developing brain. Efforts that continue to assess the factors that influence neural and cognitive development in children with T1D are critical in minimizing the deleterious effects of diabetes.

Importantly, this study indeed indicates that, in addition to the deleterious effects of hypoglycemia on the developing brain, hyperglycaemia might also impair central nervous system development, cognitive development and learning capacity in children with diabetes. It has to be stressed that both hyper- and hypoglycemia need to be avoided and optimal metabolic control is to be obtained at all ages in children with T1D.

10.14. The clinician factor: Personality characteristics of clinicians and their impact upon clinical outcomes in the management of children and adolescents with type 1 diabetes

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This diabetes team are well known for, and should be applauded for, their holistic approach to diabetes care. Also, the group always points to central aspects of daily routine clinical practice and analyzes critical issues in pediatric diabetes. The aim of this study was to examine whether or not clinician qualities influence metabolic outcomes in children and adolescents with type 1 diabetes (T1D).

Data were gathered over two 3 month periods in the large tertiary diabetes center (1500 patients, 8 clinicians) in the state of Victoria, Australia, from patients with T1D who received continuous care from each individual clinician. Clinician factors explored included target blood glucose levels, target glycosylated hemoglobin (HbA1c), Diabetes Attitude Scale and Big 5 Personality Inventory Scale. Mean HbA1c per clinician was the primary outcome variable.

Lowest to highest mean HbA1c per clinician varied by only 0.7%. There were small but statistically significant differences between clinicians with their patients' age at diagnosis, duration of diabetes, age, gender, treatment type and BMI SD score. After controlling for these differences, the clinician characteristics associated with lower mean HbA1c were having no lower limit in target HbA1c and being self-reportedly "less agreeable". Importantly, the impact of these clinician attitudinal traits was equivalent to the combined effects of patient characteristics and treatment type.

There was significant variation in metabolic outcomes between treating clinicians. After controlling for patient clinical differences, clinician mean HbA1c was associated with lower limit in target HbA1c and being "less agreeable." Clinicians who were more demanding and dogmatic appeared to have better outcomes.

These data are a worry some for those of us physicians who feel that a more democratic and liberal attitude might achieve better patient adherence and metabolic control. However, the personality analysis performed here might not give a complete picture of the physician's attitudes and whether or not the physician is liked and trusted. Other limitations are the number of physicians (8) and their possible confounders, such as age and gender.

10.15. Increased prevalence of disordered eating in the dual diagnosis of type 1 diabetes mellitus and celiac disease

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<https://www.ncbi.nlm.nih.gov/pubmed/29493097>

Disordered eating behaviors (DEBs) may lead to full blown eating disorders and these might impair patients' adherence to chronic disease management. Both type 1 diabetes mellitus (T1DM) and celiac disease (CD) are associated with DEBs. Adolescents with T1D and eating disorders have worse metabolic control and a higher rate of acute complications than T1D patients without DEBs (1).

This study from Tel Aviv, Israel examined the risk of DEBs in adolescents and young adults with a dual diagnosis of T1DM and CD, compared to individuals with only one of diagnosis.

Individuals with both T1DM and CD (T1DM+CD, $n=39$), T1DM-only ($n=97$) or CD-only ($n=267$) completed the Eating Attitude Test-26 (EAT-26) questionnaire. Those with T1DM also completed the Diabetes Eating Problem Survey-Revised (DEPS-R). There were no differences between groups in sex, age, HbA1c, age of disease diagnosis and duration.

The prevalence of DEBs was 3-fold higher in the T1DM+CD group (26.0%) than T1DM-only (8.2%) and CD-only (8.2%) groups ($P=0.003$), and in both females and males separately. In models adjusted for age, sex, and BMI, the T1DM+CD group had a higher risk for DEBs (odds ratio: 4.7, 95% CI: 1.9–11.2, $P=0.001$). Additionally, and as previously described (1) being female, older and overweight increased the risk for DEBs. Other studies from other centers have shown a clear relationship between higher HbA1c values and DEBs risk, but this was not seen here.

As might have been expected, individuals with both T1DM and CD have an increased likelihood to develop DEBs. It is mandatory to screen for the presence of eating disorders in patients with T1D and even more so in T1D patients who also have CD.

Reference

1. Reinehr T, Dieris B, Galler A, Teufel M, Berger G, Stachow R, Golembowski S, Ohlenschläger U, Holder M, Hummel M, Holl RW, Prinz N. Metabolic Control and Dynamics of Weight Status in Adolescent Girls Point to Eating Disorders in the First Years after Manifestation of Type 1 Diabetes Mellitus: Findings from the Diabetes Patienten Verlaufsdokumentation Registry. *J Pediatr*. 2019 Apr;207:205–212.e5.

10.16. BMI, mortality, and cardiovascular outcomes in type 1 diabetes: Findings against an obesity paradox

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In normal populations, high BMI is associated with higher mortality and morbidity, in particular from cardiovascular disease (CVD). In contrast, in type 1 diabetes (T1D), low body weight has been associated with increased mortality risks. This study investigated the importance of weight and weight gain/loss in patients with T1D (n = 26,125; mean age 33.3 years; 45% women) recorded in the Swedish National Diabetes Registry from 1998 to 2012.

Mean BMI in patients with T1D increased continuously from 24.7 to 25.7 kg/m² from 1998 to 2012. Over a median follow-up of 10.9 years, there were 1,031 deaths (33.2% from CVD), 1,460 major CVD events, and 580 hospitalizations for HF. Cox regression was used to calculate risk of all-cause mortality, CVD mortality, major CVD events, hospitalizations for heart failure (HF).

After exclusion of smokers, patients with poor metabolic control, and patients with a short follow-up time, there was no increased risk for mortality in those with BMI < 25, while BMI > 25 was associated with a minor increase in risk of mortality, major CVD, and HF. In women, associations with BMI were largely absent. Weight gain implied an increased risk of mortality and heart failure, while weight loss was not associated with higher risk.

As is well-established for people without diabetes, among patients with T1D the risks of major CVD events and mortality increase with increasing BMI. These associations are more apparent in men than in women. Hence, this large national study found no evidence of an obesity paradox in people with T1D. These data are important for clinical practice, since good weight control should remain an important treatment goal for all people, with and without T1D!

10.17. Relative prognostic importance and optimal levels of risk factors for mortality and cardiovascular outcomes in type 1 diabetes mellitus

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Numerous publications have reported that higher HbA1c levels relate to higher cardiovascular disease (CVD) risk in people with type 1 diabetes (T1D). However, the strength of association and optimal HbA1c levels are not established.

This analysis of T1D patients recorded in the Swedish National Diabetes Register from 1998 to 2014 examined the prognostic importance of 17 risk factors for death and CVD. Cox regression and machine learning techniques were used to identify optimal cut point levels for HbA1c, systolic blood pressure (SBP), and low-density lipoprotein cholesterol (LDL) in relation to all-cause mortality, fatal/nonfatal acute myocardial infarction, fatal/nonfatal stroke, and hospitalization for heart failure. Of 32,611 patients with T1D, 1809 (5.5%) died during follow-up over 10.4 years.

The strongest predictors for death and CVD outcomes were: high HbA1c; renal disease as indicated by albuminuria; duration of T1D; higher SBP; and higher LDL. Risk of death increased by: ~2% per 1-mmol/mol higher HbA1c (~22% per 1% HbA1c); and 35% to 50% per 1-mmol/L higher LDL. Micro- or

macroalbuminuria was associated with 2 to 4-times higher risk for CVD complications and death. HbA1c < 53 mmol/mol (7.0%), SBP < 140 mmHg, and LDL < 2.5 mmol/l were identified as optimal thresholds associated with better health across the outcomes.

In summary, the study confirms that high HbA1c levels, albuminuria, duration of T1D, SBP, and LDL are the most important predictors for mortality and CVD in patients with T1D. Furthermore, it seems to be justified that levels for HbA1c, SBP, and LDL that are even lower than current guideline recommendations appear to be associated with significantly lower disease risks. This study clearly shows that it is very well worthwhile to do the utmost to achieve good metabolic control in people with T1D.

10.18. Association of rotavirus vaccination with the incidence of type 1 diabetes in children

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For many years, it has been put forward that enterovirus infections might precede type 1 diabetes onset and that there might be a link between the two diseases. This study simply shows that it is still not the time to draw firm and clear conclusions as to whether or not enteroviruses do play a role in the pathogenesis of autoimmune diabetes. This study is mentioned since an infectious origin of autoimmune disease should be studied more diligently and more widely.

10.19. Diabetes relief in mice by glucose-sensing insulin-secreting human α -cells

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Cell engineering might be a way to reinstall insulin-production in the pancreas of people with autoimmune diabetes. This experimental mouse study achieved to switch human alpha cells to secrete insulin.

Cell-identity switches, in which terminally differentiated cells are converted into different cell types, represent a widespread regenerative strategy in animals, yet they are poorly documented in mammals. In mice, some glucagon-producing pancreatic α -cells and somatostatin-producing δ -cells become insulin-expressing cells after the ablation of insulin-secreting β -cells, thus promoting diabetes recovery. Whether human cells also display this plasticity, especially in diabetic conditions, was not previously shown.

This study shows that islet non- β -cells, namely α -cells and pancreatic polypeptide (PPY)-producing γ -cells, obtained from deceased non-diabetic or diabetic human donors, can be lineage-traced and reprogrammed by the transcription factors PDX1 and MAFA to produce and secrete insulin in response to glucose. When transplanted into diabetic mice, converted human α -cells reversed diabetes and continued to produce insulin even after six months. Notably, insulin-producing α -cells maintained expression of α -cell markers, as seen by deep transcriptomic and proteomic characterization.

These findings provide conceptual evidence and a molecular framework for a mechanistic understanding of *in situ* cell plasticity as a potential future treatment for diabetes and other degenerative diseases. Applying such approaches might even bring cure for diabetes within reach. A major challenge to such therapeutic approaches is to prevent disrupted autoimmune processes in the T1D human host from attacking newly built beta-cells – this might be possible as the cells still appeared from the outside to look like α -cells.

10.20. Reduced burden of diabetes and improved quality of life: Experiences from unrestricted day-and-night hybrid closed-loop use in very young children with type 1 diabetes

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There might be many benefits arising from the artificial pancreas and other new technologies to deliver insulin and measure glucose levels. Whether or not such technological advances will improve the lives of children and families needs to be answered.

This study surveyed the experiences of parents/caregivers of 20 young children aged 1 to 7 years with type 1 diabetes (T1D), from a multinational trial, after two 3-week periods of unrestricted day-and-night hybrid closed-loop insulin therapy at home using the Cambridge FlorenceM system. Benefits, limitations, and improvements of closed-loop technology were explored.

Families reported reduced burden of diabetes management, less time spent managing diabetes, and improved quality of sleep with closed-loop. Interestingly, 90% of respondents felt less worried about their child's glucose control using closed-loop. Size of study devices, battery performance and connectivity issues were identified as areas for improvement. Parents/caregivers wished for more options to input information to the system such as temporary glucose targets.

Parents/caregivers of young children reported important quality of life benefits associated with using closed-loop, supporting adoption of this technology. However, it is possible that these positive views might reflect the general high reliance on technology and social background of this small sample and trial setting. It remains to be explored whether or not this finding holds true in a larger cohort of families, including patients from lower education and low income settings.

11. Obesity and Weight Regulation

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Preface

We have been very happy to get around 1,500 papers out of our established search strategy in PubMed which have been saved in our 2019 yearbook EndNote database. We have then selected 15 papers (1%) which in our mind have been the most exciting ones. The highlights in this year's chapter are publications about the genetic architecture of human thinness, a new hypoleptinemic leptin-responsive form of obesity, and early childhood BMI related to sustained risk of obesity. The Yearbook chapter 2019 on obesity and weight regulation comprises further exciting articles covering a broad research area.

New Insights into Body Weight Regulation

11.1. Genetic architecture of human thinness compared to severe obesity

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<https://www.ncbi.nlm.nih.gov/pubmed/30677029>

This genome wide association study reveals new insights into the genetic basis of thinness by investigating a large cohort of healthy persistently thin individuals. In the past, it has been speculated that inheritance of thinness may constitute a protective factor against environmental factors disposing to obesity (1). Nevertheless, for many years research focused mainly on genetic causes of obesity (2, 3). Recently, some studies led our attention to the lower end of the BMI distribution curve (4–7).

In this comprehensive genome-wide association study (GWAS), genotypes of 1,471 thin healthy individuals, without chronic illness or eating disorder, were compared to individuals with early onset obesity and controls, and were followed up in replication cohorts. Heritability of thinness was shown to be comparable to that of obesity ($h^2 = 28.1\%$ vs 32.3%). Higher estimations of heritability in body weight traits had been reported by other study designs (8), although similar heritability estimations have been calculated for body fat (9). The influence of gestational, perinatal and early-childhood factors on weight homeostasis may account for lower than expected heritability (10). The authors generated a standardized genetic risk score (GRS) based on 97 already known BMI-associated loci (2). Each unit GRS-increase was found to lead to a stronger effect among obese populations than among the thin population. Moreover, the authors performed three-way association analyses showing that the established BMI-associated loci explained more phenotypic variance in the obese cohort than in the thin cohort.

The GWAS design enabled the authors to confirm associations of established BMI-associated gene loci (2, 3) in both the obese and thin cohorts, and to report new variants in known loci. Moreover, they found a novel obesity and BMI-associated locus at *PKHD1*. Importantly, the authors observed that some loci influence either the lower or the upper end of the BMI distribution, while others show effects across the entire distribution. Here, differences between the degrees of obesity and thinness should be taken into account, whereby extreme thinness may not be compatible with life.

These authors provide a solid basis for future genetic studies of thinness, which will be of great importance to further clarify the role of resistance to obesity in an obesogenic environment. Furthermore, as suggested by a later study on gain-of-function *MC4R* variants (11), knowledge of gene loci and of signaling pathways associated with lower BMI may open the door to new therapeutic strategies against obesity.

References

1. Costanzo PR, Schiffman SS. Thinness—not obesity—has a genetic component. *Neuroscience and Biobehavioral Reviews*. 1989;13(1):55–8.
2. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, *et al*. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518(7538):197–206.
3. Berndt SI, Gustafsson S, Magi R, Ganna A, Wheeler E, Feitosa MF, *et al*. Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. *Nature Genetics*. 2013;45(5):501–12.
4. Whitaker KL, Jarvis MJ, Boniface D, Wardle J. The intergenerational transmission of thinness. *Archives of Pediatrics & Adolescent Medicine*. 2011;165(10):900–5
5. Bulik CM, Allison DB. The genetic epidemiology of thinness. *Obesity Reviews*. 2001;2(2):107–15.
6. Zillikens MC, Demissie S, Hsu YH, Yerges-Armstrong LM, Chou WC, Stolk L, *et al*. Large meta-analysis of genome-wide association studies identifies five loci for lean body mass. *Nature Communications*. 2017;8(1):80.
7. Ling Y, Galusca B, Hager J, Feasson L, Valsesia A, Epelbaum J, *et al*. Rational and design of an overfeeding protocol in constitutional thinness: Understanding the physiology, metabolism and genetic background of resistance to weight gain. *Annales d'Endocrinologie*. 2016;77(5):563–9.
8. Arden NK, Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. *Journal of Bone and Mineral Research*. 1997;12(12):2076–81.
9. Chu AY, Deng X, Fisher VA, Drong A, Zhang Y, Feitosa MF, *et al*. Multiethnic genome-wide meta-analysis of ectopic fat depots identifies loci associated with adipocyte development and differentiation. *Nature Genetics*. 2017;49(1):125–30.
10. Campbell MK. Biological, environmental, and social influences on childhood obesity. *Pediatric Research*. 2016;79(1-2):205–11.
11. Lotta LA, Mokrosinski J, Mendes de Oliveira E, Li C, Sharp SJ, Luan J, *et al*. Human Gain-of-Function *MC4R* Variants Show Signaling Bias and Protect against Obesity. *Cell*. 2019;177(3):597–607.e9.

11.2. Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity

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These authors combined data from 125 cohorts comprising more than 700,000 individuals to discover rare and low-frequency (R/LF) coding single nucleotide variants (SNVs) associated with BMI, thereby identifying novel potential targets for the treatment of obesity. They identified 14 R/LF coding SNVs in 13 genes associated with BMI, of which 8 variants were in genes newly implicated in human obesity. The effect sizes of these R/LF coding variants were 10 times larger than those of common variants. Interestingly, associations with BMI at 10 of 13 SNVs were directionally consistent in 3 childhood cohorts with those observed in adults; three of these reached nominal significance: *ZBTB7B*, an early growth response gene that binds to the promoter regions of type I collagen genes; *PRKAG1*, encoding for a regulatory subunit of an important energy-sensing enzyme AMP-activated protein kinase (AMPK); and *RAB21*, encoding a protein involved in the regulation of cell adhesion and migration. Furthermore they identified 92 common coding variants, of which 41 were novel.

R/LF variants associated with BMI showed significant enrichment in neuronal pathways, confirming previous findings of common variants (1), and also a novel cluster of metabolic pathways related to insulin action and adipocyte/lipid metabolism. This latter finding is exciting since the current understanding of body weight regulation is that this takes place primarily centrally. The finding that genes involved in insulin action and lipid metabolism are relevant for body weight regulation opens doors for pharmaceutical interventions on peripheral metabolism with possibly fewer side effects than acting centrally drugs.

The strength of the present work is certainly the large sample size. Limitations are the use of exome arrays rather than sequence data, and most of the samples were from European individuals. Moreover, notice should be taken of the rather small effect size of about 7 kg for the strongest variant association in *MC4R*.

Reference

1. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, *et al.* Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518(7538):197–206.

11.3. Steroid receptor coactivator-1 modulates the function of Pomc neurons and energy homeostasis

Yang Y, van der Klaauw AA, Zhu L, Cacciottolo TM, He Y, Stadler LKJ, Wang C, Xu P, Saito K, Hinton A Jr., Yan X, Keogh JM, Henning E, Banton MC, Hendricks AE, Bochukova EG, Mistry V, Lawler KL, Liao L, Xu J, O’Rahilly S, Tong Q, UK10K Consortium, Barroso I, O’Malley BW, Farooqi IS, Xu Y

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Steroid receptor coactivator (SRC)-1 mediates nuclear hormone receptors and transcription factor-dependent transcription (1), and interacts with STAT3 (2) an important mediator of leptin-induced *POMC* expression and hence satiety (3). *Src-1* knockout mice are obese (4), however, the underlying mechanism is unclear. In a compelling line of evidence, these authors suggest that SRC-1 deficiency might impair leptin action via reduced STAT3 activation. They show that SRC-1 deficient mice have lower leptin-stimulated pSTAT3 binding to *Pomc* promoters and lower *Pomc* mRNA, become more obese on high fat diets and are resistant to the anorectic effect of leptin. In addition, 14 of 15 rare *SRC-1* variants found in individuals with severe, early-onset obesity showed reduced SRC-1-STAT3 interaction, reduced POMC-neuron activation, and reduced *POMC* expression in *in-vitro* models; only 4 rare variants were identified in normal weight controls.

Extreme obesity, especially with an early onset is highly suspicious of monogenic obesity (5), but underlying genetic causes are found in fewer than 10% of cases (6) implying that there are many yet unknown genetic causes. The finding of a new obesity gene is of high interest as knowing the diagnosis helps to lessen the disease burden for most patients and might also open up future therapeutic strategies both for the affected patients and possibly also for patients with common obesity.

The strength of the study lies in the wide variety of methods used to examine SRC-1 function and the huge cohort of patients studied by DNA sequencing. Limitations include the fact that SRC-1 deficient mice showed only a modest obesity. This is in line with the moderating function of SRC-1, but it raises the question if heterozygous variants in this gene can truly explain the extreme obesity seen in probands (mean BMI z-score = 3; age of onset < 10 years). The authors provide no co-segregation analysis to strengthen their case, thus further studies will be needed to prove if variants in SRC-1 truly cause severe obesity.

References

1. York B, O’Malley BW. Steroid receptor coactivator (SRC) family: masters of systems biology. *Journal of Biological Chemistry*. 2010;285(50):38743–50.
2. Giraud S, Bienvenu F, Avril S, Gascan H, Heery DM, Coqueret O. Functional interaction of STAT3 transcription factor with the coactivator NcoA/SRC1a. *Journal of Biological Chemistry*. 2002;277(10):8004–11.
3. Xu AW, Ste-Marie L, Kaelin CB, Barsh GS. Inactivation of signal transducer and activator of transcription 3 in proopiomelanocortin (*Pomc*) neurons causes decreased *pomc* expression, mild obesity, and defects in compensatory refeeding. *Endocrinology*. 2007;148(1):72–80.
4. Picard F, Gehin M, Annicotte J, Rocchi S, Champy MF, O’Malley BW, *et al.* SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell*. 2002;111(7):931–41.
5. Kohlsdorf K, Nunziata A, Funcke JB, Brandt S, von Schnurbein J, Vollbach H, *et al.* Early childhood BMI trajectories in monogenic obesity due to leptin, leptin receptor, and melanocortin 4 receptor deficiency. *International Journal of Obesity (2005)*. 2018;42(9):1602–9.
6. Kleinendorst L, Massink MPG, Cooiman MI, Savas M, van der Baan-Slootweg OH, Roelants RJ, *et al.* Genetic obesity: next-generation sequencing results of 1230 patients with obesity. *Journal of Medical Genetics*. 2018;55(9):578–86.

11.4. Dysregulation of a long noncoding RNA reduces leptin leading to a leptin-responsive form of obesity

Dallner OS, Marinis JM, Lu YH, Birsoy K, Werner E, Fayzikhodjaeva G, Dill BD, Molina H, Moscatti A, Kutalik Z, Marques-Vidal P, Kilpeläinen TO, Grarup N, Linneberg A, Zhang Y, Vaughan R, Loos RJF, Lazar MA, Friedman JM
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<https://www.ncbi.nlm.nih.gov/pubmed/30842678>

This paper shows that quantitative leptin expression is controlled by redundant cis elements and trans factors that interact with the proximal promoter together with a long noncoding RNA.

Friedman's research group discovered the adipocyte hormone leptin more than 20 years ago. They identified loss of leptin as the cause of the dysregulated energy homeostasis in the genetically obese ob/ob mouse. The methods used at that time (positional cloning) were innovative and led to success. Although that discovery was a major breakthrough in our understanding of the regulation of body weight, many questions remained unanswered. How is leptin regulated in the organism? The fat content of the adipocyte and the number of adipocytes are decisive for circulating leptin levels. But which molecular mechanisms regulate the quantitative expression of leptin? The current study aimed to answer these questions as well as the follow-up question, whether disruption of these regulatory mechanisms leads to obesity.

Once again, innovative methods were used: transgenic luciferase reporter mice to map *cis*-elements that regulate leptin expression; DNA affinity pulldown experiments; global run-on sequencing to investigate RNA transcription. These approaches revealed that the transcription of a long noncoding RNA can associate with the wild-type leptin promoter to quantitatively regulate expression of the leptin gene. These findings were confirmed in experimental mouse models and data from large-scale human genetic studies by demonstrating that a lack of this long noncoding RNA leads to hypoleptinemia and obesity.

These results represent another breakthrough since they show there is a subtype of human obesity due to relative leptin deficiency which could be treated by leptin therapy. Furthermore, these results suggest that there may exist other factors regulated by the cellular lipid content of the adipocyte which are able to determine cellular leptin expression.

Tracking, Programming, and Epigenetics

11.5. Acceleration of BMI in early childhood and risk of sustained obesity

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This analysis of longitudinal BMI data from early childhood to adolescents in 51,505 German subjects (CrescNet patient registry) allows a new insight into BMI development, specific dynamics and BMI trajectories from childhood to early adulthood.

The exact pattern and time course of weight gain during childhood that leads to obesity is unclear. These authors tracked BMI values, obtained by measured height and weight data in individual children from infancy to adolescence in a large population (CrescNet patient registry | Germany) recruited from resident doctors, to determine the age at which children are most vulnerable to excessive weight gain that results in obesity in adolescence. They included in the analysis children who had at least one visit with a resident pediatrician between 0 and 14.9 years of age (childhood) and another visit between 15.0 and 18.9 years (adolescence) ($n=51,505$ subjects; 336,227 data points).

They found that: (a) the majority of normal BMI adolescents had normal BMI as children; (b) the majority of overweight adolescents had normal BMI for the first 4 years life and the percentage of overweight increased from age 5 years onwards; and (c) the majority of adolescents who were obese had normal BMI as infants, and became overweight or obese by age 5 years.

Among children who were obese at 3 years of age, the probability of being overweight or obese in adolescents was almost 90%. The comparison of changes in BMI-standard-deviation score (BMI-SD) from birth onwards showed that the BMI-SD-score was much more stable among adolescents in the normal BMI group than in the overweight/obese group. For overweight/obese adolescents, the greatest BMI acceleration occurred between 2 and 6 years of age.

The strengths of this study include the population-based design, the inclusion of 51,000 children and of 300,000 measurements (weight/height). This study impressively shows that early childhood is the critical age for the development of obesity and that there are specific dynamics and patterns of BMI in this early childhood period. In daily practice, this means that BMI acceleration rather than the absolute BMI alone appears to be important in identifying children at risk for obesity in later life.

From an evolutionary point of view the genomes of hunter-gatherers were adapted to low insulin sensitivity with a lifestyle comprising intense physical activity and a high-protein and low-carbohydrate diet (1). Individuals with a still preserved “hunter-gatherers” genome who become obese at age 4–8 years may need a low-carbohydrate (CHO) diet in order to prevent excessive weight gain. By contrast individuals with a so-called ‘farmer genome’, which adapted to a CHO-rich diet, who become obese in infancy might profit more from a low-calorie diet. The present data may help to differentiate these two groups of individuals and to stimulate further genomic and metabolic investigations.

Reference

1. Hochberg Z. An evolutionary perspective on the obesity epidemic. *Trends Endocrinol Metab.* 2018; 29(12): 819–826.

11.6. Adipose tissue mitochondrial dysfunction in human obesity is linked to a specific DNA methylation signature in adipose-derived stem cells

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This epigenome-wide association study in stromal/stem cells (ASCs), derived from subcutaneous adipose tissue samples of lean and obese subjects, revealed a specific DNA methylation signature in adipocyte precursors associated with obesity, which has a significant impact on the metabolic phenotype and the mitochondrial function of mature adipocytes.

These authors hypothesized that an obesogenic environment influences the methylation status of genes in human adipose-derived stem cells (hASCs), which might contribute to the contrasting differentiation and functional capacities of adult adipocytes to promote dysfunctional white adipose tissue. First, they conducted an epigenome-wide association study in DNA from subcutaneous adipose tissue of healthy lean ($n=6$) and obese ($n=6$; mean BMI 30 kg/m²) adults, and separately considered methylation levels in hASC and adipocytes. In hASC they observed 650 differently methylated sites between lean and obese subjects. Interestingly, this methylation signature was similar in hASCs and adipocytes. They looked closer at the 10 genes with the largest methylation changes between lean and obese subjects. Of these, 5 genes (TBX15, PRDM16, ACLY, GLI2, LSP1) showed an inverse relationship between DNA methylation intensity and mRNA expression. TBX15 was examined in more detail since it was highly regulated at epigenetic level. TBX15 is a transcription factor that is essential for many developmental processes. In TBX15-silenced obese ASCs, expression levels were decreased for genes involved in fatty acid transport, oxidation, glucose uptake, glycolysis and TCA cycle.

This suggests a potential role for TBX15 in the mitochondrial and/or metabolic phenotype of mature adipocytes in an obesity context.

The strength of the study lies in the fact that the authors not only describe differences in the DNA methylation profile in ASCs derived from lean and obese subjects. But they went further by testing the relevance of DNA methylation to gene expression, and then performed experimental modulation of the gene (TBX15) with the greatest methylation difference. One limitation is that the authors examined only the effect of relatively mild obesity on mitochondrial and/or metabolic phenotype. Further studies on extreme obesity would be desirable.

11.7. Transgenerational epigenetic mechanisms in adipose tissue development

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This paper comprehensively reviews current knowledge on epigenetic mechanisms in adipose tissue that may account for transgenerational dysregulation of adipocyte formation and adipose tissue function.

There is increasing evidence that adult-onset disorders, including obesity, may derive from events that take place during fetal and early postnatal development. Today, the term ‘developmental programming’ is increasingly used to describe this phenomenon. In this process the functioning of organs or organ systems is permanently shaped (programming) during critical development phases (prenatal and neonatal) by the influence of factors such as hormones. In case of a disturbance of this programming e.g. by an oversupply of nutrients or by abnormal concentrations of hormones, it can result in ‘incorrect programming’ of the function of organs or organ systems, which in later life favour the development of chronic illnesses such as obesity or diabetes mellitus.

The authors summarize here the results of studies in animal models and humans that support the hypothesis that adipose tissue is subject to developmental programming events. They highlight the differences in adipogenesis between rodents and humans, which is important to consider in the interpretation and discussion of results of future studies. The authors show extensive graphics and figures in which they sum up the ideas about epigenetic mechanisms during adipose tissue development as well as the concept of epigenetic memory in adipose tissue by the example of malnourished dams.

Metabolic Signaling

11.8. An endothelial-to-adipocyte extracellular vesicle axis governed by metabolic state

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[https://www.cell.com/cell/pdf/S0092-8674\(18\)31177-2.pdf](https://www.cell.com/cell/pdf/S0092-8674(18)31177-2.pdf)

This paper demonstrates for the first time the existence of extracellular vesicle (EV)-mediated signaling between cell types within the adipose tissue. The results of various experiments show a network of EV-mediated exchange of cellular material in adipose tissue. This newly discovered EV trafficking in adipose tissue is regulated by the metabolic state of the body.

The authors recognized the phenomenon of EV trafficking by chance when they generated an adipocyte-specific knockout of caveolin 1 (cav1). Caveolin-1 is a protein that regulate many cell functions e.g. membrane transport, endocytosis, regulation of calcium metabolism, lipid metabolism and signal transduction in cell proliferation and programmed cell death. Mutations in the *CAVI* gene cause a rare form of lipodystrophy. Although they

effectively ablated *CAVI* in adipocytes, caveolin 1 protein remained abundant in the same adipocytes. By generating new additional mouse models, they showed *in vivo* that caveolin 1 protein is transferred in EVs from neighboring endothelial cells to adipocytes. In addition, they showed that adipose tissue-derived EVs contain proteins and lipids which can modulate cellular signaling pathways. Finally, they showed that this transfer is physiologically regulated by fasting and refeeding, and is dependent on fat mass. This suggests that EVs participate in the tissue response to changes in systemic nutrient state. These findings reveal a new mechanism which can be added to the complex signaling mechanisms between adipocytes, stromal vascular cells, and, potentially, distant organs.

11.9. Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: A randomized controlled trial

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This randomized, placebo-controlled, double blind trial showed that IL-6 is necessary for exercise-mediated loss of visceral adipose tissue mass. 53 participants (men and women) received either tocilizumab, an IL-6 signaling blocker (intervention group) or placebo (control group), every four weeks, in a 12-week intervention period combined with a bicycle routine or no exercise. In participants receiving placebo, exercise significantly reduced visceral adipose tissue mass. By contrast, in participants receiving tocilizumab and performing exercise, visceral adipose tissue mass was increased compared to the placebo group. Therefore, loss of visceral adipose tissue mass following exercise was dependent on IL-6. This IL-6 effect was specifically seen in adipose tissue. Improvements in cardiorespiratory fitness following exercise were shown to be independent of IL-6. In addition, IL-6 blockade increased cholesterol levels and this effect was not reversed by exercise.

The study shows convincingly IL-6 signalling as a mechanism by which exercise reduces visceral adipose tissue mass. Given that abdominal obesity is metabolically harmful, the findings reveal a potentially important side effect of IL-6 receptor antibodies and consolidates a physiological role of IL-6 as a beneficial lipolytic factor in humans, capable to reduce visceral fat mass. The relative small study cohort and a missing consideration of a sex-dependent fat distribution are limitations of this study.

Brown Adipose Tissue: The Story Goes On

11.10. Brown adipose tissue in prepubertal children: associations with sex, birthweight, and metabolic profile

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Studies regarding brown adipose tissue (BAT) activity in children are scarce due to the difficulties in detecting BAT by imaging and the need for elaborate techniques to measure BAT activity. Here, prepubertal children born small for gestational age (SGA, $n=41$) and children born appropriate for gestational age (AGA, $n=45$) were examined in order to investigate whether prematurity has an impact on BAT activity. This is of special interest since it has been shown that the activity of BAT is important for insulin sensitivity and a healthy lipid profile, and children with SGA have corresponding metabolic impairments.

The authors measured BAT activity using ‘thermal imaging’: the temperature at the supraclavicular region (SCR) before and after a cold stimulus was measured by infrared thermal imaging, and the area of thermally active SCR (increase after cold challenge, $\Delta\text{Area}_{\text{SCR}}$) was calculated as a surrogate index of BAT activation. They found no difference in BAT activity between SAG and AGA children. However, a sex difference was detected, with a higher BAT activity in AGA girls compared to AGA boys. Moreover, BAT activity was negatively associated with HOMA-IR, hs-CRP, liver volume, and liver fat.

BAT activation peaks during puberty and declines during adulthood (1). Recent papers have shown that BAT activity in children is positively associated with muscle volume (2) and bone mass (3), suggesting a role for BAT activity in growth and development. Until now, there was no study investigating relationships between BAT activity and metabolic health and cardiometabolic risk factors. The present paper shows for the first time positive associations of BAT activity in children with indicators of metabolic good health. These associations are present already in early childhood. Moreover, the described difference between girls and boys, which has also been described elsewhere (1), could not be seen in SGA children, possibly indicating limited activation properties in girls born preterm. Studies using more accurate techniques, such as MRI to detect BAT volume, are needed to corroborate these findings.

References

1. Gilsanz V, Smith ML, Goodarzi F, Kim M, Wren TA, Hu HH. Changes in brown adipose tissue in boys and girls during childhood and puberty. *Journal of Pediatrics*. 2012;160(4):604-9.e1
2. Gilsanz V, Chung SA, Jackson H, Dorey FJ, Hu HH. Functional brown adipose tissue is related to muscle volume in children and adolescents. *Journal of Pediatrics*. 2011;158(5):722-6.
3. Ponrartana S, Aggabao PC, Hu HH, Aldrovandi GM, Wren TA, Gilsanz V. Brown adipose tissue and its relationship to bone structure in pediatric patients. *Journal of Clinical Endocrinology and Metabolism*. 2012;97(8):2693-8.

11.11. Identification of metabolically distinct adipocyte progenitor cells in human adipose tissues

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These authors used FACS cell sorting, gene expression profiling, and metabolic and proteomic analyses to identify three distinct adipocyte progenitor cell (APCs) subtypes that reside in human white adipose tissues. Although they retain comparable differentiation capacity, they have different molecular profiles and give rise to adipocytes with divergent metabolic and endocrine features.

It is well established that different types of APCs exist, however, their molecular identities, e.g. cell surface markers, are not well defined. The International Fat Applied Technology Society (IFATS) defines an adipose derived stem cells as CD45-/CD235a-/CD31-/CD34+ cells, which represent approximately 20% of the whole stroma-vascular fraction (1) of white adipose tissue. Whilst CD34 was long used as a cell surface marker for the hematopoietic cell fraction, it is now considered to be a marker of adipose tissue stemness (2). It should be mentioned that CD34 expression is dynamic in cell culture; adherent cells lose this marker upon prolonged cultivation (2).

Here, the authors defined three subpopulations of APCs, dependent on CD34 expression before seeding: CD34 high; CD34 low; and CD34 negative APCs. Adipocytes which differentiated *in vitro* from high CD34 APCs showed extremely high rates of lipid flux compared with low CD34 APCs or CD34 negative APCs. By contrast, adipocytes derived from CD34 negative APCs displayed beige-like adipocyte properties and a unique endocrine profile. APCs were more abundant in gluteofemoral than abdominal subcutaneous and omental adipose tissues, but similar distribution patterns were found of APC subtypes between these adipose tissue depots and similar metabolic traits of these APC subtypes. The distribution of APC subtypes varied between depots and in patients with Type 2 diabetes.

In summary, this paper shows convincingly that adipocytes from three APC subtypes have distinct metabolic and endocrine profiles. The data suggest that enrichment of a certain adipocyte cell type may influence whole-body metabolism. Indeed, distribution of ASC subpopulations was altered in Type 2 diabetic patients. If this holds true, distribution of ASCs in adipose tissue might also help to predict disease development.

References

1. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, *et al.* Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*. 2013;15(6):641–8.
2. Baer PC. Adipose-derived mesenchymal stromal/stem cells: an update on their phenotype *in vivo* and *in vitro*. *World Journal of Stem Cells*. 2014;6(3):256–65.

Treatment of Severe Obesity: Next Steps

11.12. Patients with obesity caused by melanocortin-4 receptor mutations can be treated with a glucagon-like peptide-1 receptor agonist

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Pathogenic mutations in the melanocortin-4 receptor gene (*MC4R*) are the most common cause of monogenic obesity. However, very limited treatment options exist. Therefore, these authors investigated a glucagon-like peptide-1 receptor agonist (GLP-1 RA) as a treatment option for these patients. The findings are of major significance.

They assessed the effect of the GLP-1 RA liraglutide in $n=14$ obese individuals with pathogenic *MC4R* mutations and $n=28$ matched controls. Daily s.c. injections of liraglutide 3 mg for 16 weeks decreased body weight, body fat, waist circumference, and fasting and postprandial glucose levels by the same amount in both groups. Therefore GLP-1 RA likely reduces body weight independently of the *MC4R* pathway. The effect of liraglutide on weight loss in patients with pathogenic *MC4R* mutations was much higher than any previously reported intervention in this group (1–4).

This is the first study to show that monogenic obesity caused by pathogenic *MC4R* mutations can be effectively treated with a GLP-1 RA; hence the results are of major importance. Limitations of this study include the small number of participants, the fact that cases and controls were recruited within the same family or as friends, and the absence of an untreated placebo control group. Furthermore, the mechanism of GLP-1 RA-mediated weight loss is not fully understood.

References

1. Bonnefond A, Keller R, Meyre D, Stutzmann F, Thuillier D, Stefanov DG, *et al.* Eating Behavior, Low-Frequency Functional Mutations in the Melanocortin-4 Receptor (*MC4R*) Gene, and Outcomes of Bariatric Operations: A 6-Year Prospective Study. *Diabetes Care*. 2016;39(8):1384–92.
2. Collet TH, Dubern B, Mokrosinski J, Connors H, Keogh JM, Mendes de Oliveira E, *et al.* Evaluation of a melanocortin-4 receptor (*MC4R*) agonist (Setmelanotide) in *MC4R* deficiency. *Molecular Metabolism*. 2017;6(10):1321–9.
3. Danielsson P, Janson A, Norgren S, Marcus C. Impact sibutramine therapy in children with hypothalamic obesity or obesity with aggravating syndromes. *Journal of Clinical Endocrinology and Metabolism*. 2007;92(11):4101–6.
4. Reinehr T, Hebebrand J, Friedel S, Toschke AM, Brumm H, Biebermann H, *et al.* Lifestyle intervention in obese children with variations in the melanocortin 4 receptor gene. *Obesity (Silver Spring, Md)*. 2009;17(2):382–9.

11.13. Five-year outcomes of gastric bypass in adolescents as compared with adults

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These authors describe the 5-year follow-up of a multicenter patient cohort of adolescents with severe obesity who underwent gastric bypass surgery to lose weight and improve cardiometabolic risk factors (the Teen-LABS study; $n = 161$, age 13–19 years at time of surgery). They compared these data with a similar but independent study of obese adults who underwent gastric bypass surgery (the LABS study; $n = 379$, 25–50 years at time of surgery). Data on weight loss, health outcomes, and adverse events were assessed. Both, adolescents and adults achieved marked weight loss five years after surgery, but adolescents had significantly more frequent remission of pre-existing Type 2 diabetes and hypertension.

Bariatric surgery is the most effective treatment option to achieve long-term weight loss in obese adults (1). Importantly, bariatric surgery leads to resolution of major obesity-related comorbidities in a significant proportion, but not in all patients. Prognostic indicators for improvements in comorbidities are: shorter pre-surgery duration of Type 2 diabetes; higher beta-cell function; and probably also shorter pre-surgery duration of hypertension. Therefore, the authors hypothesized that bariatric surgery in obese adolescents would yield higher rates of remission of comorbidities than in adults who had been obese since their adolescent years (BMI ≥ 30 kg/m² at 18 years or younger). Indeed, comparing the outcomes of two large patient cohorts revealed important insights.

Both groups achieved significant weight loss at five years follow-up (–26% in adolescents; –29% in adults), but adolescents were more likely than adults to have remission of diabetes (relative risk 1.27, 95% CI: 1.03–1.57; $P = 0.03$). Furthermore, adolescents were 51% more likely than adults to achieve remission of presurgical hypertension (relative risk 1.51, 95% CI: 1.21–1.88; $P < 0.001$). However, these benefits came at a price: there was a small 5-year all-cause mortality rate (1.9% in adolescents; 1.8% in adults; two deaths in the adolescent cohort related to drug overdose), a higher rate of abdominal reoperations in adolescents (19/500 person years) compared to adults (10/500 person years), and a higher rate of micronutrient deficiencies in adolescents. This study adds important information for clinical decision making on the optimal age for bariatric surgery. But, still longer-term follow-up studies are needed to determine lifetime risks and benefits of bariatric surgery.

Due to the paucity of long-term data, it is impossible to fully determine the risk-benefit of bariatric surgery in adolescents. Adding to this challenge, BMI and age cutoffs for surgery are arbitrary and vary between guidelines. Most agree that the situation is complex and requires a mindful, informed approach. All adolescents should be cared for by multidisciplinary teams in centers with expertise in adolescent extreme obesity and bariatric surgery. Conventional treatment approaches should be exhausted before considering surgery. Measures to improve patient understanding of the procedure and lifestyle changes, as well as compliance, need to be implemented in a preoperative treatment program. Risk-benefit has to be considered on a case-by-case basis, keeping in mind that full information on long-term risks is currently unavailable.

Recently published recommendations by the German Working Group on Obesity in Children and Adolescents, an expert committee commissioned by the German Association for the Study of Obesity and the German Society for Pediatrics and Adolescent Medicine (2) may help to decision making in individual patients. The recommendations call for a standardized pre- and postsurgical treatment program to improve outcomes and reduce risk of adverse effects, and this has recently been implemented at five university centers in Germany.

References

1. Colquitt JL, Pickett K, Loveman E, Frampton GK. Surgery for weight loss in adults. *Cochrane Database of Systematic Reviews.* 2014(8):Cd003641.
2. M. Wabitsch B. Lennerz: Safety of Bariatric Surgery in Adolescents. In *Metabolism and Pathophysiology of Bariatric Surgery*. Edited by V.R. Preedy RR, C. R. Martin. London: Elsevier; 2016: 199–206.

11.14. Pathophysiology and individualized treatment of hypothalamic obesity following craniopharyngioma and other suprasellar tumors: a systematic review

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This paper combines a comprehensive review of current knowledge on pathophysiologic mechanisms contributing to hypothalamic obesity in craniopharyngioma patients with a systematic literature review of intervention studies for weight management in this patient population. Findings of both reviews are merged into evidence-based treatment algorithms for patients with hypothalamic obesity.

Hypothalamic obesity is a complex neuroendocrine disorder characterized by rapid weight gain, extreme obesity, hyperphagia, decreased resting energy expenditure, and reduced physical activity (1). In craniopharyngioma patients, tumor growth, neurosurgical procedures, and cranial irradiation damage key centers for the regulation of energy homeostasis located in the anterior (paraventricular nucleus), middle (arcuate nucleus, ventromedial nucleus), and posterior hypothalamus (dorsomedial nucleus, dorsal hypothalamic area). Altered eating behavior and extreme obesity develop in 40–50% of craniopharyngioma patients, and the most rapid, uncontrollable weight gain usually occurs during the first 6–12 months following neurosurgery (2). Although there are some promising reported results on the treatment of hypothalamic obesity (e.g. (3)), the overall effects on weight control are at best moderate, often heterogeneous, and have only been observed in small study populations.

As our understanding of the complex mechanisms controlling feeding behavior continue to improve, the authors of this timely review synthesize treatment paths for patients affected by hypothalamic obesity from available data of intervention studies. These treatment paths are strictly based on the underlying pathophysiology of hypothalamic obesity and cover six clinical domains, namely psychosocial problems, hyperphagia, sleeping problems, decreased energy expenditure, hyperinsulinemia, and hypopituitarism. This differentiated and evidence-based approach is not only of high value for those involved in the clinical care for this challenging patient population with highly specialized needs, but also represents a solid basis for the development of future clinical trials and state-of-the-art guideline recommendations.

References

1. Bereket A, Kiess W, Lustig RH, Muller HL, Goldstone AP, Weiss R, *et al.* Hypothalamic obesity in children. *Obesity Reviews.* 2012;13(9):780–98.
2. Muller HL, Emsler A, Faldum A, Bruhnken G, Etavard-Gorris N, Gebhardt U, *et al.* Longitudinal study on growth and body mass index before and after diagnosis of childhood craniopharyngioma. *Journal of Clinical Endocrinology and Metabolism.* 2004;89(7):3298–305.
3. Denzer C, Denzer F, Lennerz BS, Vollbach H, Lustig RH, Wabitsch M. Treatment of Hypothalamic Obesity with Dextroamphetamine: A Case Series. *Obesity Facts.* 2019;12(1):91–102.

11.15. Working toward precision medicine approaches to treat severe obesity in adolescents: report of an NIH workshop

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This paper presents the results of a multidisciplinary expert workshop to identify current knowledge and more importantly current gaps in knowledge on the epidemiological and biopsychosocial determinants of obesity and its optimal treatment approaches.

Childhood obesity is a global medical and public health threat with high prevalence rates leading to numerous health problems and comorbidities (1). While in many countries there is a plateauing in the rise of obesity rates (2, 3), a continuous rise in the rate of extreme obesity can still be noted (4). This is of special concern as extreme obesity is an intractable disease (5) associated with multiple comorbidities (6, 7).

In line with the current Endocrine Society guidelines (2), the authors recommend lifestyle intervention as the primary therapy, even though most studies showed limited or no success for most adolescents with extreme obesity (8). Concerning pharmacotherapy and weight-reducing devices, the authors point out the limited data in the pediatric population especially concerning long-term safety and efficacy. Even for bariatric surgery, which has been studied in well-designed studies in this population and is by far most effective treatment with an average weight loss of 30% (9, 10), very-long-term data are still scarce. In addition, although responses to most weight loss interventions are overall small, they vary widely between individuals.

Therefore the authors call for a standardization of measures of obesity and treatment outcomes to make (intervention) studies more comparable. This starts with the establishment of valid and accepted BMI metrics. The authors also promote the “Accumulating Data to Optimally Predict Obesity Treatment” (ADOPT) project (11) which aims to create common processes and measures for obesity research. So far, identified determinants of body weight explain only a small proportion of variations in weight status. Therefore, the authors conclude that it is necessary to develop an integrated model to allow the identification of different biopsychosocial phenotypes that predict treatment responses and will provide the basis for patient-tailored novel treatments.

In summary, this article provides an excellent lecture for clinical researchers in childhood obesity, guiding toward precision medicine approaches to treat severe obesity in adolescents. The article also defines area for future research and encourages the development of comparable and reproducible data in this challenging field of medicine.

References

1. Farpour-Lambert NJ, Baker JL, Hassapidou M, Holm JC, Nowicka P, O'Malley G, *et al.* Childhood Obesity Is a Chronic Disease Demanding Specific Health Care—a Position Statement from the Childhood Obesity Task Force (COTF) of the European Association for the Study of Obesity (EASO). *Obesity Facts*. 2015;8(5):342–9.
2. Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH, *et al.* Pediatric Obesity—Assessment, Treatment, and Prevention: An Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism*. 2017;102(3):709–57.
3. Wabitsch M, Moss A, Kromeyer-Hauschild K. Unexpected plateauing of childhood obesity rates in developed countries. *BMC Med* 2014; 12:17.
4. Hales CM, Fryar CD, Carroll MD, Freedman DS, Ogden CL. Trends in Obesity and Severe Obesity Prevalence in US Youth and Adults by Sex and Age, 2007–2008 to 2015–2016. *JAMA*. 2018;319(16):1723–5.
5. Danielsson P, Kowalski J, Ekblom O, Marcus C. Response of severely obese children and adolescents to behavioral treatment. *Archives of Pediatrics & Adolescent Medicine*. 2012;166(12):1103–8.
6. Kelly AS, Barlow SE, Rao G, Inge TH, Hayman LL, Steinberger J, *et al.* Severe obesity in children and adolescents: identification, associated health risks, and treatment approaches: a scientific statement from the American Heart Association. *Circulation*. 2013;128(15):1689–712.
7. Fox CK, Gross AC, Rudser KD, Foy AM, Kelly AS. Depression, Anxiety, and Severity of Obesity in Adolescents: Is Emotional Eating the Link? *Clinical Pediatrics*. 2016;55(12):1120–5.
8. Knop C, Singer V, Uysal Y, Schaefer A, Wolters B, Reinehr T. Extremely obese children respond better than extremely obese adolescents to lifestyle interventions. *Pediatric Obesity*. 2015;10(1):7–14.
9. Inge TH, Courcoulas AP, Jenkins TM, Michalsky MP, Helmrath MA, Brandt ML, *et al.* Weight Loss and Health Status 3 Years after Bariatric Surgery in Adolescents. *New England Journal of Medicine*. 2016;374(2):113–23.
10. Olbers T, Beamish AJ, Gronowitz E, Flodmark CE, Dahlgren J, Bruze G, *et al.* Laparoscopic Roux-en-Y gastric bypass in adolescents with severe obesity (AMOS): a prospective, 5-year, Swedish nationwide study. *Lancet Diabetes & Endocrinology*. 2017;5(3):174–83.
11. MacLean PS, Rothman AJ, Nicastro HL, Czajkowski SM, Agurs-Collins T, Rice EL, *et al.* The Accumulating Data to Optimally Predict Obesity Treatment (ADOPT) Core Measures Project: Rationale and Approach. *Obesity (Silver Spring, Md)*. 2018;26 Suppl 2:S6–s15.

12. Type 2 Diabetes, Metabolic Syndrome and Lipid Metabolism

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Preface

The increasing prevalence of Type 2 diabetes (T2DM) is well established, and includes new at-risk groups, such as children with attention deficit syndrome. It has become clear that T2DM in adolescents is more aggressive than in adults, and studies by the RISE consortium have clarified the basis of this difference. Treatment with subcutaneous liraglutide holds promise for adolescents with T2DM. Furthermore, this year the FDA approved artificial intelligence to diagnose diabetes retinopathy.

A recently recognized factor associated with pathways to cardiovascular and metabolic risk in youths is early life stress, as reflected by height-stunted growth of individuals who were in institutional care as infants, and of individuals exposed to early socioeconomic disadvantage. On the other hand, exogenous testosterone treatment to transgender men was not associated with increased cardiovascular risk. In a review of noxious effects of carbohydrates, the term carbotoxicity was coined, thus highlighting the epidemiological and experimental evidence of the negative impact of excessive carbohydrate intakes. Conversely, a prospective cohort study and meta-analysis indicated that low carbohydrate consumption (<40%) conferred greater mortality risk.

The enigma of how cholesterol is transported within cells has been solved by the discovery of the protein Aster. Orlistat therapy was demonstrated to be beneficial for children with Type 1 hyperlipoproteinemia. Supplements with omega-3 did not lower the overall incidence of adverse cardiovascular events or cancer. Novel diagnostic biomarkers were identified in the blood that can predict early fatty liver disease.

Type 2 Diabetes

12.1. Pivotal trial of an autonomous AI-based diagnostic system for detection of diabetic retinopathy in primary care offices

Abràmoff MD, Lavin PT, Birch M, Shah N, Folk JC

Nature Digital Medicine 2018;1:39.

DOI:10.1038/s41746-018-0040-6

Summary: This prospective observational study evaluated the performance of a diabetic retinopathy diagnostic system (IDx-DR) compared to the gold standard diagnostic for diabetic retinopathy. Nine hundred individuals with diabetes but without a history of diabetic retinopathy were examined. Retinal images of the patients were obtained using a robotic camera, and a clinical diagnosis was made in 20 seconds by an artificial intelligence (AI) diagnostic system and compared to images read by three experienced and validated readers. The AI system correctly identified 173 of the 198 individuals with more than mild diabetic retinopathy, (a sensitivity of 87%), and 556 of the 621 disease-free individuals (a specificity of 90%).

Comment: This breakthrough study describes the first FDA authorized autonomous AI diagnostic system in any field of medicine. AI is the simulation of human intelligence processes by computer systems. These processes include learning (the acquisition of information and rules for using the information), reasoning

(using rules to reach approximate or definite conclusions) and self-correction. One of the techniques used in AI is referred to as “deep learning”.¹ These methods have dramatically improved the state-of-the-art in visual object recognition, object detection speech recognition, language translation and many other domains such as drug discovery, genomics, robotics and even self-driving cars.

In medicine, the impact of AI is categorized into three branches²: 1) for clinicians, 2) for health systems and 3) for patients. For clinicians, deep learning is helpful in interpreting medical scans, pathology slides, skin lesions, electrocardiograms, endoscopy and faces. For health systems that use electronic record data, deep-learning algorithms enable predicting several health outcome parameters and improving workflow algorithms. For patients, deep-learning algorithms enable accessing their own data, such as smartwatch algorithms to detect atrial fibrillation and continuous sensing of blood-glucose. Despite the controversy involving AI, this article attests to the beginning of a new and exciting era.

References

1. LeCun Y, Bengio Y, Hinton G. Deep learning. *Nature*. 2015; **521**(7553): 436–44.
2. Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. *Nature Medicine*. 2019; **25**(1): 44–56.

12.2. Risk of type 2 diabetes in adolescents and young adults with attention-deficit/hyperactivity disorder: a nationwide longitudinal study

Chen MH, Pan TL, Hsu JW, Huang KL, Su TP, Li CT, Lin WC, Tsai SJ, Chang WH, Chen TJ, Bai YM
Department of Psychiatry, Taipei Veterans General Hospital, Taipei, Taiwan

J Clin Psychiatry 2018;79.

DOI:10.4088/JCP.17m11607

Summary: In a population-based prospective cohort study, based on the Taiwan National Health Insurance Research Database, 35,949 adolescents and young adults with attention-deficit/hyperactivity disorder (ADHD) had a higher risk of developing T2DM and had a shorter duration between enrollment and onset of T2DM than did 71,898 age- and sex-matched controls. Long-term use of atypical antipsychotics was associated with a higher likelihood of subsequent T2DM.

Comment: ADHD is the most common neurodevelopmental disorder in childhood. The estimated prevalence of diagnosed ADHD in US children & adolescents increased from 6.1% in 1997–1998 to 10.2% in 2015–2016.¹ In parallel, the prevalence of childhood obesity rose by alarming rates. Meta-analysis evidence shows a significant association between ADHD and obesity, regardless of possible confounding factors such as psychiatric comorbidities. Several possibilities may explain this observation. Firstly, ADHD increases the risk of obesity. Both the deficient inhibitory control, as well as the inattention and poor planning that characterize ADHD might cause difficulties in adhering to regular eating patterns and healthy food intake. In addition, children with ADHD have been shown to watch more television and engage less in physical activity than those without ADHD. Secondly, factors associated with obesity such as sleep-disordered breathing, and shorter or later sleep may manifest with ADHD-like symptoms. Thirdly, ADHD and obesity share common biological risk factors, for example, severely obese mothers and mothers with diabetes have increased risks of having a child with ADHD, as well as an obese child. In the current study, adolescents with ADHD had a 2.83 (95% CI, 1.96–4.09) fold risk and young adults a 3.28 (95% CI, 1.41–7.63) fold risk of developing T2DM compared to the controls. Of note, long-term use of atypical antipsychotics was associated with a higher likelihood of subsequent T2DM. Young adults with ADHD and comorbid hypertension, dyslipidemia, and obesity were more susceptible to T2DM.

References

1. Xu G, Strathearn L, Liu B, Yang B, Bao W (2018) Twenty-Year Trends in Diagnosed Attention-Deficit/Hyperactivity Disorder Among US Children and Adolescents, 1997–2016. *JAMA Netw Open* 1:e181471.
2. Cortese S, Tessari L (2017) Attention-Deficit/Hyperactivity Disorder (ADHD) and Obesity: Update 2016. *Curr Psychiatry Rep* 19:4.
3. Xiang AH, Wang X, Martinez MP, Getahun D, Page KA, Buchanan TA, Feldman K (2018) Maternal Gestational Diabetes Mellitus, Type 1 Diabetes, and Type 2 Diabetes During Pregnancy and Risk of ADHD in Offspring. *Diabetes Care* 41:2502–2508.

12.3. Metabolic contrasts between youth and adults with impaired glucose tolerance or recently diagnosed type 2 diabetes: i. observations using the hyperglycemic clamp

RISE Consortium

Diabetes Care 2018;41:1696–1706.

doi: [10.2337/dc18-0244](https://doi.org/10.2337/dc18-0244)

URL <http://www.ncbi.nlm.nih.gov/pubmed/29941497>

Summary: In a case control study, age-related differences were compared between youth and adults with impaired glucose tolerance (IGT) or recently diagnosed diabetes. Youth had lower insulin sensitivity, hyperresponsive β -cells and reduced insulin clearance compared with adults.

Comment: This is one of two studies presenting detailed comparisons of glucose metabolism, insulin sensitivity and insulin secretion at baseline in adolescent versus adult cohorts using hyperglycemic clamps and oral glucose tolerance tests¹.

Adolescents with IGT and T2DM differed significantly in their physiological parameters from adults. Specifically, insulin sensitivity was 46% lower in youth than in adults, and youth had higher fasting and stimulated levels of C-peptide than the adults. All beta-cell responses were significantly greater in youth. Interestingly, hepatic insulin clearance was reduced in youth. These results suggest a different pathophysiology in adolescents and adults with abnormal glucose tolerance, and support the notion that puberty augments insulin resistance. In light of the evidence that sustained demand on the β -cell may be an important predictor of progression of β -cell dysfunction, it is logical to assume that this hypersecretion is a critical contributor to the unique pathophysiology of youth-onset T2DM.²

References

1. Consortium R. Metabolic Contrasts Between Youth and Adults With Impaired Glucose Tolerance or Recently Diagnosed Type 2 Diabetes: I. Observations Using the Hyperglycemic Clamp. *Diabetes care*. 2018; **41**(8): 1696–706.
2. Zeitler, P. *World J Pediatr* (2019). <https://doi.org/10.1007/s12519-019-00247-1>

12.4. Impact of insulin and metformin versus metformin alone on beta-cell function in youth with impaired glucose tolerance or recently diagnosed type 2 diabetes

RISE Consortium

Diabetes Care 2018;41:1717–1725.

DOI: [10.2337/dc18-0787](https://doi.org/10.2337/dc18-0787)

<http://www.ncbi.nlm.nih.gov/pubmed/29941500>

Summary: In a randomized, open label clinical trial of 91 adolescents with IGT or new onset T2DM, one-year early interventions with long-acting insulin followed by metformin, or with metformin alone failed to prevent deterioration in beta-cell function.

Comment: Among adults with IGT or recent-onset T2DM, treatment with metformin has previously been reported to improve β -cells function and reduced diabetes progression by 31% over 3 years; and 2 weeks of intensive insulin therapy improved and maintained beta-cell function, resulting in prolonged remission from requiring diabetes medication.

T2DM in adolescents appears to be more aggressive than in adults, therefore interventions to preserve or improve beta-cell function in youth are highly important. The Restoring Insulin Secretion (RISE) Pediatric Medication Study assessed whether initial short-term treatment with insulin glargine for 3 months followed by metformin for 9 months would preserve or improve beta-cell function compared with metformin alone, with a sustained effect after withdrawal of therapy.

No significant differences were observed between treatment groups at baseline, 12 months or 15 months in beta-cell function, BMI percentile, HbA1c, fasting glucose, or oral glucose tolerance test 2-h results. In both treatment groups, clamp-measured beta-cell function was significantly lower at 12 and 15 months versus baseline. BMI was higher in the glargine followed by metformin versus metformin alone group between 3 and 9

months. These findings may indicate a more aggressive disease course for T2DM in younger patients, and highlight the need for alternate approaches to preserve beta-cell function in youth.

12.5. Liraglutide in children and adolescents with type 2 diabetes

Tamborlane WV, Barrientos-Perez M, Fainberg U, Frimer-Larsen H, Hafez M, Hale PM, Jalaludin MY, Kovarenko M, Libman I, Lynch JL, Rao P, Shehadeh N, Turan S, Weghuber D, Barrett T, Ellipse Trial I

N Engl J Med 2019; 10.1056/NEJMoa1903822.

DOI: [10.1056/NEJMoa1903822](https://doi.org/10.1056/NEJMoa1903822)

<http://www.ncbi.nlm.nih.gov/pubmed/31034184>

Summary: In a double-blind, randomized, phase 3 trial, 135 overweight and obese adolescents, aged 10 to 17 years with T2DM, were randomly assigned to receive subcutaneous liraglutide (up to 1.8 mg per day) or placebo, both in addition to metformin treatment. At the 26-week analysis, mean HbA1c decreased by 0.64 percentage points with liraglutide and increased by 0.42 percentage points with the placebo, resulting in a -1.06 percentage point difference ($P < 0.001$); the difference increased to -1.30 percentage points by 52 weeks.

Comment: Despite the evidence that the disease course of T2DM is more aggressive among adolescents than adults, treatment options for adolescents are limited. Liraglutide (marketed as Saxenda® and Victoza®), a human glucagon-like peptide-1 (GLP-1) receptor agonist, was approved one decade ago to treat adults with T2DM with insufficient glycemic control. In addition, the FDA approved liraglutide injection for chronic weight management in individuals with obesity or overweight and a weight-related comorbid condition, based on clinical trials that repeatedly demonstrated the ability of GLP-1 receptor agonists to induce weight loss.¹ Appetite suppression and delayed gastric emptying are thought to be responsible for the weight lowering effects of GLP-1.

In the current study, liraglutide 1.8 mg injection showed superiority to placebo in decreasing HbA1c levels among adolescents; a significantly higher proportion of children and adolescents treated with liraglutide (63.7%) achieved HbA1C $< 7\%$ at week 26 than did those treated with placebo (36.5%). Of note, there was no difference in weight loss between the two adolescent groups, despite the known weight-reducing effects of liraglutide in adults with or without diabetes. This is possibly because only about 50% of the adolescents received the approved dose for weight loss in adults, 3.0 mg/day.

The results of this ELLIPSE trial have been submitted to the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for evaluation of liraglutide as a potential treatment option for children aged 10 years and above with T2DM.

Reference

1. Mehta A, Marso SP, Neeland JJ. Liraglutide for weight management: a critical review of the evidence. *Obes Sci Pract*. 2016; 3(1): 3–14.

Metabolic Syndrome

12.6. Early life adversity with height stunting is associated with cardiometabolic risk in adolescents independent of Body Mass Index

Reid BM, Harbin MM, Arend JL, Kelly AS, Dengel DR, Gunnar MR

J Pediatr 2018;202:143–149.

<https://www.ncbi.nlm.nih.gov/pubmed/30146113>

Summary: In a case control study of 30 post-institutionalized youths compared with 90 age- and BMI percentile-matched youths living in the Minneapolis-Saint Paul metropolitan area, early life stress, as reflected by height-stunted growth in institutional care, was associated with cardiovascular and metabolic risk in youth, even after their moving into well-resourced homes early in life, and in the absence of increased adiposity.

Comment: It is well known that institutionalization in early childhood results in impaired physical growth.¹ This effect seems most pronounced when deprivation coincides with early developmental sensitive periods. Of note, height seems to be more susceptible to the adverse effects of institutional care than weight.² Even in the presence of adequate nutritional provision, psychosocial deprivation may cause inhibition of growth hormone production and cell resistance to growth factors.³ This is usually reversible upon removal from the depriving environment.

In the current study, we learn about the impact of institutionalization within the first 2 years of life, a critical window for children's healthy development, on components of the metabolic syndrome. Children who were adopted from orphanages at a mean age of 18 months, and who had a significant growth-delay at adoption, were evaluated at age 13 years, and compared with age- and BMI percentile-matched youths. Independent of body composition and BMI, post-institutionalized children had evidence of arterial stiffening, higher systolic blood pressure, a higher proportion of trunk tissue fat, and higher levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides, insulin and HOMA-IR scores; and lower values of total lean mass and gynoid lean mass.

References

1. Berens AE, Nelson CA. The science of early adversity: is there a role for large institutions in the care of vulnerable children? *Lancet*. 2015; **386**(9991): 388–98.
2. Martins C, Belsky J, Marques S, Baptista J, Silva J, Mesquita AR, et al. Diverse physical growth trajectories in institutionalized Portuguese children below age 3: relation to child, family, and institutional factors. *Journal of Pediatric Psychology*. 2013; **38**(4): 438–48.
3. Dobrova-Krol NA, van Ijzendoorn MH, Bakermans-Kranenburg MJ, Cyr C, Juffer F. Physical growth delays and stress dysregulation in stunted and non-stunted Ukrainian institution-reared children. *Infant Behavior & Development*. 2008; **31**(3): 539–53.

12.7. Prospective associations between socioeconomically disadvantaged groups and metabolic syndrome risk in European children. Results from the IDEFICS study

Iguacel I, Michels N, Ahrens W, Bammann K, Eiben G, Fernandez-Alvira JM, Marild S, Molnar D, Reisch L, Russo P, Tornaritis M, Veidebaum T, Wolters M, Moreno LA, Bornhorst C, IDEFICS consortium

Int J Cardiol 2018;272:333–340.

<https://www.ncbi.nlm.nih.gov/pubmed/30017513>

Summary: In a multi-center prospective cohort study of 2401 European children, early life exposure to socioeconomic disadvantages, particularly living in low-educated families, having a non-traditional family structure, parental unemployment and the accumulation of > 3 disadvantages were associated with higher risk for the metabolic syndrome (MetS), already during childhood.

Comment: Socioeconomic status (SES) is an important predictor of health and a major determinant for inequalities in health care. Among adults in developed countries, lower SES has been found to be associated with a higher risk of cardiovascular disease. Furthermore, lower SES in childhood showed a residual effect 31 years later, and was associated with clinically significant increased risks for MetS, IFG and T2DM in adulthood.¹ In the current study, the impact of low SES on MetS components was demonstrated already in early childhood.

SES variables including: social network, family structure, parental income, education, employment status and origin, as well as psychosocial factors and lifestyle were assessed in 2401 European children aged 2.0–9.9 from eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden) at baseline and two years later at ages 4.0–11.9.

Education and income were found to act as 'causes-of-causes', and unemployment and non-traditional family structure were associated with a higher metabolic risk in children. Suggested possible mechanisms for the impacts of SES on MetS include living in more deprived neighborhoods with lower availability of fresh products, more fast-food outlets, a greater consumption of cheaper but calorie-dense foods, physical inactivity due to few recreational opportunities, limited knowledge regarding healthy lifestyle, and limited accessibility to health services. Another possible underlying mechanism is the stress-mediated pathway. Lower SES has been shown to be associated with greater stress hormone levels, catecholamines and cortisol.

Poverty and poor health worldwide are inextricably linked. It is crucial to tackle the root causes of poor health and address the factors that sustain the cycle of poor health, such as lack of education and poor nutrition.

Reference

1. Puolukka E, Pahkala K, Laitinen TT, Magnussen CG, Hutri-Kähönen N, Tossavainen P, *et al.* Childhood Socioeconomic Status in Predicting Metabolic Syndrome and Glucose Abnormalities in Adulthood: The Cardiovascular Risk in Young Finns Study. *Diabetes care.* 2016; **39**(12): 2311–7.

12.8. Carbotoxicity-noxious effects of carbohydrates

Kroemer G, Lopez-Otin C, Madeo F, de Cabo R

Cell 2018;175:605–614.

doi: [10.1016/j.cell.2018.07.044](https://doi.org/10.1016/j.cell.2018.07.044).

<http://www.ncbi.nlm.nih.gov/pubmed/30340032>

Summary: Epidemiological, clinical intervention trials and experimental evidence indicate the negative impact of excessive carbohydrate uptake. This review details the history of carbohydrate consumption and mechanisms of carbotoxicity, as well as the beneficial effects of reducing carbohydrates in the diet.

Comment: After we adopted the idea of lipotoxicity and glucotoxicity, a new term is coined, “carbotoxicity”. It refers to toxicity secondary to the excessive intake of different types of carbohydrates: glucose, fructose and mannose.

The history of dietary carbohydrates includes three major, transformative steps. The first was the transition from hunter-gatherers to agriculture, which shifted carbohydrate intake from fruits, seeds, nuts and roots, to a range of cereals (in Europe), rice (in Asia), corn (in Mesoamerica) and potatoes (in South America). After this transition, carbohydrate intake was estimated as contributing 15–30% of total energy intake. The second major change in carbohydrate intake occurred in the 19th and 20th centuries, and is marked by the mass production and consumption of refined sugars, which changed the eating habits of Europeans as they started consuming jams, candy and processed foods. The third and most significant surge in carbohydrate intake was after World War II, and is linked to the rising ingestion of ultra-processed food items, sodas and high-fructose corn syrup; consequently, carbohydrate intake reached 45%–65% of total energy intake.

In contrast to beliefs that have been held for decades, the authors state that digestible carbohydrates are more toxic than lipids, since high carbohydrate intake is associated with increased risk of total mortality. The molecular, cellular and neuroendocrine mechanisms of carbotoxicity are described, specifically the impact of advanced glycation end-products, high dose fructose and uric acid.

Several types of diet that curb carbohydrate intake, and thus combat carbotoxicity, are reviewed. These include the low-carb diet and the ketogenic diet, in which carbohydrates comprise <20% and 5% of calorie intake, respectively. In addition, the impact of several pharmacological strategies for reducing carbotoxicity are suggested, such as acarbose, SGLT2 inhibitors and metformin. This review is of particular interest in light of the following article (see 12.9).

12.9. Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis

Seidelmann SB, Claggett B, Cheng S, Henglin M, Shah A, Steffen LM, Folsom AR, Rimm EB, Willett WC, Solomon SD

Lancet Public Health 2018;3:e419–e428.

DOI [10.1016/S2468-2667\(18\)30135-X](https://doi.org/10.1016/S2468-2667(18)30135-X)

URL: <http://www.ncbi.nlm.nih.gov/pubmed/30122560>

Summary: The long-term effects on health outcomes of dietary carbohydrate intake was investigated in a large prospective cohort of 15,428 US adults aged 45–64 years with 25 years follow-up. Both low carbohydrate consumption (<40%) and high carbohydrate consumption (>70%) conferred greater mortality risk, with minimal risk observed at 50–55% carbohydrate intake. Of note, mortality increased when carbohydrates were exchanged for animal-derived fat or protein, and mortality decreased when the substitutions were plant-based.

Comment: It appears that the very basic and seemingly simple thing in life, to eat, has become complicated and controversial. Fierce debate about the right way to eat pervades in social networks, with diverse opinions. Professional recommendations from the fields of *medicine and nutrition* have shifted from one extreme to another – *low fat – low carb – low protein* –diets have all been claimed as means of improving our health and losing weight. After many years during which fats were considered the number one enemy, the current fad is to cut back on carbohydrates.

The association of the percentage of energy intake from carbohydrates with all-cause mortality was assessed in 15,428 adults aged 45–64 years, based on a dietary questionnaire administered at two intervals, spanning a 6-year period. In addition, data from seven multinational prospective studies including 432,179 participants were analyzed.

A U-shaped relationship was observed between carbohydrate intake and mortality. Low carbohydrate diets, which generally result in lower intake of vegetables, fruits and grains, and increased intake of protein from animal sources, have been hypothesized to stimulate inflammatory pathways, biological ageing and oxidative stress. The substitution of animal compared with plant sources of fat and protein was found to increase the risk of mortality. On the other end of the spectrum, high carbohydrate diets confer a chronically high glycemic load that can lead to negative metabolic consequences.

The science behind fad diets is extremely important, and as concluded here, the anti-carb fad should be viewed, as any extreme fad – i.e. with caution.

12.10. Exogenous testosterone does not induce or exacerbate the metabolic features associated with PCOS among transgender men

Chan KJ, Liang JJ, Jolly D, Weinand JD, Safer JD

Endocr Pract 2018;24:565-572.

<http://www.ncbi.nlm.nih.gov/pubmed/29624102>

Summary: According to this retrospective chart review of 34 transgender men, testosterone therapy in this population, across a wide range of doses and over many years, did not result in the profile of dyslipidemia and dysglycemia that is observed in women with polycystic ovary syndrome (PCOS). Instead, treatment of transgender men with testosterone resulted in a shift of metabolic biomarkers toward the average physiological male.

Comment: PCOS in women is characterized by menstrual irregularity, metabolic derangement and increased androgen levels. It was suggested that the androgen excess aggravates cardiovascular and metabolic aberrations in these women.

In the current study, transgender men who received cross-sex hormone therapy with testosterone for 6 years showed no significant changes in levels of HbA1c, triglycerides and low-density-lipoprotein cholesterol. With increasing testosterone levels, BMI and HDL levels decreased significantly. Blood pressure was not assessed.

The findings of the current study have two important implications; firstly, treatment of transgender men with cross-sex hormone therapy is not associated with worsening of cardiovascular risk factors. Secondly, the findings might be extrapolated to imply that hyperandrogenism does not explain the metabolic derangements in PCOS.

Lipid Metabolism

12.11. Aster proteins facilitate nonvesicular plasma membrane to ER cholesterol transport in mammalian cells

Sandhu J, Li S, Fairall L, Pfisterer SG, Gurnett JE, Xiao X et al.

Cell. 2018; **175**(2): 514–29 e20.

URL: <http://www.ncbi.nlm.nih.gov/pubmed/30220461>

Summary: The enigma of how cholesterol is transported within cells has been solved in these experimental mouse models and structural imaging studies by the discovery of the proteins Aster A, B and C, which serve

as a molecular bridge for the transfer of cholesterol from the plasma membrane (PM) to the endoplasmic reticulum (ER).

Comment: Newly synthesized cholesterol is transported from the ER to the PM; this occurs via nonvesicular pathways by several lipid transfer proteins. In parallel, cholesterol that is obtained from extracellular sources is transported back from the PM to the ER, where it is esterified or converted to oxysterols, bile acids and steroid hormones.¹ However, until now it was not known how cholesterol is transported from the PM to the ER. Three proteins were discovered, named Aster-A, -B and -C (Greek for 'star'), which have a role in cholesterol trafficking. These proteins have remarkable similarity to the sterol-binding START (steroidogenic acute regulatory protein domain, which regulates cholesterol transfer within the mitochondria) but have higher affinity.

In a series of elegant studies, the authors determined the crystal structure of the Aster proteins, characterized the mode of their binding, and then determined their functional importance in cholesterol trafficking and homeostasis. First, they showed that Aster proteins are recruited in response to cholesterol levels. In standard lipid-poor conditions, the Aster proteins are located throughout the ER. Upon addition of cholesterol to the media, the Aster proteins quickly redistribute to ER tubules and form bridges from the PM to the ER, and the sterol-binding ASTER domain extracts cholesterol from the PM and moves it down the concentration gradient to the ER.

The authors subsequently showed that antisense oligonucleotide against Aster-A causes a delayed response to exogenous cholesterol, as demonstrated by higher levels of SREBP targets, and reduced cholesterol ester synthesis, both of which can be explained by a reduction in cholesterol transport from the PM to the ER. Finally, Aster-B knockout mice by CRISPR/Cas9 editing were established. Aster-B is the only Aster protein expressed in the adrenal gland, a tissue that relies on uptake of HDL cholesterol to generate steroid hormones and cholesterol esters. Remarkably, in the adrenal glands of the knockout mice, levels of the cholesterol esters were dramatically reduced and lipid droplets were completely lacking. Serum corticosterone levels were also reduced.

Future studies should investigate the importance of Aster proteins in diseases associated with alterations in cholesterol metabolism, including dyslipidemia, atherosclerosis, neurodegeneration and sexual development.²

References

1. Rapoport T and Sever N: In F1000Prime, 22 Oct 2018; [10.3410/f.734007092.793551775](https://doi.org/10.3410/f.734007092.793551775).
2. Kutyavin VI, Chawla A. Aster: A New Star in Cholesterol Trafficking. *Cell*. 2018; **175**(2): 307–9.

12.12. Orlistat therapy for children with type 1 hyperlipoproteinemia: a randomized clinical trial

Patni N, Quittner C, Garg A

Journal of Clinical Endocrinology and Metabolism. 2018; 103(6): 2403–7.

URL: <http://www.ncbi.nlm.nih.gov/pubmed/29659879>

Summary: Orlistat therapy reduced serum triglycerides by 50–60% in two children with Type 1 hyperlipoproteinemia (T1HLP) in a randomized, open-label, crossover trial with four periods and two sequences. The treatment was safe and is suggested as first-line therapy in conjunction with an extremely low-fat diet and fat-soluble vitamin supplementation.

Comment: T1HLP, also known as familial chylomicronemia syndrome, is a rare, autosomal recessive condition characterized by extreme hypertriglyceridemia due to a deficiency in the enzyme lipoprotein lipase (LPL) or other proteins necessary for proper LPL function, such as LMF1, APOC2, APOA5 and GPIHBP1. Triglyceride (TG) levels may range from 1,500 to 15,000 mg/dl, and result in eruptive or tuberous xanthomas, recurrent pancreatitis, lipemia retinalis and hepatosplenomegaly. In 2012, an adeno-associated virus gene therapy (alipogene tiparvovec) was the first gene therapy approved in Europe for LPL deficiency. This treatment is administered by multiple intramuscular injections (>40) in the legs given at a single visit, under spinal anesthesia. As patients developed antibodies to the capsid proteins, the lowering effect of TGs was transient and this treatment was withdrawn from the market in 2017. The only currently available effective therapy is an extremely low-fat diet with 10% to 15% of the total energy as fat.

Orlistat is a gastric and pancreatic lipase inhibitor that can lower dietary fat absorption by 30%, and therefore, may reduce serum TG levels in patients with T1HLP by decreasing the substrate available for chylomicron

formation. Two children participated in an alternating, repeated crossover trial with four periods and two sequences ('orlistat' and 'off' for 3 months each). Orlistat therapy reduced mean fasting serum TGs by 50%, without any serious adverse effects.

Longer-term clinical trials with a larger number of patients are warranted to determine the efficacy and safety of orlistat with prolonged use in patients with T1HLP for preventing acute pancreatitis

12.13. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer

Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, Gibson H, Albert CM, Gordon D, Copeland T, D'Agostino D, Friedenberg G, Ridge C, Bubes V, Giovannucci EL, Willett WC, Buring JE, VITAL Research Group

N Engl J Med 2019;380:23-32.

<https://www.ncbi.nlm.nih.gov/pubmed/30415637>

Summary: In this randomized primary prevention placebo-controlled trial of 25,871 US adults, with a two-by-two factorial design, supplements with omega-3 did not lower the overall incidence of adverse cardiovascular events or cancer compared to a placebo.

Comment: Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs). Three types of omega-3 fatty acids are involved in human physiology: α -linolenic acid (ALA), found in plant oils; and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both commonly found in marine oils. Mammals are unable to synthesize the essential omega-3 fatty acid ALA and must obtain it through diet; they can then use ALA to form the long-chain omega-3 fatty acids, EPA and then from EPA make DHA. Omega-3 fatty acids play important roles in the body as components of the phospholipids that form the structures of cell membranes. DHA, in particular, is especially high in the retina, brain and sperm. EPA acts as a precursor for prostaglandin-3 which inhibits platelet aggregation,

Laboratory and animal studies suggest that omega-3 fatty acids have antithrombotic, hypotriglyceridemic, blood-pressure-lowering and antiinflammatory effects; and thus may impede growth of atherosclerotic plaques, slow heart rate and reduce susceptibility to cardiac arrhythmias. It is no wonder, therefore, that 7.8% of U.S. adults (18.8 million) consume fish oil/omega-3/DHA, EPA fatty acids supplements.

The current study examined effects of such supplements among 26,000 adults (mean age, 67 years) with no history of cardiovascular disease, cancer or other serious disorders who received either daily fish-oil capsules (containing 460 mg of eicosapentaenoic acid and 380 mg of docosahexaenoic acid) or placebo. Supplementation with n-3 fatty acids did not result in a lower incidence of major cardiovascular events or cancer than placebo. There still may benefit to n-3 supplementation than with placebo among participants with low fish consumption.

12.14. An integrative systems genetic analysis of mammalian lipid metabolism

Parker BL, Calkin AC, Seldin MM, Keating MF, Tarling EJ, Yang P, Moody SC, Liu Y, Zerenturk EJ, Needham EJ, Miller ML, Clifford BL, Morand P, Watt MJ, Meex RCR, Peng KY, Lee R, Jayawardana K, Pan C, Mellett NA, Weir JM, Lazarus R, Lusis AJ, Meikle PJ, James DE, de Aguiar Vallim TQ, Drew BG

Nature 2019;567:187-193.

URL <https://doi.org/10.1038/s41586-019-0984-y>

Summary: Novel diagnostic and prognostic biomarkers in the blood that can predict early fatty liver disease were identified, using a proteomic and lipidomic-wide systems genetic approach in 107 genetically distinct mouse strains. In addition, the novel lipid-regulatory protein, PSMD9, was identified as a regulator of lipid metabolism, with potential therapeutic implications.

Comment: Although nonalcoholic fatty liver disease (NAFLD) is not one of the defining criteria for the MetS, it is a common manifestation and a risk factor for T2DM and cardiovascular disease, liver cancer and liver failure. However, fatty liver disease usually has no early symptoms and diagnosis is often late.¹ To predict individuals at a higher genetic risk of developing hepatic lipotoxicity, Genetic Reference Panels (GRP) techniques were used; these enable differentiating the impact of genetics and the environment on complex traits. Allowing accurate control of the environment, as well as access to critical metabolic tissues, GRPs of 107 inbred mouse strains were engaged, and lipidomics and proteomics were performed. Several lipids that were significantly correlated

between the liver and plasma were identified. This discovery might lead to a blood test to avoid invasive biopsy or surgery, to determine persons at increased risk of advanced fatty liver disease.

In addition, correlations between the lipidomic and proteomic datasets identified proteins associated with the proteasome and proteolysis that were strongly correlated with hepatic lipid abundance. A proteosomal chaperone protein, non-ATPase regulatory subunit 9 (PSMD9), strongly correlated with multiple hepatic and plasma lipid species.

Silencing of PSMD9, by using antisense oligonucleotides, reduced plasma lipids and prevented hepatic steatosis. Overexpression of hepatic PSMD9 using adenovirus promoted lipid accrual. The identification of PSMD9 as a regulator of lipid metabolism has potential therapeutic implications for new drug targets.

Reference

1. Moore JB, Thorne JL. Predicting and reducing hepatic lipotoxicity in non-alcoholic fatty liver disease. *Lab Animal*. 2019; **48**(5): 143-4.

Most Amusing Article of the Year

12.15. Is it time to start using the emoji in biomedical literature?

O'Reilly-Shah VN, Lynde GC, Jabaley CS

BMJ 2018;363:k5033.

doi:10.1136/bmj.k5033

Don't skip this article!

Lack of time, *workload volume*, the *huge amounts of medical data*..... who has time to read all the information and medical literature?

The authors introduce and discuss the pros and cons of using emoji in biomedical literature “*allowing for vast swaths of the human experience to be communicated by a single character*”.

The advantage of using emoji is that they can add inflection and subtext in a manner not previously possible, for example instead of “regrettably, your submission did not receive a high enough priority rating to warrant its publication.” just use



Take a look at their suggestions to use emoji as an alternative to denotation of statistical significance, and don't miss how the conclusions are expressed in emoji(s)! url: sci-hub.tw/10.1136/bmj.k5033.

13. Global Health for the Paediatric Endocrinologist

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Preface

Welcome to the 4th edition of this section on Global Health in Pediatric Endocrinology and Diabetes. We again found a vast array of articles that are relevant to the ambitious 2030 Agenda for Sustainable Development adopted in 2015 by all United Nations member states. Pediatric Endocrinology specifically fits with Sustainable Development Goal 3: "Ensure healthy lives and promote well-being for all at all ages", a Goal that focuses on non-communicable diseases (NCDs).

As we are nearing the 100th anniversary of the discovery of insulin by Frederick Banting (and Charles Best) and John MacLeod (and James Collip), articles on diabetes highlight directly or indirectly the need for better access to affordable insulin. While all aspects of pediatric endocrinology are discussed in the 2018-2019 literature, a number of articles focus on disorders of sexual development and on the relationship between nutrition and stunting.

Diabetes

13.1. Effect of Novartis Access on availability and price of non-communicable disease medicines in Kenya: a cluster-randomised controlled trial

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Lancet Glob Health 2019;7: e492–502. DOI: [10.1016/S2214-109X\(18\)30563-1](https://doi.org/10.1016/S2214-109X(18)30563-1)

- The authors evaluated the effect of a Novartis programme that provides metformin at a wholesale price of US\$1 per month in Kenya.
- This cluster-randomized controlled trial significantly increased the availability of metformin at health facilities, but not at patient households.

The pharmaceutical industry is often blamed for excessive prices of medicines that prevent the people most in need to access them. This interesting trial (funded by Novartis, a manufacturer of metformin) reports the outcomes of the Novartis-Access program, an initiative designed to make essential medicines available at an affordable price. A portfolio of 14 medicines, including metformin, that are prescribed for treatment of non-communicable diseases (NCDs) such as hypertension, heart failure, dyslipidaemia, type 2 diabetes, asthma and breast cancer, was made available for purchase by a main distributor to public and non-profit health facilities in Kenya, at a wholesale price of US\$1 per treatment per month. Patients with known diabetes treated with metformin could in turn buy the drug at this low price. The results of this Novartis-Access program show that the initiative did increase availability of metformin at the health facility level but not at the patient level.

The reasons for these somewhat disappointing results are discussed in depth by the authors. These included lack of awareness of the program, relatively short duration of the trial (although a longer-term evaluation is planned), the fact that patients had to be previously diagnosed, and delayed registration of the medicines by the Kenyan

Health Authorities. However, industry-led access-to-medicines programmes are one of several important avenues that can be pursued to improve affordable access to essential medicines and outcome evaluation of these initiatives is therefore a key step. Other sustainable avenues include for instance pooled procurement (whereby a large quantity of medicines is bought at a lower price and distributed between several countries), local manufacturing of drugs and easier approval of medicines in order to increase competition between manufacturers and to decrease drug prices (1).

Reference

1. Manji I, Pastakia SD. Novartis Access: a small step towards increased access for non-communicable disease care. *Lancet Glob Health* 2019;7: e398.

13.2. Challenges associated with providing diabetes care in humanitarian settings

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- Practical challenges associated with diabetes care in humanitarian contexts in low- and middle- income countries abound.
- In this position paper, the authors articulate the important needs around diabetes care in the context of the 6 building blocks proposed in the WHO Health Systems Framework (1).

Three articles included in this chapter focus on access to insulin, metformin and blood glucose monitoring tools. Managing diabetes in a humanitarian setting markedly differs from care provided in otherwise stable low-resource settings. With more than 400 million people suffering from (mainly Type 2) diabetes, it is not surprising that diabetes, an NCD that has become a priority on the United Nations agenda, is a common cause of health consultations in refugee camps. This important article highlights several practical issues that go well beyond access to insulin and glucose strips. It makes us reflect on specific needs in emergency, high-risk situations and how to address them.

Key questions include: How to integrate diabetes care in general primary healthcare where expertise is usually unavailable? How to ensure self-management in conditions of food insecurity? How to store insulin and other temperature-sensitive medicines when electricity may be discontinued without warning? How to ensure treatment continuity when the unstable political situation may result in medication interruption (lessons learned from experience in HIV and tuberculosis treatment include the use of buffer stocks and runaway packs to help minimise interruption to medication)? Where to find protocols adapted to these particular conditions (Médecins Sans Frontières has developed guidelines to this effect (2)).

References

1. Monitoring the building blocks of health systems: a handbook of indicators and their measurement strategies. World Health Organization 2010. https://www.who.int/healthinfo/systems/WHO_MBHSS_2010_full_web.pdf (accessed June 28, 2019).
2. Médecins Sans Frontières. Non-communicable diseases: programmatic and clinical guidelines, version 3. 2018. <https://fieldresearch.msf.org/handle/10144/619084> (accessed June 28, 2019).

13.3. Levels of type 1 diabetes care in children and adolescents for countries at varying resource levels

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- The quality and availability of pediatric diabetes management varies from setting to setting depending on the available resources.
- In this position paper, the authors propose a ‘levels of care’ concept with three tiers that stratifies the existing levels of care into minimal care, intermediate care, and comprehensive care.

This article acknowledges the reality: in many low- and middle-income countries (LMICs), diabetes care is suboptimal and is associated with high mortality and morbidity. The authors propose a “Levels of Care” framework for T1D care that can guide health authorities to focus their efforts on appropriate objectives for better diabetes care. This article builds on previous work by the first author who developed a standardized, reproducible Child Program Index of diabetes care measure that can be used to assess critical factors influencing diabetes treatment outcomes (1). Indeed, while we are all familiar with the “gold standard” approach as described in the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines, we do not always appreciate the “next step” approach that will lead to an improvement in diabetes care.

The authors propose nine levels of diabetes care, from a “Minimal care” (characterized by poor insulin access, poor education and uncommon blood glucose testing and associated with elevated HbA1c values and high mortality) to “Intermediate care” (characterized by appropriate access to human insulin and blood glucose strips and associated with low incidence of complications) and “Comprehensive care” (characterized by access to insulin analogs or insulin pumps, multidisciplinary diabetes team and optimal blood glucose monitoring and associated with the lowest HbA1c and prevalence of long term complications). The association between the various components of diabetes care (insulin access, blood glucose monitoring, HbA1c measures, complications screening and quality of diabetes education) and the clinical outcomes (mean HbA1c and prevalence of mortality and complications) at each level of care will serve as a guide for health authorities wishing to implement additional actions for diabetes management and encourage them to reach the next level of care.

Reference

1. Ogle GD, Middlehurst AC, Silink M. The IDF Life for a Child Program Index of diabetes care for children and youth. *Pediatr Diabetes*. 2016 Aug;17(5):374–84.

13.4. Why are we failing to address the issue of access to insulin? A national and global perspective

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Diabetes Care 2018;41:1125–1131. DOI: [10.2337/dc17-2123](https://doi.org/10.2337/dc17-2123)

(Erratum in *Diabetes Care* 2018; 41: 2048. DOI: [10.2337/dc18-er09a](https://doi.org/10.2337/dc18-er09a))

- Many people currently in need of insulin are unable to access it.
- Unaffordable price of insulin is a major barrier.
- These authors analyse the causes of unsatisfactory access to insulin from a U.S. and global perspective.

This article identifies the various components of a global framework that regulates insulin affordability: the private sector, the government and a plural sector that includes NGOs, academia, research organisations and patient groups. Presently, the private sector has, by far, the greatest influence on insulin cost. One of the reasons

is that 99% of the value and 96% of the volume of the insulin market are controlled by only 3 pharmaceutical companies (Novo Nordisk, Eli Lilly and Sanofi), which contributes to keeping insulin prices high.

The article discusses 2 important points. First, the emergence of biosimilar insulins, which would be expected to increase competition and consequently improve affordability (1). However, those benefits remain to be seen. It is true that an increasing number of smaller pharmaceutical companies are now manufacturing biosimilar insulins (defined as human or analog insulins that are almost identical to a reference product or comparator). However, with a few exceptions, biosimilar insulins have not been successfully evaluated according to the stringent criteria of agencies such as the FDA (USA), PMDA (Japan) or the EMA (Europe). The WHO is presently working on a prequalification process that could contribute to increasing penetration of insulin in more countries. Secondly, they discuss whether the use of analog insulins, which remain more expensive than human insulins, should be promoted in low resource settings. Indeed, there is a concern that high use of analog insulins could contribute to keeping the overall cost of insulin high. The authors feel that the marginal improvement in metabolic control offered by insulin analogs does not justify their higher price. Whether other potential advantages of analog insulins compared to human insulin (such as convenience and decreased risk of hypoglycemia in settings where glucagon is mostly unavailable) is worth the high price remains to be evaluated (2–3).

As health professionals, our primary goal should be to ensure that patients get universal access to at least human insulin.

References

1. McCall C. Biosimilars for insulin: a cost-saving alternative? *Lancet* 2018 392; 463-464.
2. Beran D, Hemmingsen B, Yudkin JS. Analogue insulin as an essential medicine: the need for more evidence and lower prices. *Lancet Diabetes Endocrinol* 2018; 7: 338.
3. Beran D, Laing RO, Kaplan W, Knox R, Sharma A, Wirtz VJ, Frye J, Ewen M. A perspective on global access to insulin: a descriptive study of the market, trade flows and prices. *Diabet Med* 2019; 1–8.

13.5. Blood glucose meters and test strips: Global market and challenges to access in low-resource settings

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Lancet Diabetes Endocrinol 2019; 7: 150–60. DOI [10.1016/S2213-8587\(18\)30074-3](https://doi.org/10.1016/S2213-8587(18)30074-3)

- Access to blood glucose monitoring is often poor in resource-limited settings.
- The authors analyse the reasons for suboptimal access, with a focus on cost, availability, system accuracy, competitive bidding, technological trends, and non-financial barriers. Urine glucose monitoring is an alternative where there are cost considerations.

Emphasis has been placed mostly on insulin access for the management of Type 1 diabetes in children. However, blood glucose monitoring is a key component of diabetes management and is typically very expensive in resource-limited settings. Looking at 15 low- and middle-income countries, these authors found that the median cost of 2 glucose strips was 1.00 USD, which is more than twice the median cost of daily insulin needs, making blood glucose monitoring unaffordable for the most patients.

This was acknowledged in the 2018 edition of the ISPAD Clinical Practice Guidelines, which now includes a section for diabetes management in resource-limited settings. The guidelines state that “glucose monitoring is very expensive. We recognize that in many countries the cost of these assessments relative to the cost of living may make this technology unavailable... All centers caring for young people with diabetes should urge nations, states, and health care providers to ensure that children and adolescents with diabetes have adequate glucose monitoring supplies” (1).

In 2017, the World Health Organization (WHO) released the first edition of the “Model List of Essential In Vitro Diagnostics” (“essential diagnostics list”, EDL), which is intended “to provide evidence-based guidance to

countries for creating their own lists of essential *in vitro* diagnostic tests” (2). Blood glucose (determined by glucometer) was included in this original list to “diagnose and screen for diabetes and intermediate hyperglycaemia, to diagnose hypoglycaemia”. Although the concept of *ongoing* blood glucose monitoring is not clearly spelled out, this is an important first step that highlights for national health authorities the importance of blood glucose determination by glucometer.

The solutions proposed by the authors are similar to those proposed to improve access to insulin. These include preferential pricing for countries with limited resources, regional pooled procurement programs (whereby a larger quantity of strips are bought at a lower price and distributed between countries), and a World Health Organization (WHO) prequalification scheme, whereby affordable meter and strip systems undergo quality assessment procedures with the aim of increasing market competition and, as a consequence, decrease the price of strips.

References

1. Codner E, Acerini CL, Craig ME, Hofer SE, Maahs DM. ISPAD Clinical Practice Consensus Guidelines 2018: Limited Care Guidance Appendix. *Pediatr Diabetes* 2018; 19 (Suppl. 27): 328–338.
2. First WHO Model List of Essential In Vitro Diagnostics. Geneva: World Health Organization; 2019 (WHO Technical Report Series, No. 1017). Available at: https://www.who.int/medical_devices/diagnostics/WHO_EDL_2018.pdf (Accessed June 26, 2019).

Endocrinology: Newborn Screening

13.6. Newborn screening in the developing countries

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Curr Opin Pediatr 2018, 30:734–739. DOI: [10.1097/MOP.0000000000000683](https://doi.org/10.1097/MOP.0000000000000683)

- Congenital hypothyroidism is the most cost-effective screened condition.
- Screening for hemoglobinopathies and glucose-6-dehydrogenase deficiency can be cost-effective in sub-Saharan Africa, India and some parts of Asia where there is a high incidence of these diseases.
- Screening for metabolic conditions should be considered in areas of high consanguinity.
- Review article.

This article provides an overview of the various developing newborn screening (NBS) programs around the world. Slow progress is noted in most continents, with low priority given by health authorities for the funding of nationwide programs in low- and middle-income countries. On the positive side, an increasing number of programs are looking for synergies between various diseases to be screened for. At the present time, the NBS programs are being developed according to protocols used in high-income countries. However, specific issues in low- and middle-income countries such as a high percentage of home births, the absence of reliable transportation of the samples and the lack of reference laboratories make the development of point of care testing (at the bedside) techniques desirable. This research is ongoing (including for congenital hypothyroidism) but is not yet available.

13.7. A pilot study on newborn screening for congenital adrenal hyperplasia in Beijing

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- 44,360 neonates were screened for CAH as part of a pilot screening programme in Beijing.
- In this prospective study, a CAH incidence of 1:7393 was found, and the most common 21 OHase mutation was c.293-13C/A > G.

The authors describe the results of a pilot neonatal screening program for congenital adrenal hyperplasia (CAH) in Beijing. Six neonates with CAH were identified (five of them with severe salt wasting), corresponding to an incidence of 1:7393. Although the authors state that this incidence is higher than the national average, this estimate should be taken with caution as the number of patients enrolled in the study was relatively small (n=44,360), meaning that the 95% confidence interval of the incidence is very large (1:3450 to 1:33,300). This is a reminder that studies assessing incidence for a relatively uncommon disease need to be appropriately powered.

The authors acknowledge two weaknesses of the screening process that illustrate the practical difficulties of developing a newborn screening program for CAH in a country where many families live far from hospitals. First, more than 25% of the neonates with an elevated 17OHP could not be contacted for follow up, meaning that their incidence of CAH may be underestimated (although the majority of those who could not be recalled had relatively lower 17OHP values). Second, the neonates with a positive screening test could be seen in clinic for retesting only within 13 to 83 days after the screening test. As a consequence, all neonates with salt wasting CAH had already presented with severe hyponatremia and hyperkalemia at the time of diagnosis. The authors of this important work are already working on an improved screening process with a shorter turnaround time, involving faster transportation and processing of the samples.

Endocrinology: Disorders of Sexual Development

13.8. Malaysian females with congenital adrenal hyperplasia: surgical outcomes and attitudes

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- The outcomes of feminizing genitoplasty of 46,XX individuals with CAH were reviewed in this cross-sectional study.
- The study highlights the importance of cultural sensitivities, access to medical treatment and timing of the diagnosis on attitudes toward feminizing genitoplasty in Malaysia.

The authors should be commended for offering an open-minded analysis of genitoplasty outcomes in a large number (n=59) of female patients with congenital adrenal hyperplasia (CAH) in Malaysia and for discussing their findings in the context of the Malaysian society and culture. We found the comparison of the differences in “shyness/embarrassment” (in the context of decision-making and perception around DSD) between Malay and Western societies highly interesting. In the Malay society, such shyness is called “Malu” and is considered as a

demonstration of respect towards the elders and the elite. It does not have the negative connotation as found in Western societies.

In 3/4 of the cases, genitoplasty was performed by a surgeon trained in DSD repair. Overall, and this is similar to other reports, there was a wide range of outcomes in terms of satisfaction with the cosmetic results (42% were considered as poor) and preferred age for the genitoplasty (half of the parents prefer to have it performed early in life). Interestingly, the authors reported that little attention was given to clitoris preservation until 2006, but, on the positive side, that this has now become an integral part of surgery. Among the 18 participants who were older than 18 years, the authors reported that only one participant was married and only two were sexually active. This very low number is consistent with other reports of poor social outcomes among female CAH patients. However, this has to be interpreted in the context of a progressively older age of marriage among Malaysian women (25.7 years in 2010) (1). Finally, the patient population investigated in this study is quite young and it is very important to obtain long-term follow up data on fertility, sexual satisfaction and overall quality of life.

Reference

1. Malaymail, April 9 2017. Marrying young? Poll shows women consider financial stability, men think sex. www.malaymail.com/news/malaysia/2017/04/09/marrying-young-poll-shows-women-consider-financial-stability-men-think-sex/1352621 (Accessed June 27, 2019).

13.9. Incidence of disorders of sexual development in neonates in Ghana: prospective study

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- Prospective cohort study of 9255 neonates at a tertiary care center in Ghana to determine the incidence of disorders of sexual development (DSD) using physical examination, ultrasound and 17-hydroxyprogesterone measurements.
- The estimated incidence of a DSD was 28/10,000 live births. Congenital adrenal hyperplasia (CAH) was most common and showed poor survival (3 of 4 identified children died).

Rare endocrine conditions such as DSDs are largely underdiagnosed in settings where the majority of women deliver at home, routine newborn exams are not performed, and health care professionals with expertise in DSDs are not available. As a result, epidemiologic information on DSDs in many low- and middle-income countries is scarce to unavailable, while excess morbidity and premature mortality (such as from CAH) prevail.

This study from Ghana is the first to evaluate the incidence of DSDs in newborns in a sub-Saharan African country. Its relatively large sample size allowed for an informative incidence estimate that suggests DSDs may be relatively frequent in sub-Saharan Africa. Beyond such epidemiologic data, the study teaches several important lessons. First, examination of the newborn genitalia is paramount for the ascertainment of DSDs, and this task can be given to lay health workers if properly trained. Next, a history of consanguinity, also obtainable by lay health workers, should increase the level of alertness for congenital adrenal hyperplasia. Further, when pediatric endocrinologists are available in-country, a clinical examination combined with relatively simple diagnostic tools such as a 17-hydroxyprogesterone, electrolytes, and pelvic ultrasound can provide most patients with a diagnosis (1). Lastly, the study highlights the limitations of care for patients with DSDs in low-resource settings: Lack of access to more sophisticated diagnostic tools such as a karyotype and genetic testing limits diagnostic certainty for a proportion of patients, and lack of essential medicines such as hydrocortisone and fludrocortisone (2) likely underlies the excess mortality in patients with CAH.

As capacity for pediatric endocrinology increases in low- and middle-income countries, more studies such as this one will provide valuable information on global epidemiology and setting-adapted care delivery for patients with DSDs.

References

1. Odundo GO, Ngwiri T, Otuoma O, *et al.* The Impact and Successes of a Paediatric Endocrinology Fellowship Program in Africa. *Int J Endocrinol* 2016;2016:1–6.
2. Rowlands A, Ameyaw E, Rutagarama F, *et al.* Insights from the WHO and National Lists of Essential Medicines: Focus on Pediatric Diabetes Care in Africa. *Horm Res Paediatr* 2018;90:82–92.

13.10. Women with amenorrhea and men with menstruation: the qualitative experiences of people with disorders of sex development in Nigeria

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- This qualitative study evaluated the physical and emotional experiences of 13 adults living with disorders of sexual development (DSD) at a tertiary care center in Nigeria.
- Diagnosis of DSD is frequently delayed in LMIC resulting in development of ambiguous physical traits and features.

This is one of the first studies to report on patients' experiences of living with a DSD in a West African country where diagnoses are frequently delayed until pubertal development or sexual ambiguity becomes obvious in late adolescence or adulthood, and where a male gender assignment in patients with a 46 XX DSD may be more common. Participants in this study had CAH (n=6, 3 raised as female, 3 raised as male), androgen insensitivity syndrome (n=3, all raised female), ovo-testicular DSD (n=2, both raised male), Mayer-Rokitansky-Kuster-Hauser syndrome (n=1, raised female) and Turner syndrome (n=1, raised female).

In this setting where sex education is not routine and lay knowledge about normal female and male puberty is limited, menses is seen as a tell-tale sign of being a “real” or “normal” woman and emerged as the central theme for both female and male participants. In females, the absence of menstruation was mostly experienced as a disappointment and elicited feelings of incompleteness. Women associated amenorrhea to a lack of fertility and, as such, a lack of the ability to function as a woman in society. The six males seemed to fare worse than the females in that onset of menses elicited fear, anxiety, depression, and suicidal ideations. This was linked to perceptions of stigma and fear about social labelling and their fate in society. Positive coping in a minority of women resulted from beliefs, such as God having a special plan for them, or women with amenorrhea being “special”, and likened to women in paradise who do not need to menstruate.

The study highlights the high level of distress and stigma experienced by patients with DSDs in Nigeria, a finding that is likely to translate to many other settings. Further research is needed to determine whether increased awareness of DSDs, earlier diagnosis, and improved psychosocial support of patients and families can improve physical, mental and social health outcomes in this population.

13.11. Outcome of feminizing genital reconstruction in female sex assigned disorder of sex development in a low-income country

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- This retrospective review of the surgical and psychosocial outcomes included 25 patients who underwent feminizing genital reconstructive surgery in Nigeria.
- Barriers to optimal care delivery for disorders of sexual development (DSDs) in LMICs include late presentations, inadequate diagnostic and treatment facilities, a social desirability of male sex, stigmatization, and high frequency of late sex reassignment.

Care for DSDs that results in optimal bio-psycho-social health outcomes is difficult to achieve anywhere but presents even greater challenges in LMICs. In settings where lay and health professional awareness of DSDs is low, diagnosis is often delayed, and sex of rearing decided upon regardless of the underlying chromosomal, gonadal and phenotypic sex, sexual function, or prospects of fertility. Rather, religious beliefs, cultural norms and societal pressures may predominate the decision-making process. Further, in the absence of sophisticated diagnostic tools providers need to rely on clinical exam, pelvic ultrasound, and minimal hormonal (17-hydroxyprogesterone) and genetic (Barr body detection) evaluation to make a diagnosis and decide on a care plan.

Diagnoses made as late as during teenage years to young adulthood led to gender reassignment in a striking 10 of 25 patients evaluated here (including 8 of 21 with likely 46 XX DSD due to CAH). However, multi-disciplinary care teams are beginning to emerge, and they are reviewing their practice, using small, but feasible and meaningful research studies to improve the quality of their care and health outcomes for their patients. They conclude that timely evaluation, more adequate diagnostic tools, reliable access to hormone treatments, improvements in timing of surgery and operating technique, as well as gender equality and stigma reduction can help to reduce barriers and improve care outcomes.

Growth and Nutrition

13.12. Exposure to improved nutrition from conception to age 2 years and adult cardiometabolic disease risk: a modelling study

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- Perinatal chronic undernutrition plays a role in adult-onset cardiometabolic disease.
- In this 40-year, longitudinal cohort in Guatemala, protein-energy nutritional supplementation during the first 2 years of life reduced the odds of diabetes but increased the risk of obesity and several obesity-related conditions in adulthood.

The Barker hypothesis proposes that intrauterine growth retardation plays a causal role in the origins of hypertension, coronary heart disease, and non-insulin-dependent diabetes in adulthood. In this study, the Barker hypothesis was tested in an original manner. Forty years ago, a randomized trial tested the effect of a nutritional supplement, made from dry skimmed milk, sugar, and a vegetable protein mixture (protein-rich, 90 kcal per 100 mL) compared to a low-energy beverage made from sugar and water (all calories from sugar; 33 kcal per 100 mL) on growth during the first 2 years of life in rural Guatemala. Forty years later, the adults who participated to the study as infants were evaluated from a cardiometabolic risk point of view. The authors found that early exposure to a high protein/high calorie diet was associated with a 50% decrease in the risk of diabetes but with a significant increase in BMI, obesity and total and non-HDL cholesterol.

This study is important as it highlights the importance of early nutritional exposure in children (1). Of course, many environmental changes that may affect the results of this study can occur over 40 years but high protein intake in infants has been shown in prospective studies to lead to increased weight gain and higher adiposity in childhood. Recent data (not available when the original study was performed) have led pharmaceutical companies to progressively decrease the protein content of formula to match the lower protein content of breastmilk. Although the quality of the protein (humans vs cow) remains different, this quantitative change may decrease the risk of later obesity in formula-fed infants. This study is also an opportunity to remember that breastfeeding remains the first choice for infant nutrition.

- (1) Prentice AM. Early life nutritional supplements and later metabolic disease. *Lancet Glob Health* 2018;6: e816.

13.13. Independent and combined effects of improved water, sanitation, and hygiene, and improved complementary feeding, on child stunting and anaemia in rural Zimbabwe: a cluster-randomised trial

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Lancet Glob Health 2019;7: e132–47. DOI: [10.1093/cid/civ844](https://doi.org/10.1093/cid/civ844)

- This cluster-randomised, community-based trial investigates whether the combination of a water, sanitation and hygiene (WASH) intervention and of improved infant and young child feeding (IYCF) intervention improves stunting and anemia in children living in rural Zimbabwe.
- Stunting decreased with IYCF, but WASH had no additional effect. Prevalence of diarrhea was not affected by the intervention.

The WASH intervention aims at improving conditions of water, sanitation, and hygiene. The rationale is that WASH will decrease fecal ingestion and as a consequence improve chronic inflammation and environmental enteric dysfunction (EED), regarded as a major underlying cause of both stunting and anemia. The IYCF intervention provides 20 g/d of Nutributter from 6 to 18 months and promotes optimal use of locally available foods for complementary feeding (1). The IYCF intervention alone increased haemoglobin concentrations, reduced stunting by 21%, reduced anaemia by 24%, and increased weight for height, confirming previous studies. WASH, in contrast with a study in Bangladesh but consistent with other trials, was ineffective either by itself or in combination with IYCF.

The authors discuss the possible reasons for the failure of the WASH intervention. First, although there was a good uptake of WASH at a household level, this was much more modest at the community level. Second, previous research has shown that the intensity of WASH implementation is an important factor, and its intensity may not have been high enough in this trial. We also wonder whether the beneficial effects of breastfeeding (immunoprotection) could have masked the effects of WASH. These children were breastfed exclusively until age 6 months and >97% were still at least partially breastfed at 18 months. Finally, the authors only report height SD, not height velocities which could show different results, in particular after exclusive breastfeeding stopped. These data are available and will likely be reported in a subsequent paper. Thus, the lack of effect of WASH observed here does not necessarily mean that it is generally ineffective but maybe its implementation needs to be optimized (2).

References

1. The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) Trial Team. The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) Trial: Rationale, Design, and Methods. *Clin Infect Dis* 2015;61(S7):S685–702.
2. Maleta KM, Manary MJ. WASH alone cannot prevent childhood linear growth faltering. *Lancet Glob Health* 2019;7: e16.

13.14. As tall as my peers – similarity in body height between migrants and hosts

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- This literature review evaluated the phenomenon of faster growth, earlier maturation and often taller adult height in migrant youth as compared to their non-migrant relatives.
- The authors propose a new framework to understand growth regulation and determinants of adult height that includes social networks as a growth regulating entity.

Nutrition, social conditions (housing, water, sanitation), economic status, psychosocial health and environmental factors are well-recognized determinants of human growth. This paper provides an anthropological perspective, arguing that social peer group and social status position of dominance or subordination are regulators of growth. They propose a new framework of determinants of adult height whereby nutrition, health and living conditions are merely *prerequisites* of growth, whereas social mechanisms function as *regulators*.

While this hypothesis is not scientifically proven in this paper, the authors carefully review historic and recent data that support it. By examining immigrant populations who moved from low- to high-income settings, the authors show a significant increase in the immigrant population's mean height by as much as 2 s.d.s. (about 10 cm) over the period of one generation. They revisit previously described observations, that colonial populations who moved from high- to low-income settings where they assumed a dominant social status position grew taller than their peers in their country of origin. In the traditional concept of growth regulation, these marked changes in *mean height* are attributed to improved conditions affecting each individual's growth. However, the population's height *distribution* typically remains unchanged, suggesting that social- and community-based growth adjustment rather than individual factors may underlie the increase in mean height.

While the exact physiologic mechanisms that mediate the hypothesized social growth regulation remain elusive and hypothesis driven at best, the data and arguments presented raise the question whether the concept of stature as a social signal may be a missing link in our current model of growth determinants. This shift in conceptualization of growth regulation may be relevant for childhood stunting interventions in low- and middle-income countries (1). Formally testing this hypothesis is a key next step.

Reference

1. Hossain M, Choudhury N, Adib Binte Abdullah K, Mondal P, Jackson AA, Walson J, Ahmed T. Evidence-based approaches to childhood stunting in low and middle income countries: a systematic review. *Arch Dis Child.* 2017 Oct;102(10):903–909. doi: [10.1136/archdischild-2016-311050](https://doi.org/10.1136/archdischild-2016-311050).

13.15. The obesity transition: stages of the global epidemic

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- This study uses quantitative data from reputable global data sets to demonstrate that the epidemiology of obesity can be framed as a conceptual model of obesity transition.
- A new framework to classify the obesity epidemic is proposed that may assist policy makers and researchers to improve surveillance and develop targeted preventative obesity interventions.

As pediatricians and endocrinologists, we tend to consider the interplay of biological factors such as genetics, epigenetics and the microbiome, and environmental factors such as sociocultural and economic conditions, policies and the built environment as the most important determinants of obesity. While these factors likely account for the varying distribution and intensity of obesity between geographical regions, it remains that obesity has become a global epidemic across populations.

In this paper, akin to the well-known concept of the epidemiological transition, the authors develop the concept of obesity transition whereby populations predictably transition through four distinct phases of obesity over time: In stage 1, obesity is overall low but at around 5% highest in women, greater in adults than in children, and greater in persons of high vs. low socioeconomic status (SES). All very large low-income countries such as India are currently at this stage. In stage 2, the prevalence increases to 25–40% among adults and to around 10% among children, and the gap between sexes and between SES narrows. All countries that were at stage 1 in 1975 (e.g., Mexico) were at stage 2 by 2016. In stage 3, the adult prevalence stalls while childhood obesity increases slightly, however the sex gap closes and there is a reversal in SES differences. Most European and North American countries are at this stage. Stage 4 is a hypothetical stage, yet to be attained by any country, where the obesity prevalence curve flattens in children, such that eventually leaner children enter adulthood, leading to eventual reductions in the prevalence of adult obesity. Trends towards this stage may be seen in some high-SES subpopulations.

This new concept provides the means for future identification of obesity transition stages in any given population, anticipation of obesity risk in subpopulations, and introduction of proactive measures that may attenuate transition. For instance, understanding a potential mismatch between today's high carbohydrate and high calorie diet and the origin of a population (hunter-gatherers with low insulin sensitivity vs farmers with high insulin sensitivity) might help design individual-specific therapeutic approaches (low carbohydrate diets vs low calorie diets) (2). If future research can identify factors that determine the underlying drivers of transition between stages, might populations be able to attain stage 4?

References

1. Stanaway JD, Afshin A, Gakidou E, *et al.* Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990, 2013, 2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018; 392: 1923–94.
2. Hochberg Z. An Evolutionary Perspective on the Obesity Epidemic. *Trends Endocrinol Metab.* 2018 Dec;29(12): 819–826.

Micronutrients

13.16. Global prevalence and disease burden of vitamin D deficiency: a roadmap for action in low- and middle-income countries

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- This technical report reviews the global prevalence and public health disease burden of vitamin D deficiency.

- Funded by the Bill & Melinda Gates foundation, the working group of content experts from North America and Europe provide a roadmap outlining population-based strategies to improve vitamin D status in low- and middle-income countries (LMICs).

Population representative data on vitamin D status in LMICs are scarce, particularly in youth. Despite predominantly low-quality literature, the available evidence to date suggests that vitamin D deficiency and nutritional rickets may be widespread globally (1), especially in regions where fortification programs do not exist. Most affected are populations residing in Asia, the Middle East, and Africa, as well as immigrants from these regions living in countries at higher latitudes. This report gathers evidence on global prevalence estimates and on functional consequences of vitamin D deficiency, outlines criteria to define vitamin D deficiency as a public health problem and provides an approach to reduce the associated health burden. While there are no surprising or new conclusions with regards to the recommended method to determine vitamin D status (25-OHD measurements), the cut-off to define deficiency (<30 nmol/L), the availability of reliable sources of vitamin D (most foods and UVB radiation are not), or the known consequences of vitamin D deficiency, novelty lies in the clearly outlined roadmap for action to address the global burden.

The suggested approach is a collaborative action between national ministries of health and international organizations. The roadmap starts with an assessment of vitamin D status, whereby the population status is deemed insufficient warranting public health interventions if more than 20% of the populations have 25-OHD levels <30 nmol/L, or in the absence of available 25-OHD data if the prevalence of rickets is >1%. Next, intervention via introduction of mandatory fortification of staple foods and/or supplementation of at-risk subgroups is recommended as appropriate based on the assessment. Options for vehicles for food fortification including dairy products, edible oils, and flour are mentioned. Lastly, monitoring and evaluation processes accompany the roadmap. The report ends with a list of research opportunities that reflect the many knowledge gaps that are still to be filled. Much is still to be learned, but a first step at tackling vitamin D deficiency and its complications in LMICs has been made.

References

1. Cashman KD, Sheehy T, O'Neill CM. Is vitamin D deficiency a public health concern for low middle income countries? A systematic literature review. *Eur. J. Nutr.* 2019 Feb;58(1):433-453. doi: [10.1007/s00394-018-1607-3](https://doi.org/10.1007/s00394-018-1607-3).
2. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? *J. Steroid Biochem.* 2014; *Mol. Biol.* 144: 138–145.

13.17. Improved micronutrient status and health outcomes in low- and middle-income countries following large-scale fortification: evidence from a systematic review and meta-analysis

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- This systematic review and meta-analysis aimed to determine the impact of large-scale food fortification (LSFF) on health and nutrition outcomes in low- and middle-income countries (LMIC).
- The authors demonstrate that LSFF increases serum micronutrient concentrations including iodine, with a positive impact on functional outcomes such as a 74% reduction in the odds of goiter.

Micronutrient malnutrition is prevalent in LMIC and associated with the global burden of poverty and disease. Iodine deficiency disorders are the most common cause for preventable neurodevelopmental delay. While salt iodization has long been established as an effective strategy to eliminate iodine deficiency and its disorders, according to the most recent 2017 Iodine Global Network score card, 20 countries in the world remain iodine deficient (1).

This systematic review provides real-world evidence that LSFF increases micronutrient concentrations and reduces adverse health outcomes. The significant reductions in iodine deficiency disorders by means of universal salt iodization are highlighted as a success. While this is indeed encouraging, our challenge remains to

eliminate iodine deficiency on a global scale. As much as salt iodization and other LSFF seem like straight forward interventions, as per the WHO/CDC logic model for micronutrient interventions (2), they do require financial and infrastructure resources to be established; policy frameworks, adequate production and supply, quality control, delivery mechanisms, communication and behavior change strategies to run successfully; and adequate program access and coverage to reach the target population. All of these processes are subject to vulnerability, with issues such as poor vehicle choice, challenges with large-scale distribution, and non-adherence to fortification recommendations being common. Recent experience with iodine status re-evaluation in Haiti (unpublished data) and Tanzania, two countries that are still iodine-deficient, highlight that challenges remain in almost every aspect of LSFF such as salt iodization. Thus, while additional research is needed to inform LSFF program priorities and tackle coverage and access issues among the poor and most vulnerable, the study's findings should encourage widespread use of LSFF, an intervention already largely contributing to alleviating micronutrient malnutrition.

References

1. Iodine Global Network. Global Scorecard of Iodine Nutrition in 2017 in the general population and in pregnant women (PW). IGN: Zurich, Switzerland. 2017. www.ign.org/cm_data/IGN_Global_Scorecard_AllPop_and_PW_May2017.pdf (Accessed June 30, 2019).
2. WHO/CDC. Logic model for micronutrient interventions in public health. [Internet]. Geneva (Switzerland): WHO; 2016. Available from: <http://apps.who.int/iris/bitstream/10665/250746/3/WHO-NMH-NHD-EPG-16.1-colour-eng.pdf> (Accessed June 30, 2019).

14. The Year in Science and Medicine 2019

Ze'ev Hochberg, Ken Ong

14.1. Could artificial intelligence make doctors obsolete?

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<https://www.bmj.com/content/363/bmj.k4563>

Machines that can learn and correct themselves already perform better than doctors at some tasks. This opinion article maintains that machines will never be able to replicate the inter-relational quality of the therapeutic nature of the doctor-patient relationship.

Artificial intelligence (AI) has the potential to be more accurate than doctors at making diagnoses and performing surgical interventions, and can do this at a speed that humans cannot match. Today's physicians cannot approximate this knowledge by keeping up-to-date of current medical research, while maintaining close contacts with their patients. Machine learning is also not subject to the same level of potential bias seen in human learning that reflects cultural influences and links with particular institutions.

Doctors form relationships with patients on the basis of trust; but machines and systems can be more trustworthy than humans if they can be regarded as unbiased and without conflicts of interest. Many patients rate correct diagnosis higher than empathy or continuity of care. Moreover, AI-driven systems could be cheaper than hiring and training new staff. The authors raise the argument that "Doctors as we now know them will become obsolete eventually."

Others think that the inter-relational quality of the doctor-patient relationship is vital and cannot be replicated. They agree that machines will increasingly perform tasks that human doctors do today, such as diagnosis and treatment, but say doctors will remain because they are better at dealing with the patient as a whole person. Doctors can relate to the patient as a fellow human being and can gain holistic knowledge of their illness as it relates to the patient's life.

"Computers aren't able to care for patients in the sense of showing devotion or concern for the other as a person, because they are not people and do not care about anything. Sophisticated robots might show empathy as a matter of form, just as humans might behave nicely in social situations yet remain emotionally disengaged because they are only performing a social role."

Most importantly there is no cure for some patients. Care is about helping them have the best quality of life possible with their condition and for the longest time. "Who wants to receive a terminal diagnosis from a robot?"

14.2. Gene-silencing technology gets first drug approval after 20-year wait

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<https://www.nature.com/articles/d41586-018-05867-7>

For the first time, a drug based on the mechanism gene-silencing through RNA interference (RNAi) has received regulatory approval. We can expect that this is a forerunner of a new class of drugs targeting disease-causing genes.

It has been known for 20 years that short RNA molecules can attach to messenger RNA that carries a gene's message and disrupt its translation to protein. This advance won Andrew Fire and Craig Mello the Nobel Prize, but efforts to turn it into medicine quickly hit hurdles. Scientists struggled to keep the fragile RNA molecules intact and direct them to the right tissue. The method was tried for hereditary transthyretin amyloidosis, where mutated forms of the protein transthyretin accumulate and sometimes impair heart and nerve function. Initially it did not work, but a more potent formulation worked in human trials; in a clinical trial in 225 people with

hereditary transthyretin amyloidosis who showed signs of nerve damage, average walking speed significantly improved in those who received the treatment. This became the intravenous drug Patisiran, which was approved by the U.S. and E.U. regulators this year.

Many RNAi researchers are now working on a newer delivery method: attach chemically stabilized RNA onto a sugar molecule that homes in on the liver, the eye and central nervous system, or the heart.

14.3. A membrane transporter is required for steroid hormone uptake in *Drosophila*

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<https://www.ncbi.nlm.nih.gov/pubmed/30293839>

Steroid hormones were believed to enter target cells via passive diffusion through the plasma membrane. This article shows that, at least for *Drosophila*, steroid hormones require a protein transporter to enter cells.

Genomic functions of steroid hormones are mediated by intracellular nuclear receptors, which regulate the transcription of target genes in the nucleus upon binding of steroid ligands. It is widely accepted that lipophilic steroid hormones can freely enter and exit cells by simple diffusion across lipid bilayers. In insects, the primary steroid hormone ecdysone enters its target cells and binds to the ecdysone receptor (EcR), which forms a heterodimer with another nuclear receptor Ultraspiracle and activates transcription of multiple genes. The same group of researchers has shown that in *Drosophila* ecdysone is released from an endocrine gland against the concentration gradient through a vesicle-mediated process, not by simple diffusion. Thus, insect steroid hormone ecdysone requires a membrane transporter to enter cells.

Here, they identify and characterize a *Drosophila* solute carrier (SLC) transporter, which they named Ecdysone Importer (Ecl), involved in cellular uptake of ecdysone. Ecl is highly conserved among insects and other arthropods that utilize ecdysteroids, and its tagged protein localizes to the plasma membrane of the cells in tissues that receive ecdysone.

Transporter-mediated steroid hormone trafficking across cell membranes have been demonstrated in many biological systems against their concentration gradient, such as when cells actively eliminate steroid hormones out of the cytoplasm. Such active transport necessarily requires energy, which is often provided by ABC transporters that can couple ATP hydrolysis to substrate transfer.

Collectively, these results challenge the simple diffusion model of ecdysteroid transport across cell membranes, and instead suggest a transporter-mediated, facilitated diffusion mechanism. Evolutionary conservation of solute carrier organic anion (SLCO) superfamily in metazoans may call for a reconsideration of the simple diffusion model of steroid hormone transport beyond arthropods. If these transporters are found in humans, it will represent a paradigm shift in endocrinology. It would also open up the possibility of developing chemical reagents that manipulate steroid hormone entry into cells.

14.4. A reprogramming human T cell function and specificity with non-viral genome targeting

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<https://www.nature.com/articles/s41586-018-0326-5>

This article describes a CRISPR-Cas9 genome-targeting system that does not require viral vectors, allowing rapid and efficient insertion of large DNA sequences at specific sites in the genomes of primary human T cells, while preserving cell viability and function.

The common approach to genetically reprogram T cells for therapeutic purposes is through recombinant viral vectors. However, this fails to target transgenes to specific genomic sites. New methods of genome editing such as CRISPR–Cas9 enable more specific insertion of large transgenes into target cells.

The authors used a CRISPR–Cas9 genome-targeting approach that does not require viral vectors to rapidly and efficiently insert large DNA sequences at selected sites in the genomes of human T cells. They applied this strategy to correct a pathogenic *IL2RA* mutation in cells from patients with monogenic autoimmune disease, and demonstrate improved signalling function. They replaced the endogenous T cell receptor (TCR) locus with a new TCR that redirected T cells to a cancer antigen. The engineered TCR T cells specifically recognized tumor antigens, and provided anti-tumor cell responses *in vitro* and *in vivo*.

The technique may open up new possibilities for treating cancer, infections such as H.I.V., and also autoimmune diseases. There is no therapeutic immunotherapy product based on the new technique. Yet, it has been announced that the researchers also corrected – in the lab – the T-cells of three children with a rare mutation that causes autoimmune diseases. The plan now is to return these corrected cells to the children, expect them to function normally and suppress the defective immune cells, and thereby cure these children.

14.5. Horizontal transfer of *BovB* and *L1* retrotransposons in eukaryotes

Ivancevic AM, Kortschak RD, Bertozzi T, Adelson DL

Genome Biol. 2018 Jul 9;19(1):85.

<https://genomebiology.biomedcentral.com/articles/10.1186/s13059-018-1456-7>

Analyzing 759 plant, fungal and animal genomes, this article describes multiple possible horizontal gene transfer events in bat and frog, finding new parasite vectors of transfer such as bed bug, leech and locust.

Junk DNA sequences that can multiply and change their position within a genome are called Jumping Genes (Transposons), the so-called horizontal transfer, as opposed to the normal linear transfer of genes. They can sometimes create or reverse mutations. It was shown that transposons can jump to the genome of another species using a virus or a parasite (like ticks) as a vector. It is predicted that up to 25% of a cow's and sheep's genomes are comprised of jumping genes.

The paper describes two transposons called *BovB* and *L1* that reached the mammalian genome from other species. *BovB* is present in the genomes of cows, reptiles bat, frog and elephants. *L1* was involved in the rapid evolution of mammals, including humans, in creating new traits. Across 759 species of plant, fungal and animal genomes, they identified multiple possible *L1* horizontal transfer events in eukaryotic species. They show multiple transfer events of *BovB*, describing new parasite vectors such as the bed bug, leech and locust. Such transposable elements have colonized more than half of the genome sequence in today's mammals.

Jumping genes seem to be a general genetic phenomenon, and are likely to be identified in even more species and multiple organs.

14.6. A late middle pleistocene Denisovan mandible from the Tibetan Plateau

Fahu Chen, Frido Welker, Chuan-Chou Shen, Shara E. Bailey, Inga Bergmann, Simon Davis, Huan Xia, Hui Wang, Roman Fischer, Sarah E. Freidline, Tsai-Luen Yu, Matthew M. Skinner, Stefanie Stelzer, Guangrong Dong, Qiaomei Fu, Guanghui Dong, Jian Wang, Dongju Zhang, Jean-Jacques Hublin

Nature. 2019 May;569(7756):409–412.

<https://www.nature.com/articles/s41586-019-1139-x>

A Denisovan mandible, identified by ancient protein analysis, was found on the Tibetan Plateau. It is at least 160,000 years old and provides direct evidence of the Denisovans outside Siberia.

The enigma of the archaic Denisovan started in 2010 when a fraction of a finger was discovered in the Denisovan cave in Siberia, which gave this species its name. This species split from the Neanderthals about 400,000 years ago. Last year's Yearbook cited a paper describing the tooth of an individual whose father was Neanderthal and the mother a Denisovan, and was dated to 160,000 years ago. The lack of informative Denisovan fossils hinders our ability to connect them geographically and temporally to recent Asian populations.

In 1980 the right half of a hominin mandible was found in Baishiya Karst Cave on the plains of Tibet at 3,280-meter altitude. A recent excavation revealed the presence of abundant Palaeolithic stone artefacts and cut-marked animal bones in the cave. Its mandibular and dental anatomy relates other Chinese fossil hominins to the Denisovans.

This mandible is the first to be found outside of Siberia. It provides unique insights into Denisovan mandibular and dental morphology and DNA analysis. 160,000 years ago, life was even tougher than today's Tibet. It was colder and had less oxygen. Denisovans successfully adapted to the high-altitude hypoxic environment, long before the regional arrival of modern *Homo sapiens*. The researchers believe that a unique Denisovan gene allowed them to survive such altitude, cold and hypoxic conditions, and they report that Denisovan genes are common among contemporary Tibetans.

14.7. A large impact crater beneath Hiawatha Glacier in northwest Greenland

Kurt H. Kjær, Nicolaj K. Larsen, Tobias Binder, Anders A. Bjørk, Olaf Eisen, Mark A. Fahnstock, Svend Funder, Adam A. Garde, Henning Haack, Veit Helm, Michael Houmark-Nielsen, Kristian K. Kjeldsen, Shfaqat A. Khan, Horst Machguth, Iain McDonald, Mathieu Morlighem, Jérémie Mouginot, John D. Paden, Tod E. Waight, Christian Weikusat, Eske Willerslev, Joseph A. MacGregor

Sci Adv. 2018 Nov 14;4(11):eaar8173

<https://advances.sciencemag.org/content/4/11/eaar8173>

The discovery of a 31-kilometer-wide impact crater in northwest Greenland, beneath up to a kilometer of ice, implies an instantly vaporizing rock that sent shock waves across the Arctic. Its impact must have had a powerful effect on global climate; a thousand-year global cooling event.

You may be familiar with the 200-kilometer-wide Chicxulub crater in Mexico, carved out by a dinosaur-killing asteroid 66 million years ago. Here, the authors report the discovery of a somewhat smaller impact crater beneath the Hiawatha Glacier in northwest Greenland. They identify a 31-kilometer-wide, circular bedrock depression beneath up to a kilometer of ice. Meltwater from the impact, pouring into the north Atlantic Ocean, could have markedly lowered temperatures by halting a conveyor belt of currents that brings warmth to northwest Europe. The crater was created by a fractionated iron asteroid, which must have been more than a kilometer wide. The more recent 12,000 years depth of ice is continuous and conformable, but all the deeper and older ice appears to be rich in debris and heavily disturbed. So, while the exact age of this impact crater is presently unknown, it might have occurred between 12,000 to 100,000 years ago, so that there is a chance that the event was seen by the new Americans who arrived from Asia 12,000 years ago, by European Neanderthals or by European modern men.

14.8. Temperature-dependent hypoxia explains biogeography and severity of end-Permian marine mass extinction

Justin L. Penn, Curtis Deutsch, Jonathan L. Payne, Erik A. Sperling

Science 07 Dec 2018: Vol. 362, Issue 6419, eaat1327

<https://science.sciencemag.org/content/362/6419/eaat1327>

These authors report the frequencies of Metabolic Index traits in living species and used these values to define a set of model ecophysiotypes. They then populated the model Permian ocean with each ecophysiotype, and found that ocean warming increases the metabolic O₂ demand amid declining supply; this removes the aerobic habitats for the vast majority of ecophysiotypes and implies a high likelihood of extinction.

We are in the midst of an extinction crisis - the so-called 6th extinction, but compared to the largest extinction in Earth's history, which occurred at the end of the Permian Period, ours is slow. That "Great Dying," (~252 million years ago) saw the loss of up to 96% of all marine species and 70% of terrestrial species. They conclude that rapid global warming and accompanying ocean oxygen loss were responsible for the majority of recorded extinctions. Tolerances of marine animals to warming and oxygen loss are physiologically related and are represented in the ratio of temperature-dependent oxygen supply and demand rates, termed the Metabolic Index (ϕ). If climate warming and oxygen loss reduce ϕ below the species-specific minimum requirement, the ocean would no longer support active aerobic metabolism and long-term population persistence.

Using a model of the Earth's climate and coupled geochemical proxy data, the imposed increase in atmospheric greenhouse gas levels raises near-surface ocean temperatures. Extinction intensity should have been lower in the tropics than at high latitudes. Across diverse taxonomic groups, the observed extinction intensity increases with latitude, consistent with the predicted signature of aerobic habitat loss. Temperature-dependent hypoxia can account for more than half of the observed magnitude of regional extinction. These results highlight the future extinction risk arising from a depletion of the ocean's aerobic capacity that is already under way.

An important factor not considered in this study is the rate of climate change during the end-Permian event. If warming and oxygen loss were imposed slowly, perhaps high-latitude organisms could adapt to warming and oxygen loss, whereas if these changes happened quickly, massive die-off would occur. Existing data suggest that the rates of these changes were rapid.

14.9. Did our species evolve in subdivided populations across Africa, and Why does it matter?

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Trends in Ecology & Evolution, 2018. 33 (8); 582–594.

<https://www.sciencedirect.com/science/article/pii/S0169534718301174>

This opinion piece argues that *Homo sapiens* evolved within interlinked groups living across Africa, and not in a single region of East Africa. Millennia of separation gave rise to diversity of human forms, and a complex mix of archaic and modern features in different places and at different times over the last 300,000 years ultimately shaped our species.

The view that *Homo sapiens* evolved from a single region/population within Africa has long been given primacy in studies of human evolution. However, evidence from several research fields is no longer consistent with this view. Instead, our human ancestors were dispersed throughout Africa, and were kept apart by diverse and shifting habitats, such as forests and deserts. Millennia of separation gave rise to a staggering diversity of human forms.

Many of the inhospitable regions in Africa today, such as the Sahara desert, were once wet and green, with interwoven networks of lakes and rivers, and abundant wildlife. Similarly, some tropical regions that are humid and green today were once arid, meaning that human populations would have gone through many cycles of isolation through local adaptation followed by genetic and cultural mixing.

14.10. Comparing folic acid dosage strengths to prevent reduction in fetal size among pregnant women who smoked cigarettes: A randomized clinical trial

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JAMA Pediatr 2019; 173(5):493–494.

<https://jamanetwork.com/journals/jamapediatrics/fullarticle/2728105>

This double-blind, randomized controlled trial, in 345 US women who smoked cigarettes during pregnancy, showed that high (4 mg/day) versus standard dose (0.8 mg/day) folic acid increased mean birth weight by 140 grams, had a 31% lower risk of having a SGA baby, and a 35% lower risk of fetal growth restriction.

It is well known that smoking during pregnancy reduces fetal growth and birth weight, by roughly 200 grams; offspring subsequently show rapid 'catch-up' weight gain during infancy leading to higher risk of overweight and obesity during childhood (1). While there may be various possible mechanisms, here the authors tested a hypothesis based on observational evidence of lower serum folate levels in smokers. The findings are clear – high dose of folic acid (similar to the dose that some recommend to obese women for prevention of neural tube defects) prevented most of the birth weight reduction normally seen in infants of maternal smokers. This is the first such trial, the protective effect on SGA did not quite reach statistical significance, and confirmation in larger

samples is needed. However, the apparent large effect sizes are highly promising for a new and safe way to improve the health of such babies.

But is it controversial to find a way to allow mothers to smoke more safely? Could this be seen as encouraging mothers not to stop smoking in pregnancy? The same issue applies to use of medical therapies to prevent co-morbidities of obesity. Of course we should encourage lifestyle behaviour change, and in this trial both study arms received smoking cessation counselling. But we should also aim to understand the mechanisms that link unhealthy lifestyles to disease and use this information to improve preventive strategies.

Reference

1. Ong KK, Preece MA, Emmett PM, Ahmed ML, Dunger DB. Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast-feeding: longitudinal birth cohort study and analysis. *Pediatric Research* 2002;52:863–7.

14.11. Neonatal selection by Toll-like receptor 5 influences long-term gut microbiota composition

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Nature 2018;560:489–493.

<https://www.nature.com/articles/s41586-018-0395-5>

This paper reports competitive gut colonization experiments in mice. Compared to adult mice, neonatal mice showed much higher expression of the flagellin receptor *Tlr5* in their intestinal epithelial cells, and exposure to this protein during the first 2 weeks of life, before weaning, was crucial in determining lifelong gut microbiota composition with relevance to metabolic health.

TLR5 binds to flagellin, a protein that some pathogenic *Salmonella* bacteria use for motility; thereby TLR5 limits early-life colonization by such bacteria. Here, the authors show that, compared to normal wild-type mice, *Tlr5*-deficient neonatal mice have a higher rate of colonisation by flagellated *Salmonella*. These early differences lead to life-long ‘dysbiotic’ changes in gut microbiota, which are also associated with poorer metabolic health. Furthermore, by experimentally transferring the dysbiotic gut microbiota from these mice, they showed that transient early life expression of *Tlr5* is crucial in determining the outcome. Normal wild-type pups, who express high levels of *Tlr5*, gradually eliminated the dysbiotic microbiota, whereas *Tlr5*-deficient pups and adult mice (both with low or absent *Tlr5*) retained high levels of dysbiotic microbiota.

These findings highlight a remarkable mechanism by which metabolic health can be programmed for the long-term by very short-term exposures interacting with genetic variation. There is much research interest in how gut microbiota is established and evolves with changes in diet in human infants. So far, there is no evidence that the marked gut microbiota differences seen between breastfed and formula fed infants persists after weaning. Possibly we will find such hypothesised persisting effects from other early life dietary exposures or gastrointestinal infections, and their interactions with genotypes.

14.12. Genome amplification and cellular senescence are hallmarks of human placenta development

Velicky P, Meinhardt G, Plessl K, Vondra S, Weiss T, Haslinger P, Lendl T, Aumayr K, Mairhofer M, Zhu X, Schutz B, Hannibal RL, Lindau R, Weil B, Ernerudh J, Neesen J, Egger G, Mikula M, Rohrl C, Urban AE, Baker J, Knofler M, Pollheimer J

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PLoS Genet 2018;14:e1007698.

<https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1007698>

These authors studied human placental and decidual tissues obtained from elective pregnancy terminations (6–12 weeks gestation). Placental extravillous trophoblasts (EVTs), the cells that rapidly invade the mother’s endometrium, undergo an initial stage of genomewide amplification leading to a ‘tetraploid’ chromosomal state (XXYY or XXXX) followed by cellular senescence. By contrast, cells from androgenic complete hydatidiform moles (CHM) fail to show normal senescence.

These studies illuminate the marked and rapid cellular changes that occur during early placental formation. Initially, EVT_s replicate extensively and rapidly invade the mother's endometrium, yet the extent of placental invasion is a remarkably well-controlled balance between the fetus's needs and mother's self-protection. Here, the authors describe a further genomewide amplification process separate to mitosis and cell division, which leads to 'tetraploidy' (2 pairs of chromosomes). The benefit of having an excess of normal chromosome number was not examined, but might allow a brief and extensive burst of gene expression in these highly active cells. Subsequently, on invasion on the endometrium, EVT_s quickly undergo growth arrest and cellular senescence, likely as a way to limit the extent of their invasion. By contrast, androgenic hydatidiform moles (cells with diploid chromosomes only from spermatozoa not the ovary) are rare highly aggressive placental tumours – these cells, which lack any DNA of maternal origin, fail to undergo normal cell senescence after invasion.

This crucial balance between the fetus and mother is also upset in some fetal growth disorders. The authors discuss that cases of Beckwith-Wiedemann syndrome characterized by mutations in the cell cycle regulation gene *CDNK1C* show placental features, such as hyperplasia and excessive EVT formation, that are similar to those seen in hydatidiform moles. It seems likely that more subtle genetic variations in the fetal and maternal genomes may also shift this exquisite balance and explain their separate contributions to variation in birth weight (1).

Reference

1. Warrington NM, Beaumont RN, Horikoshi M, Day FR, *et al.* Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. *Nat Genet.* 2019 May;51(5):804–814.

14.13. Effect of genetic diagnosis on patients with previously undiagnosed disease

Splinter K, Adams DR, Bacino CA, Bellen HJ, Bernstein JA, Cheatle-Jarvela AM, Eng CM, Esteves C, Gahl WA, Hamid R, Jacob HJ, Kikani B, Koeller DM, Kohane IS, Lee BH, Loscalzo J, Luo X, McCray AT, Metz TO, Mulvihill JJ, Nelson SF, Palmer CGS, Phillips JA, 3rd, Pick L, Postlethwait JH, Reuter C, Shashi V, Sweetser DA, Tiff CJ, Walley NM, Wangler MF, Westerfield M, Wheeler MT, Wise AL, Worthey EA, Yamamoto S, Ashley EA, Undiagnosed Diseases Network Harvard Medical School, Brigham and Women's Hospital, and Massachusetts General Hospital, Boston, USA
N Engl J Med 2018;379:2131–2139.
<https://www.nejm.org/doi/full/10.1056/NEJMoa1714458>

The authors reviewed data on 1519 patients referred to the Undiagnosed Diseases Network (UDN), a US NIH funded network linking seven clinical sites. 53% of patients were female and their symptoms were neurologic (40%), musculoskeletal (10%), immunological (7%), gastrointestinal (7%), or rheumatological (6%). Of the 382 patients who had a complete evaluation, the UDN was able to establish the diagnosis in 132 (35%), many of which resulted in changes in management. 31 new syndromes were defined.

The cost of human whole-genome or whole-exome genome sequencing is falling drastically. Having been solely a research tool for some well-funded investigators, it now has a rapidly increasing place in clinical practice with tangible and even cost-effective benefits. In this study, changes in management occurred in 21% of those patients with a positive diagnosis, including use of medications, vitamin, coenzyme therapy and organ transplantation. Other types of benefit include genetic counselling, changes in prognosis and avoidance of other possibly extensive and invasive diagnostic testing. Furthermore, the benefits of genome sequencing will be cumulative – many patients with genome sequence results will initially have no diagnosis, but as patterns build across patients with similar phenotypes and gene mutations, new syndromes will be defined and new genes will be established as causes of existing disorders.

A particular strength of the network described here was the inclusion of a model organism screening facility, to test the functional relevance of new human mutations. For example, a novel mutation in *NR5A1* (steroidogenic factor-1) was shown to alter gene function when experimentally introduced into drosophila, and hence confirmed as a novel cause of 46,XX DSD in the proband and additional patients.

14.14. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study

Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, Prigmore E, Keelagher R, Best SK, Carey GK, Mellis R, Robart S, Berry IR, Chandler KE, Cilliers D, Cresswell L, Edwards SL, Gardiner C, Henderson A, Holden ST, Homfray T, Lester T, Lewis RA, Newbury-Ecob R, Prescott K, Quarrell OW, Ramsden SC, Roberts E, Tapon D, Tooley MJ, Vasudevan PC, Weber AP, Wellesley DG, Westwood P, White H, Parker M, Williams D, Jenkins L, Scott RH, Kilby MD, Chitty LS, Hurler ME, Maher ER, Prenatal Assessment of Genomic Exomes Consortium

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[https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(18\)31940-8/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(18)31940-8/fulltext)

This large prospective cohort study recruited from 34 UK fetal medicine units to evaluate the use of prenatal whole genome sequencing in 610 fetuses with a structural abnormality detected on antenatal ultrasound scanning and no chromosomal abnormality. Overall, a diagnostic genetic mutation was identified in 8.5% of fetuses, and more commonly in those fetuses with multisystem anomalies (15.4%), skeletal anomalies (15.4%), or cardiac anomalies (11.1%). The lowest yield, only 3.2%, was in fetuses with isolated increased nuchal translucency in the first trimester.

Currently, when a structural abnormality is found on antenatal ultrasound, it is routine practice to offer testing of fetal DNA, obtained from chorionic villi, amniotic fluid, or fetal blood, for chromosomal aneuploidy and other copy number variants. The current study collected DNA samples left over from those routine tests and showed that whole genome sequencing adds significantly to the yield of genetic diagnoses. However, the overall detection rate here (8.5%) is much lower than that reported elsewhere for genome sequencing of children with neuro-developmental disorders and other undiagnosed postnatal disease (30–40%).

Furthermore, the use antenatal genetic testing throws up specific ethical issues. None of the genetic diagnoses would have led to a prenatal treatment, and would have only rarely enabled better postnatal management. Instead, if the genetic information had been given in real-time, it could have negatively affected decisions to proceed with the pregnancy – two-thirds of diagnostic genetic variants were additionally associated with learning disabilities.

14.15. Recovery of trait heritability from whole genome sequence data

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bioRxiv March 25, 2019. doi: <https://doi.org/10.1101/588020>

The *Yearbook* does not usually report on studies that are yet published only on ‘preprint’ servers, i.e. author-deposited versions of papers that have not been accepted or even peer-reviewed. However, *Nature* also made an exception for this paper (1)!

These authors analysed whole genome sequence data from the TOPMed study. Among 21,620 unrelated individuals > 18 years old of European ancestry, they calculated that the 47 million genetic variants detected could explain 79% (SE 9%) of the population variation in height and 40% (s.e. 9%) for BMI, consistent with estimates from family based studies.

‘Missing heritability’ has puzzled genetics researchers for several years. Genome-wide association studies (GWAS) have identified hundreds of common genetic variants with robust associations with human diseases and traits. However, effect sizes are small and together it is computed that all current and future GWAS associations account for only 30–50% of heritability estimated from studies of such traits in families. This has even led to serious doubts over the over-estimation of heritability, typically derived from comparing the concordance of

traits between monozygous and dizygous twins. Hence the current study provides great reassurance and promise. Firstly, reassurance that our long-held estimates of heritability are indeed correct; that genetics does have a substantial impact on many human traits. Secondly, promise that whole genome sequencing will pave the way to one day fully understanding the genetic basis for common variation of human traits among normal populations, with many new lessons for understanding disease mechanisms, treatments and prevention.

Reference

1. News. Genetic study homes in on height's heritability mystery. *Nature* 23 April 568, 444–445 (2019).

14.16. Insights into imprinting from parent-of-origin phased methylomes and transcriptomes

Zink F, Magnusdottir DN, Magnusson OT, Walker NJ, Morris TJ, Sigurdsson A, Halldorsson GH, Gudjonsson SA, Melsted P, Ingimundardottir H, Kristmundsdottir S, Alexandersson KF, Helgadóttir A, Gudmundsson J, Rafnar T, Jonsdóttir I, Holm H, Eyjolfsson GI, Sigurdardóttir O, Olafsson I, Masson G, Gudbjartsson DF, Thorsteinsdóttir U, Halldorsson BV, Stacey SN, Stefansson K

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<https://www.ncbi.nlm.nih.gov/pubmed/30349119>

The authors analyse whole blood samples collected in participants of the Icelandic deCODE genetics studies in order to distinguish maternal genotype versus paternal genotype effects on gene expression and methylation in blood. The results provide a new map of imprinted methylation and gene expression patterns across the human genome with greater resolution and power than previously reported.

Paediatric endocrinologists are familiar with imprinted gene disorders, such Prader-Willi and Angelman syndromes, where exactly the same genetic mutation leads to completely different clinical disorders depending on the parent-of-origin of the mutation. This is due to imprinted genes, which retain an epigenetic memory of which parent they came from and show selective activation on that basis. Relatively few genes are imprinted, possibly ~100/20,000 genes, however it is thought that imprinted genes can also lead to slightly imbalanced gene expression at many other genes through shared gene networks.

As well as providing a highly valuable new reference database of human imprinting in peripheral blood cells, the current study shows that many imprinted genes show ‘polymorphic imprinting’ – where the pattern of imprinting varies considerably between individuals. Notably, the highest variability was seen at *VTRNA2-1*, where imprinting has been famously reported to vary in response to peri-conceptual nutrition in rural Gambians (1). Variability was also significant at several other sites, including *DLK1*, *IGF2*, *HTR2A*, and *IGF2R*. Through these and other modifying mechanisms, the authors show how imprinting contributes to the normal continuous variation in human traits rather than binary characteristics.

Reference

1. Silver MJ, Kessler NJ, Hennig BJ, Dominguez-Salas P, Laritsky E, Baker MS, Coarfa C, Hernandez-Vargas H, Castolino JM, Routledge MN, Gong YY, Hecceg Z, Lee YS, Lee K, Moore SE, Fulford AJ, Prentice AM, Waterland RA. Independent genomewide screens identify the tumor suppressor *VTRNA2-1* as a human epiallele responsive to periconceptual environment. *Genome Biol.* 2015 11;16:118.

14.17. Whole-genome sequencing of Atacama skeleton shows novel mutations linked with dysplasia

Bhattacharya S, Li J, Sockell A, Kan MJ, Bava FA, Chen SC, Avila-Arcos MC, Ji X, Smith E, Asadi NB, Lachman RS, Lam HYK, Bustamante CD, Butte AJ, Nolan GP

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Genome Res 2018;28:423–431

<https://genome.cshlp.org/content/28/4/423>

The discovery of a mummified humanoid female skeleton on a shelf in a building in La Noria, Chile in 2003 created enormous scientific and popular interest. Its highly unusual appearance included a length of only 6

inches despite a bone age of 6–7 years old, marked turricephaly (a cone-shaped top of the skull), and reduced number of ribs bilaterally. See photographs at: <https://genome.cshlp.org/content/28/4/423/F1.expansion.html>.

This extraordinary phenotype led to wide and even bizarre speculation about its origin. The 2013 documentary film ‘*Sirius*’, based on UFO theorist Steven Greer’s book, claimed that this specimen represented evidence of alien life!

Here, we read that science has come to the rescue. From only one cubic millimetre of bone, the US research team extracted high quality DNA and performed whole-genome sequencing (WGS). They confirmed that this was a modern Chilean female and found multiple mutations in many genes with known links to bone development and ossification disorders and musculoskeletal abnormalities, including: *COL1A1* (Ehlers-Danlos syndrome and osteoporosis), *COL2A1* (various osteochondrodysplasias), *KMT2D* (Kabuki syndrome), *FLNB* (ectopic and premature ossification), *ATR* (Seckel syndrome), *TRIP11* (achondrogenesis), and *PCNT* (microcephalic osteodysplastic primordial dwarfism type II, MOPDII). This study shows that WGS can bring a powerfully scientific approach to the understanding of archaeological and anthropological history, in the same way as WGS is unearthing the diagnosis in our clinically undiagnosed patients.

A key remaining question is whether this fetal specimen resulted from a statistically highly extreme coincidence of multiple known and novel mutations. Alternatively, the authors speculate that the discovery site, La Noria, an abandoned nitrate mining town in the Atacama Desert, points to a possible role for prenatal nitrate exposure as a possible cause of multiple de novo mutations.

14.18. Darolutamide in nonmetastatic, castration-resistant prostate cancer

Fizazi K, Shore N, Tammela TL, Ulys A, Vjaters E, Polyakov S, Jievaltas M, Luz M, Alekseev B, Kuss I, Kappeler C, Snapir A, Sarapohja T, Smith MR, Investigators A

N Engl J Med 2019;380:1235–1246

<https://www.nejm.org/doi/full/10.1056/NEJMoa1815671>

This paper reports a randomized, double-blind, placebo-controlled, phase 3 trial of darolutamide, a novel oral androgen-receptor antagonist, in 1509 men with non-metastatic, castration-resistant prostate cancer. Median metastasis-free survival was significantly longer with darolutamide (40.4 months) than placebo (18.4 months; hazard ratio for metastasis or death: 0.41; 95% CI, 0.34 to 0.50; $P < 0.001$).

It is well established that androgens drive prostate cancer growth and metastasis. Hence, chemical castration using GnRH agonist therapy is first line treatment. However, full suppression of the hypothalamic-pituitary axis does not completely suppress androgen levels and most hormone dependent cancers become resistant to treatment after only a few years, presumably due to increased sensitivity to androgens. Darolutamide is a structurally unique non-steroidal androgen-receptor selective antagonist that can be taken orally. Unlike other selective androgen-receptor antagonists, it does not cross the blood-brain barrier and does not induce cytochrome P450, hence its apparent good side-effect profile.

Highly selective and effective novel sex hormone antagonists have been developed and are showing highly promising results for treatment of hormone sensitive cancers – see also recent results for fulvestrant, an oestrogen receptor antagonist in metastatic breast cancer (1). Currently their costs are too high, even for acceptance onto some national cancer guidelines. However, in time, with reducing costs, future genetic versions and hopefully ongoing good safety profiles, we hope that these agents will open up new effective options for the management of various disorders of growth, puberty, and DSD.

Reference

1. Mehta RS, Barlow WE, Albain KS, Vandenberg TA, Dakhil SR, Tirumali NR, Lew DL, Hayes DF, Gralow JR, Linden HH, Livingston RB, Hortobagyi GN. Overall Survival with Fulvestrant plus Anastrozole in Metastatic Breast Cancer. *N Engl J Med*. 2019 Mar 28;380(13):1226–1234.

15. Editors' Choice

Ken Ong, Ze'ev Hochberg

15.1. A Copeptin-based approach in the diagnosis of diabetes insipidus

Fenske W, Refardt J, Chifu I, Schnyder I, Winzler B, Drummond J, Ribeiro-Oliveira A Jr., Drescher T, Bilz S, Vogt DR, Malzahn U, Kroiss M, Christ E, Henzen C, Fischli S, Tonjes A, Mueller B, Schopohl J, Flitsch J, Brabant G, Fassnacht M, Christ-Crain M
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N Engl J Med 2018;379:428–439
<https://www.nejm.org/doi/full/10.1056/NEJMoa1803760>

In this multi-centre cohort of 156 patients with hypotonic polyuria, direct measurement of hypertonic saline-stimulated plasma copeptin had much greater diagnostic accuracy than a standard water-deprivation test, as judged by the final reference diagnosis, which was determined on the basis of medical history, test results (with copeptin levels masked), and treatment response at a 3-month follow-up visit.

Water-deprivation tests are used commonly to distinguish between (central) Diabetes Insipidus (DI), Renal DI and Primary Polydipsia. However, these tests are often challenging to perform. Patients with Primary Polydipsia are often well-hydrated at the start and it may take much more than 12 hours of water deprivation to reach appropriate urine concentrations; conversely this test is potentially dangerous in patients with DI who quickly become severely dehydrated. Hence these tests require carefully monitoring over a long period – often with a very grumpy child! Furthermore, the existence of (central) ‘Partial DI’ complicates matters; it can be difficult to distinguish this from insufficient duration of testing in Primary Polydipsia.

Copeptin is co-secreted with vasopressin as part of its pre-pro-hormone. It has no known biological function, but the authors show that it serves as an accurate surrogate marker for vasopressin levels, for which an accurate assay has long proved challenging. A plasma copeptin cutoff level of 4.9 pmol/l or less indicated complete or partial central DI, and a level > 4.9 pmol/l indicated Primary Polydipsia.

The main disadvantage of the protocol reported here is the hypertonic saline infusion test. Although much shorter than the water deprivation test, it requires close monitoring of plasma sodium levels to achieve the target level of at least 150 mmol/l, and common side-effects are nausea, vertigo, and headache. The authors have presented and will soon publish an alternative stimulation test based on the copeptin level 60-minutes after intravenous administration of Arginine. I suspect that our future junior fellows will be amused by our tales of conducting (hopefully superseded) water deprivation tests.

15.2. Vitamin D supplements and prevention of cancer and cardiovascular disease

Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, Gibson H, Gordon D, Copeland T, D'Agostino D, Friedenberg G, Ridge C, Bubes V, Giovannucci EL, Willett WC, Buring JE, VITAL Research Group
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N Engl J Med 2019;380:33–44
<https://www.ncbi.nlm.nih.gov/pubmed/30415629>

This paper describes a large randomized, placebo-controlled trial of vitamin D3 (cholecalciferol) 2000 IU per day in 25,871 US adults. After median follow-up of 5.3 years, supplementation with vitamin D did not alter the risk of the primary end points, invasive cancer of any type hazard ratio, 0.96; 95% confidence interval [CI], 0.88–1.06; $P=0.47$) or major cardiovascular events (hazard ratio, 0.97; 95% CI, 0.85–1.12; $P=0.69$).

We routinely (and should continue to) recommend that infants and young children receive vitamin D supplements in order to avoid effects of vitamin deficiency on bone and muscle health. In addition, observational studies have reported a very large number of other health benefits associated with higher circulating vitamin D

levels, ranging from Type 2 diabetes, cardiovascular disease autoimmunity, inflammation, cancer and innate immunity. However, a major limitation of those observational studies is ‘residual confounding’. Vitamin D is lipophilic, accumulates substantially in adipose tissue, and therefore apparent associations with lower circulating vitamin D levels can be artificially created, or strengthened, by higher levels of adiposity. Statistical adjustments can be performed but are dependent on studies having collected accurate measures of adiposity. The current paper joins other growing trial evidence that such associations may not be causal (1). The trial was very large, well performed, tested a relatively high dose of vitamin D (equivalent to five-times the daily dose recommended in the UK) and found convincingly no benefit on the primary outcomes.

Some believers of widespread benefits of vitamin D supplementation might remain optimistic. In this US setting, 43% of participants were already taking a vitamin D supplement at baseline, so the majority may have been vitamin D replete.

Reference

1. Pittas AG, Dawson-Hughes B, Sheehan P, Ware JH, Knowler WC, Aroda VR, *et al.* Vitamin D Supplementation and Prevention of Type 2 Diabetes. *N Engl J Med* 2019. Epub ahead of print Jun 7.

15.3. Assessment of safety and outcome of lateral hypothalamic deep brain stimulation for obesity in a small series of patients with Prader-Willi syndrome

Franco RR, Fonoff ET, Alvarenga PG, Alho EJJ, Lopes AC, Hoexter MQ, Batistuzzo MC, Paiva RR, Taub A, Shavitt RG, Miguel EC, Teixeira MJ, Damiani D, Hamani C
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JAMA Network Open 2018;1:e185275
<https://www.ncbi.nlm.nih.gov/pubmed/30646396>

The authors describe a small case series of four patients, age range 18–28 years, with Prader-Willi syndrome and obesity. All had received childhood growth hormone therapy, two had previous bariatric surgery, and all had psychiatric comorbidities before the current intervention. All received deep brain stimulation, via electrodes bilaterally implanted in the lateral hypothalamic area, over a 6-month protocol. The intervention was ineffective in reducing body weight or BMI, which actually increased by on average 9.6% and 5.8%, respectively.

It is well recognised that patients with Prader-Willi syndrome invariably develop hyperphagia during childhood, which often leads to severe obesity. Hyperphagia is extremely distressing – even modest hunger is uncomfortable for many of us! Many treatments have been tried to reduce patients with Prader-Willi syndrome but yet without good evidence of effectiveness. The neural mechanism for hyperphagia in this condition is not established; high circulating ghrelin levels are typical and could drive appetite, but patients also have multiple other hormone abnormalities (hypogonadism, growth hormone dysfunction, hypothyroidism, central adrenal insufficiency), as well as behavioural and psychiatric problems.

Deep brain stimulation (DBS) is widely accepted to be an effective treatment for advanced stages of Parkinson’s disease, and other types of movement disorders. Its use in other conditions, such as obsessive-compulsive disorder, is being explored. The rationale for deep brain stimulation as an intervention in severe obesity is that it mimics the reward circuitry stimulated by binge-like feeding. Unfortunately no such benefits were obvious in this small case series, but the paper highlights the importance of reporting negative findings. Results of studies of other interventions, such as GLP-1 receptor agonists, are eagerly awaited.

15.4. Association of long-term child growth and developmental outcomes with metformin vs insulin treatment for gestational diabetes

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JAMA Pediatr 2019;173:160–168
<https://jamanetwork.com/journals/jamapediatrics/article-abstract/2716403>

This paper describes a large population-based cohort of women in New Zealand who received metformin ($n=1996$ women) or insulin ($n=1932$) for treatment of gestational diabetes mellitus (GDM). There were no differences between the two groups in any measure of offspring childhood height, weight for height, or behavioural development.

There have been substantial changes in the detection and management of GDM in recent years. Implementation of the 2010 International Association of the Diabetes and Pregnancy Study Group (IADPSG) criteria has led to the diagnosis of many more women as having GDM, possibly up to 6–11 times more, compared to previous criteria (1). This has created a major challenge how to best manage this condition which affects an estimated 10% of all pregnancies worldwide. A large proportion of GDM women receive only lifestyle advice combined with glucose monitoring. Subcutaneous insulin therapy is a far more intensive option. It seems sensible that, somewhere between those two options, oral anti-hyperglycaemic agents, such as Metformin, should have an important role. While Metformin is being used increasingly for GDM in many countries, in several settings it is little used, possibly due to concerns about possible risks of birth defects and other pregnancy complications – its mechanism of action is not fully understood and there are concerns from *in vitro* studies that Metformin impairs mitochondrial function, by inhibiting Complex I of the respiratory chain. In this light, the findings of the current large study are highly reassuring.

Reference

1. Behboudi-Gandevani S, Amiri M, Bidhendi Yarandi R, Ramezani Tehrani F. The impact of diagnostic criteria for gestational diabetes on its prevalence: a systematic review and meta-analysis. *Diabetol Metab Syndr*. 2019 Feb 1;11:11.

15.5. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis

Voerman E, Santos S, Patro Golab B, Amiano P, Ballester F, Barros H, Bergstrom A, Charles MA, Chatzi L, Chevrier C, Chrousos GP, Corpeleijn E, Costet N, Crozier S, Devereux G, Eggesbo M, Ekstrom S, Fantini MP, Farchi S, Forastiere F, Georgiu V, Godfrey KM, Gori D, Grote V, Hanke W, Hertz-Picciotto I, Heude B, Hryhorczuk D, Huang RC, Inskip H, Iszatt N, Karvonen AM, Kenny LC, Koletzko B, Kupers LK, Lagstrom H, Lehmann I, Magnus P, Majewska R, Makela J, Manios Y, McAuliffe FM, McDonald SW, Mehegan J, Mommers M, Morgen CS, Mori TA, Moschonis G, Murray D, Chaoimh CN, Nohr EA, Nybo Andersen AM, Oken E, Oostvogels A, Pac A, Papadopoulou E, Pekkanen J, Pizzi C, Polanska K, Porta D, Richiardi L, Rifas-Shiman SL, Ronfani L, Santos AC, Standl M, Stoltenberg C, Thiering E, Thijs C, Torrent M, Tough SC, Trnovec T, Turner S, van Rossem L, von Berg A, Vrijheid M, Vrijkotte TGM, West J, Wijga A, Wright J, Zvinchuk O, Sorensen TIA, Lawlor DA, Gaillard R, Jaddoe VVW

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<https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1002744>

These investigators painstakingly collated individual-level data from 37 pregnancy and birth cohort studies from Europe, North America, and Australia, comprising 162,129 mothers and their children, in order to identify the optimal levels gestational weight gain in relation to risk of offspring obesity. They confirm excessive gestational weight gain as an independent risk factor to childhood obesity, but its additional effects are small compared to the impact of maternal overweight or obesity before pregnancy.

Guidance on optimal gestational weight gain is difficult to set with confidence. Many countries follow the US Institute of Medicine (IOM) 2009 guidelines, which were based on a combined consideration of various maternal and infant outcomes. The IOM defined separate thresholds of excessive weight gain depending on mother's pre-pregnancy BMI status. Other countries, such as the UK, set no specific weight gain targets.

The current paper revisits the question of optimal gestational weight gain, specifically in relation to the risk of childhood obesity. Unfortunately for those hoping for a simple answer, there does not appear to be any obvious single threshold. Overall the risk of childhood obesity increased linearly with increasing gestational weight gain (adjusted for pre-pregnancy BMI status) and this association was scarcely changed after adjustment for birth weight. Furthermore, gestational weight gain may not be the most effective target in relation to childhood obesity; within each maternal BMI category, excessive gestational weight gain only slightly increased the risk of childhood overweight/obesity.

15.6. Late-pregnancy dysglycemia in obese pregnancies after negative testing for gestational diabetes and risk of future childhood overweight: An interim analysis from a longitudinal mother-child cohort study

Gomes D, von Kries R, Delius M, Mansmann U, Nast M, Stubert M, Langhammer L, Haas NA, Netz H, Obermeier V, Kuhle S, Holdt LM, Teupser D, Hasbargen U, Roscher AA, Ensenauer R
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PLoS Med 2018;15:e1002681.

<https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1002681>

These authors highlight a novel pregnancy risk factor, 'late-pregnancy dysglycaemia' in women who are obese but had normal glucose tolerance when they were tested for gestational diabetes mellitus (GDM) earlier in pregnancy. In a prospective cohort study of obese women without GDM ($n=448$), high maternal glycated haemoglobin at delivery (HbA1c $\geq 5.7\%$ [39 mmol/mol]) was associated with high third-trimester gestational weight gain and large-for-gestational-age birth weight, and was a major risk factor for higher childhood BMI and later diabetes or prediabetes in the mothers.

The detection and management of GDM has deservedly received much attention in recent years. However, most of us assume that those pregnant women who test negative for GDM are 'in the clear'. However, the current paper clearly shows that this is not the case. Here, the authors report a unique prospective cohort study with data on trimester-specific glucose levels. Among obese mothers who tested negative testing for GDM at the end of the 2nd trimester, compared to those with normal HbA1c at delivery, those with 'late-pregnancy dysglycemia' (30.1%) had higher risks of total and third-trimester excessive gestational weight gain and a 4-fold (RR 4.01, 95% CI 1.97–8.17) higher risk of future prediabetes or diabetes. Their offspring had higher risk for large-for-gestational-age birth weight, and had greater weight gain during early childhood (Delta BMI z -score per year 0.18, 95% CI 0.06–0.30) and higher BMI z -score at 4 years (Delta 0.58, 95% CI 0.18–0.99). Late-pregnancy dysglycemia in GDM-negative mothers accounted for about one-quarter of the association of maternal obesity with offspring BMI at age 4 years. Importantly, the authors conclude that negative GDM testing in obese pregnancies is not an 'all-clear signal'. Instead attention is needed to monitor and control weight gain and glucose levels in obese women in the third-trimester.

15.7. Association of early introduction of solids with infant sleep: A secondary analysis of a randomized clinical trial

Perkin MR, Bahnsen HT, Logan K, Marrs T, Radulovic S, Craven J, Flohr C, Lack G
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JAMA Pediatr 2018;172:e180739.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=29987321>

This randomised controlled trial of 1303 exclusively breastfed 3-month-old UK infants was primarily performed to test the impact of early introduction of solid foods, from age 3 months, on the risk of food allergies (1). Sleep was measured as a secondary trial outcome: the trial showed that early introduction of solids increased infant sleep duration, reduced the frequency of waking at night, and reduced the risk of reported very serious sleep problems.

There is currently debate as to the optimal age to introduce solid foods into infant diets. The main disadvantage of early introduction is the higher risk of gastrointestinal and respiratory tract infections, primarily due to displacement of breast milk. At age 3 months most babies lack the coordination to move solid food from the front of the mouth to the back for swallowing and are at risk of choking. Early introduction of solids is also consistently associated with higher infant weight gain and childhood obesity risk, although some studies show this is association is due to reverse causality (i.e. parents give solids earlier to hungrier infants) (2). Recently, the UK restated its agreement with WHO guidance that solids should be introduced at around age 6 months (3). US and European guidance states 'at 4–6 months', but the European guidance is under review.

The current paper reminds us that when we set guidance for health reasons, we need to be aware of the issues that families are actually concerned about. Longer sleep duration and less frequent night waking are consistent

with the higher energy density contained in solids. Whenever we discuss lifestyle advice, e.g. in diabetes or obesity clinics, we should be aware of the obvious trade-offs between convenience and health outcomes, and acknowledge that these choices are difficult for many families.

References

1. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, Brough H, Marrs T, Radulovic S, Craven J, Flohr C, Lack G; EAT Study Team. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. *N Engl J Med*. 2016 May 5;374(18):1733–43.
2. Vail B, Prentice P, Dunger DB, Hughes IA, Acerini CL, Ong KK. Age at Weaning and Infant Growth: Primary Analysis and Systematic Review. *J Pediatr*. 2015 Aug;167(2):317–24.e1.
3. The Scientific Advisory Committee on Nutrition (SACN). Feeding in the first year of life. www.gov.uk/government/publications/feeding-in-the-first-year-of-life-sacn-report (2018).

15.8. Variation in the heritability of child body mass index by obesogenic home environment

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JAMA Pediatr 2018;172:1153–1160.
<https://www.ncbi.nlm.nih.gov/pubmed/?term=30285028>

This paper describes a gene-environment interaction twin study in 925 UK families (1850 twins). The heritability of childhood BMI was calculated by comparing the concordance in BMI at mean age 4.1 years between monozygous and dizygous twins; heritability was much higher among more obesogenic households ($h^2 = 86\%$) compared to low risk households ($h^2 = 39\%$), as assessed by parent-reported food, physical activity, and media-related influences in the home.

In recent years, we have seen substantial advances in our understanding of the genetic basis of obesity, both severe early onset monogenic obesity with hyperphagia and also the more common susceptibility to overweight and ‘typical’ obesity. It is very important that, alongside these scientific advances, we promote a mature understanding of the role of genetics and heritability in health. One of the most common fallacies is the ‘deterministic’ idea; that having a genetic susceptibility to obesity means there is little you can do to avoid becoming obese. Conversely, the findings of the current study robustly show that carriers of genetic susceptibility to obesity have significantly more to gain by following a healthy lifestyle.

Specifically, the findings of this paper show the particular importance of a healthy home environment. It may be that this makes it more likely that healthy changes in an individual’s behaviour will occur, that the changes will be larger or possibly more sustained. However, this study was of young children, mean age 4.1 years, who may be more influenced by the home environment than older children and adults, whereas overweight and obesity common occurs in older age groups. Similar studies in such older populations are needed.

15.9. Association of youth triponderal mass index vs body mass index with obesity-related outcomes in adulthood

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JAMA Pediatr 2018;172:1192–1195.
<https://www.ncbi.nlm.nih.gov/pubmed/30326018>

The large prospective Cardiovascular Risk in Young Finns Study included 3596 participants aged 3 to 18 years at baseline, and followed the large majority up to 20–30 years later. Comparing various measures of adiposity at baseline, Body Mass Index (BMI) outperformed the Triponderal Mass Index (TMI) and skinfold thicknesses in predicting later Type 2 diabetes, hypertension and LDL cholesterol levels, and carotid intimal thickness.

A paper we described in Yearbook 2017 reported that the TMI was more accurate than BMI as an indicator of childhood adiposity (1). The biological rationale to that observation was that, in children (but not in adults), BMI

is positively related to height – hence an index with a stronger numerical correction for height ($TMI = \text{weight}/\text{height}^3$, compared to $BMI = \text{weight}/\text{height}^2$) should correlate more strongly with body ‘ponderance’ and adiposity. However, as we stated in 2017, accurate prediction of adiposity only partially addresses the definition of obesity: ‘a state of excess fat related to adverse health’.

The current paper now addresses the second part of this definition. Intriguingly, it shows that there is a clear disconnect between accurate prediction of adiposity (TMI is better than BMI) and prediction of adverse metabolic health (BMI is much better than TMI). In our opinion, the reason why childhood BMI better predicts later health is that it is a combined marker not only of childhood adiposity, but also of taller stature, possibly related to a faster tempo of growth and pubertal maturation, which we know is promoted by increased nutrition. So we are happy to continue to use BMI to define thresholds of overweight and obesity in children.

Reference

1. Peterson CM, Su H, Thomas DM, Heo M, Golnabi AH, Pietrobelli A, Heymsfield SB. Tri-Ponderal Mass Index vs Body Mass Index in Estimating Body Fat During Adolescence. *JAMA Pediatr* 2017; 171(7):629–636.

15.10. Growth hormone regulates neuroendocrine responses to weight loss via AgRP neurons

Isadora C Furigo, Priscila DS Teixeira, Gabriel O de Souza, Gisele CL Couto, Guadalupe García Romero, Mario Perelló, Renata Frazão, Lucila L Elias, Martin Metzger, Edward O List, John J Kopchick, J Donato
Nature Communications, 2019; 10 (1); 662
<https://www.nature.com/articles/s41467-019-08607-1>

This paper highlights the brain as a key target for growth hormone (GH) signaling affecting mostly energy conservation. To identify GH response neurons, C57BL/6 mice received intraperitoneal injection of saline or GH and their brains were processed to detect the phosphorylation of pSTAT5 as a marker of GH receptor activation. They found that GH acts directly on brain GH receptors to conserve energy when the body loses weight. It influences the metabolic responses that conserve energy when we are hungry or on a diet with important implications in terms of understanding why it is so hard to lose weight.

Leptin has until now been considered the main hormone that acts to conserve energy when we are hungry. GH acts on the brain in a similar way to leptin. Only that leptin levels fall, and GH levels rise in response to weight loss, while GH receptors, GH-binding proteins and circulating IGF1 levels decrease. Brain GH receptors are located in the hypothalamus. Signals from the hypothalamus influence the cells of the autonomic nervous system and activate Agouti Related Protein (AgRP), which acts to increase appetite and to diminish energy metabolism and expenditure.

Here, the authors studied genetically modified mice with AgRP-specific GH receptor ablation (KO). When subjected to a diet with 60% food restriction, energy expenditure decreased in control mice, but significantly less so in AgRP GHR KO mice, suggesting that they did not save energy as efficiently, and so displayed a higher rate of weight loss, primarily greater loss of fat mass (energy reserves) but also loss of lean mass and the mass of vital organs, i.e. bone, muscle, ligaments, tendons, and body fluids.

So, evolution has endowed humans with two energy conserving mechanisms: one activates by decreasing peripheral leptin; the other by increasing pituitary GH. These findings help to explain why leptin replacement does not completely reverse the neuroendocrine adaptations induced by weight loss, since both GH and leptin play a role informing the brain about energy deficiency. Thus, pharmacological compounds that block GH signaling may prevent the compensatory decrease in energy expenditure during dieting and consequently represent a promising approach to facilitate weight loss.

15.11. A neural circuit for gut-Induced reward

Han W, Tellez LA, Perkins MH, Perez IO, Qu T, Ferreira J, Ferreira TL, Quinn D, Liu ZW, Gao XB, Kaelberer MM, Bohórquez DV, Shammah-Lagnado SJ, de Lartigue G, de Araujo IE
Cell. 2018 Oct 18;175(3):665–678.e23.
<https://www.ncbi.nlm.nih.gov/pubmed/30245012>

This article reports a critical role for the vagal gut-to-brain axis in motivation and reward amongst the sensory cells of the right vagal nerve. Optogenetic stimulation of the mouse vagal gut-to-brain axis produced reward behaviors. Stimulation of gut-innervating vagal sensory neurons recapped the hallmark effects of stimulating the right, but not left, vagal sensory ganglion, induced dopamine release from the *Substantia nigra*, sustained self-stimulation behavior, and conditioned both flavour and place preferences.

It challenges the long-held assumption that vagal sensory neurons only inhibit reward circuits and thereby suppress motivational behavior. Transneuronal optic labeling identified the glutamatergic neurons of the dorsolateral parabrachial pons (relays information from the taste area of the solitary nucleus to the ventral posteromedial nucleus of the thalamus) as the obligatory communication linking the right vagal sensory ganglion to dopamine cells in *Substantia nigra*.

These findings more specifically imply that food reinforcement and satiation should not be considered mutually exclusive physiological processes. Consistently, activating the hypothalamic AgRP-positive “drive” neurons counteract parabrachium-mediated satiety, while conveying negative attractiveness (averseness). Thus, nodose neurons (the inferior ganglion of vagus nerve) mediate reward signals. The right nodose neurons may be particularly sensitive to nutritive signals, whereas the left nodose neurons induced satiation, independently of reward, may preferentially display responses to mechanical distention.

Intuitively we suspected, but it now has scientific evidence: the gut is a major regulator of motivational and emotional states. Moreover, vagal stimulation may become an approach to affective disorders. Accordingly, one possible approach around cardiac complications of satiety drugs may be implanting the stimulator on vagal nerve segments located at the vicinity of the upper gut.

15.12. Ghrelin enhances food odor conditioning in healthy humans: An fMRI study

Han JE, Frasnelli J, Zeighami Y, Larcher K, Boyle J, McConnell T, Malik S, Jones-Gotman M, Dagher A

Cell Reports. 2018 Dec 4;25(10):2643–2652.e4.

<https://www.sciencedirect.com/science/article/pii/S2211124718317728>

More on the gut and the reward response: Ghrelin has been reported to encourage eating through dopamine that is important for the reward response. Here, the authors injected 38 subjects with ghrelin, while exposing them to various odors, both food and non-food based. They were also shown random images of objects, so that over time subjects associated the images with the odors. Using functional magnetic resonance imaging (fMRI), they monitored activity in brain regions known to be involved in reward response from dopamine.

They found that activity in these regions was higher in subjects injected with ghrelin, but only when responding to the images associated with food smells. This means that ghrelin controls the extent to which the brain associates reward with food odors. Following ghrelin injection, participants responded faster to food odor-associated cues and perceived them to be more pleasant, but ghrelin had no effect on their reaction to images associated with non-food odors. Ghrelin also increased functional connectivity between the hippocampus and the ventral striatum.

Obesity is associated with enhanced hypothalamic responses to food, but also to an abnormal reward response to food-related cues that are abandoned in our environment, for example fast food advertising. In 1930, Newburgh and Johnston wrote: “All obese persons are alike in one fundamental respect; they literally overeat”. This study shows that ghrelin may be a major factor in our intensified response to food cues. The brain regions identified have been linked to a vulnerability to obesity, suggesting a genetically-based hypersensitivity to food-associated images and smells.

Reference

1. Newburgh LH, Johnston MW. The Nature of obesity. *J Clin Invest*. 1930 Feb;8(2):197–213.

15.13. Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free fetal DNA

Zhang J, Li J, Saucier JB, Feng Y, Jiang Y, Sinson J, McCombs AK, Schmitt ES, Peacock S, Chen S, Dai H, Ge X, Wang G, Shaw CA, Mei H, Breman A, Xia F, Yang Y, Purgason A, Pourpak A, Chen Z, Wang X, Wang Y, Kulkarni S, Choy KW, Wapner RJ, Van den Veyver IB, Beaudet A, Parmar S, Wong LJ, Eng CM

Nat Med. 2019 Mar;25(3):439–447.

<https://www.nature.com/articles/s41591-018-0334-x>

Prenatal screening for trisomy 21, trisomy 18, trisomy 13, and sex chromosome aneuploidies can be performed using next-generation sequencing of cell-free DNA (cfDNA) in the maternal circulation. This article describes a new non-invasive prenatal screening (NIPS) approach for the detection of de novo or paternally inherited disease-causing variants in 30 genes associated with frequent human dominant monogenic disorders.

Fetal DNA that is circulating in the blood of pregnant women is now frequently extracted noninvasively and screened to detect common fetal chromosome aneuploidies, such as trisomy of chromosome 21. But there are numerous other syndromes that are caused by single gene mutations that are not detected on routine tests.

These authors developed a next-generation DNA sequencing approach for circulating fetal DNA that can detect alterations in 30 genes that cause monogenic disorders, mostly in the *FGFR3* and *FGFR2* genes (causes of skeletal disorders), as well as Noonan spectrum disorders. They developed a single nucleotide polymorphism (SNP)-based fetal fraction calculation method using informative transmitted parental alleles in the fetal cfDNA present in maternal plasma. Of the 422 women tested, 151 (35.8%) had a reported abnormal prenatal ultrasound finding indicative of a fetal developmental abnormality, 3 (0.7%) had abnormal routine serum screening results, and 43 (10.2%) reported a positive family history of genetic disease. Among 151 cases with an abnormal prenatal ultrasound, 28 yielded a positive result for a de novo pathogenic or likely pathogenic variant in one of the 30 genes on the screening panel.

Overall, this new NIPS approach provides valuable molecular information on the fetus for these not uncommon dominant monogenic disorders. The findings will help guide physicians and parents regarding further evaluation and management of their pregnancy.

15.14. Enteroendocrine cells switch hormone expression along the crypt-to-villus BMP signalling gradient

Joep Beumer, Benedetta Artegiani, Yorick Post, Frank Reimann, Fiona Gribble, Thuc Nghi Nguyen, Hongkui Zeng, Maaïke Van den Born, Johan H Van Es, Hans Clevers

Nature Cell Biology 20,909–916 (2018)

<https://www.nature.com/articles/s41556-018-0143-y>

Produced by scattered Enteroendocrine cells (EECs) found along the length of the intestinal epithelium, gut hormones generate signals related to the rate of nutrient absorption, the composition of the luminal milieu and the integrity of the epithelial barrier. To study these rare cells, these authors combined the technology of single-cell sequencing (Science Breakthrough of the Year 2018) with a method to determine the age of each cell. As a result, they could study the development of EECs.

Fewer than 1% of the cells in the intestinal lining are EECs. Scattered throughout the stomach and intestine, EECs control a wide range of physiological processes linked to metabolism. Products of food digestion (i.e., glucose, amino acids and fatty acids) and microbial fermentation act as stimuli for local EECs. Such hormones act as the GI tract communication and coordination with the brain and pancreas and the more distant parts of the digestive tract. In response to defined stimuli, different EECs produce different hormones, which coordinate movement of intestinal muscles, stimulate the repair of the intestine's protective cell layer, induce hunger or satiety or promote the secretion of insulin. Indeed, one of the most successful treatments for diabetes is based on the gut hormone Glucagon-like peptide-1 (GLP1).

Similar to the growth plate columns, EECs are continuously produced and live for several weeks, while changing their position along the villi-crypts structures. While moving and aging, EECs change their hormone expression. BMP4 signals alter the hormone expression profiles of individual EECs to resemble those found in the villus. Accordingly, BMP4 induces hormone switching of EECs migrating up the crypt–villus axis.

Once we understand the signals that control hormone expression, we may be able to stimulate the intestine to differentially generate specific hormones to treat diabetes, obesity or inflammatory bowel disease. But also, bariatric surgery rearranges intestinal anatomy, resulting in markedly elevated postprandial concentrations of GLP 1 and peptide YY, which contribute substantially to postsurgical weight loss and resolution of T2D mellitus.

15.15. Irisin mediates effects on bone and fat via αV integrin receptors

Kim H, Wrann CD, Jedrychowski M, Vidoni S, Kitase Y, Nagano K, Zhou C, Chou J, Parkman VA, Novick SJ, Strutzenberg TS, Pascal BD, Le PT, Brooks DJ, Roche AM, Gerber KK, Mattheis L, Chen W, Tu H, Bouxsein ML, Griffin PR, Baron R, Rosen CJ, Bonewald LF, Spiegelman BM

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Irisin, a cytokine secreted by muscle during exercise, effects bone resorption and the differentiation of preadipocytes into thermogenic brown fat cells by displacing tethers between the cell and extracellular matrix molecules. Physical activity can reverse age-dependent decline in skeletal muscle, preventing osteoporosis, regenerative neurogenesis, hippocampal function, cognitive ability, and neuromuscular junction formation, and the age-dependent recession correlates with Wnt signaling pathway (1). The current study shows that these effects may be mediated by Irisin.

Irisin is a hormone that muscles release in greater amounts during exercise. It binds to bone cells, where it helps new bone cells to take the place of old ones, and mediates certain favorable effects of physical activity. In particular, irisin has been shown to have beneficial effects in bone, adipose tissues, and also brain. These effects are consistent with the known benefits of exercise, such as strengthening bones, increasing energy expenditure, and improving cognition. This study shows that irisin binds to proteins of the αV class of integrins, which has previously been reported to contribute to bone remodeling, and identifies interacting surfaces between irisin and $\alpha V/\beta 5$ integrin. Chemical inhibition of the αV integrins blocks signaling and function by irisin in osteocytes and fat cells. Irisin increases both osteocyte survival and production of sclerostin, a local modulator of bone remodeling. Genetic ablation of irisin completely blocks osteocyte osteolysis induced by ovariectomy, preventing bone loss and supporting an important role of irisin in skeletal remodeling.

The findings show that irisin binds to specific receptors on fat tissue and on the surface of osteocytes. When applied to cultured osteocytes, irisin protected the cells against certain types of cellular damage. Mice that received irisin injections showed elevated levels of sclerostin, an important regulator of the process by which old bone cells break down and are replaced by new ones.

These and previous data suggest that irisin could be a useful target for the treatment of osteoporosis. Although irisin targets bone resorption, intermittent treatment with irisin improves bone density and strength, similar to the dual effects of PTH. There is much evidence that exercise brings improvements in mood and cognition, and irisin might mediate some of these effects in the brain.

Reference

1. Hu S, Yang L, Wu C, Liu TY. Regulation of Wnt signaling by physical exercise in the cell biological processes of the locomotor system. *Physiol Int*. 2019 Mar 1;106(1):1–20.

15.16. Early maturity as the new normal: a century-long study of bone age

Melanie E Boeyer, Richard J Sherwood, Chelsea B Deroche, Dana L Duren

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The century-long Fels Longitudinal Study of human growth and development aimed to track when growth plate fusion started and completed in children born as far back as 1915. Among 1292 children, each with between 1 to 30 serial left hand-wrist radiographs, children born in the 1990s reached skeletal maturity faster and sooner than those born in the 1930s.

In the dialectics of physical anthropology and auxology, ‘bone age’ is erroneously understood to be an expression of the biological maturity of a child. Inferring from a ‘bone age’ film of the hand and wrist, the

clinician may contemplate diagnostic possibilities and predict height potential. He may recommend types of physical activity, or the timing of orthodontic procedures and orthopedic surgery.

The ‘age’ in ‘bone age’ insinuates the concept of precision and unity, which it does not provide, and the assignment of ‘years’ as its units makes little sense. We now have data to suggest that bone maturation is subject to environmental circumstances. The study does not address what might be the cause of faster maturation but hints to exposure to environmental hormones and hormone mimickers. However, it is more likely that the change is due to the earlier and faster puberty that is widely reported in contemporary children. It is surprising that while sex steroids, mostly estrogen, are secreted today much earlier than in the 1930s and menarche occur earlier by as much as 2 years, bones mature faster by only 7 and 10 months in boys and girls, respectively.

These findings directly impact the timing of the clinical care of certain pediatric orthopedic conditions, such as correction of leg-length differences, scoliosis and the timing of growth hormone therapy.

The authors suggest that there is a “new normal” for timing when kids’ skeletons reach full maturity, but says nothing about developmental milestones before epiphyseal fusion. For the pediatric endocrinologist, who is used to individual variability in bone maturation, this is not much of a surprise and we doubt that it requires new norms (1).

Reference

1. Hochberg Z. Diagnosis of endocrine disease. On the need for national-, racial-, or ethnic-specific standards for the assessment of bone maturation. *Eur J Endocrinol.* 2016 Feb;174(2):R65–70.

15.17. Spray dried smectite clay particles as a novel treatment against obesity

Dening TJ, Joyce P, Kovalainen M, Gustafsson H, Prestidge CA

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<https://www.ncbi.nlm.nih.gov/pubmed/30519891>

Smectite purified from bentonite clay, and laponite SD-LAP, a synthetic Smectite were fed to rodents on a high-fat diet. Over a two-week period both the engineered clay and orlistat had weight loss effects.

The unanticipated discovery that clay has a unique ability to “soak up” fat droplets in the gut might have led to a new treatment for obesity. Clay minerals are very common in soils, in fine-grained sedimentary rocks such as shale, mudstone, and siltstone and have been detected at several locations on Mars and asteroids as well as the Jupiter’s moon Europa. Smectite clay minerals have long been investigated as adsorbent materials in the environmental science field and have been used for oral drug delivery owing to their excellent biocompatibility. The strong adsorptive capacity of clay minerals for ionized, polar and/or hydrophobic molecules has been widely investigated for the absorption of environmental contaminants/pollutants from soil, including lipids and fatty acids as byproducts of industrial processes. These investigators exploited the highly adsorptive nature of montmorillonite SD-MMT, a natural Smectite purified from bentonite clay, and laponite SD-LAP, a synthetic Smectite, to develop a novel anti-obesity treatment.

The clay was prepared via spray drying. The ability of SD-MMT and SD-LAP particles to inhibit lipid digestion kinetics and adsorb lipid species from solution was assessed during *in vitro* lipolysis. They were fed to rodents on a high-fat diet and their effect on body weight gain was evaluated. Over a two-week period both the engineered clay and orlistat had weight loss effects, but the clay outperformed the drug. SD-MMT and SD-LAP particles adsorbed 42% and 94% of all lipid species, respectively. They also reduced the rodent weight gain relative to the negative control treatment group and performed similarly to orlistat.

These particles may be developed as novel anti-obesity treatments with fewer adverse effects than currently marketed treatment options. Whereas the orlistat blocks enzymatic digestion of fat molecules, the clay particles trap these fats so they are excreted out of the body without causing gastrointestinal disturbances.

15.18. The gravitostat regulates fat mass in obese male mice while leptin regulates fat mass in lean male mice

Ohlsson C, Hägg DA, Hammarhjelm F, Dalmau Gasull A, Bellman J, Windahl SH, Palsdottir V, Jansson JO
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<https://www.ncbi.nlm.nih.gov/pubmed/29800288>

The authors compared the effects of leptin versus weight loading using intraperitoneal weighted capsules ('gravitostat stimulation') on changes in fat mass in mice. Leptin infusion suppressed body weight and fat mass in lean but not in overweight or obese mice. Weight loading decreased body weight in overweight and obese mice.

We thought that leptin was the major regulator of fat mass. These authors found evidence for another potent regulator, named the 'gravitostat'. They found that increased loading using weighted capsules decreased body weight and fat mass. We are familiar with a similar concept from studies showing the role of bone sensors to weight on skeletal strength. Now we learn that the gravitostat regulates fat mass independently of fat-derived leptin secretion and circulating levels.

Leptin infusion suppressed body weight and fat mass in lean mice given normal chow but not in overweight or obese mice given a high-fat diet. The same disappointment was evident years ago when leptin failed to reduce human obesity. The intraperitoneal implantation of weight capsules had only a nonsignificant tendency to reduce body weight in lean mice, but was more effective in obese mice. The gravitostat regulates fat mass via a circuit based on weight sensors in the osteocyte that amplified the expression of the obesity-promoting neuropeptides AgRP and NPY in the *arcuate nucleus*. These findings demonstrate that the gravitostat regulates fat mass in obese mice, whereas leptin regulates fat mass only in lean mice with low endogenous serum leptin levels.

Thus, leptin and the gravitostat are preferentially active in mice with relatively low and high body weight, respectively. Leptin suppressed body weight and fat mass in lean mice. Increased loading using intraperitoneal capsules with different weights decreased body weight in overweight and obese mice.

It sounds so simple: add external weight and lose body weight.

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