Yearbook of Paediatric Endocrinology 2021

Editors
Ken Ong
Ze'ev Hochberg

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Paediatric Endocrinology 2021

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Preface

While this last year was undoubtedly dominated by COVID-19, it was another inspiring year (June 2020 to May 2021) for publications relating to Paediatric Endocrinology. Among other reports:

– New treatments were described: vosoritide for achondroplasia; 6-monthly leuprolide acetate for precocious puberty; osilodrostat for Cushing's disease; semaglutide for nonalcoholic steatohepatitis and also for severe obesity due to LEPR or POMC mutations; a new 20-kDa Human Placental growth hormone; and the green Mediterranean diet for metabolic syndrome

– New evidence showed that: infliximab decreases the risk of thyroid disorders; dose titration is better than 'block and replace' in Graves' disease; high dose cyproterone acetate increases the risk of intracranial meningioma in women; the long-term safety of rhGH therapy; better than expected heat-stability of various insulins; and the impact of COVID on type 1 diabetes management

– New genes were identified for hypopituitarism, familial short stature, androgen insensitivity syndrome, hypophosphatemic rickets and congenital diabetes with infantile obesity, and susceptibility to chemotherapy-related reduced ovarian function

– New mechanisms include: ceramide signaling in obesity-induced precocious puberty; Kisspeptin deficiency in excess adrenal steroid hormone secretion; the structure and function of the satiety receptor, MC4R, and the high prevalence of clinically relevant variants; and the cellular basis of distinct thirst modalities

When considering our future, our history deserves to be remembered. 100 years ago, Dr. Frederick Banting of the University of Toronto had the idea to make a pancreatic extract that had anti-diabetic qualities. Also in 1921, the first BCG vaccination against tuberculosis was reported, and the American biochemist, Elmer McCollum, identified a component in cod liver oil which cured rickets - he called this vitamin D.

The Yearbook has been coedited by Ze'ev Hochberg ever since its beginning in 2004. Ze'ev originated this concept with Jean-Claude Carel and it has evolved from hardback only, to paperback only, to online only, and is now available in multiple formats (online, pdf, or paperback from Amazon Kindle). We are sorry to announce that Ze'ev will retire from the Yearbook after this year. In his place, we welcome Christa Flück as coeditor from 2022. Looking to the future, we welcome your feedback and comments on the Yearbook format and contents

We warmly acknowledge the generous endorsement and support by ESPE, which makes the Yearbook possible, and the efficient and professional work of Bioscientifica, who turn our raw materials into the polished output. The enthusiasm and hard work of our expert Associate Editors and coauthors makes this an indispensable resource for pediatric endocrinologists and clinical researchers to keep updated with the key findings in our field.

Ze'ev Hochberg (Haifa)
Ken Ong (Cambridge)

1. Pituitary and Neuroendocrinology

Yafei Wang[1], Johanna Hietamäki[1,2], Tero Varimo[2], Matti Hero[2], Päivi J Miettinen[1,2], Taneli Raivio[1,2]
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Preface

In this first chapter of the Yearbook, we present recent advances in the development/ontogeny of the pituitary, genetics of hypopituitarism, clinical and translational aspects, and lastly we describe two papers on the relationship between pituitary function and viral disease. We hope that our selection of papers is both educational and interesting to all readers.

A Comprehensive Review of Hypopituitarism

1.1. Insights into non-classic and emerging causes of hypopituitarism

Prodam F, Caputo M, Mele C, Marzullo P, Aimaretti G
Nat Rev Endocrinol. 2021 Feb;17(2):114–129.
doi: 10.1038/s41574-020-00437-2. PMID: 33247226.

We highly recommend this comprehensive review by Prodam *et al.* to everyone as a starter to this chapter. How many of you knew that hypopituitarism in humans was first described just over 100 hundred years ago? The authors of this excellent review divide the causes of hypopituitarism to (i) pituitary and non-pituitary tumours, (ii) brain damage, (iii) infarction, (iv) autoimmune disorders, (v) genetic causes, (vi) infiltrative diseases, (vii) infections, (viii) drug-induced hypopituitarism, (ix) paraneoplastic syndromes (with anti-PIT1 and anti-POMC autoantibodies), and (x) other causes (such as snake bite!). The current understanding of the genetic causes underlying pituitary hormone deficiency is also summarized.

Development/Ontogeny

1.2. Single nucleus multi-omics regulatory landscape of the murine pituitary

Ruf-Zamojski F, Zhang Z, Zamojski M, Smith GR, Mendelev N, Liu H, Nudelman G, Moriwaki M, Pincas H, Castanon RG, Nair VD, Seenarine N, Amper MAS, Zhou X, Ongaro L, Toufaily C, Schang G, Nery JR, Bartlett A, Aldridge A, Jain N, Childs GV, Troyanskaya OG, Ecker JR, Turgeon JL, Welt CK, Bernard DJ, Sealfon SC
Nat Commun. 2021 May 11;12(1):2677.
doi: 10.1038/s41467-021-22859-w. PMID: 33976139

This work generated an integrated single nucleus multi-omics resource to elucidate the epigenetic mechanisms that regulate transcriptional networks in the murine pituitary. The authors identified epigenetically defined cell type composition, cell type-specific and sex-specific differences in transcriptional and epigenetic programs, an experimentally supported cis-regulatory domain, and epigenetic mechanisms contributing to cell type-specific and sex-specific regulon composition.

Based on this atlas, the authors focused on the *Fshb* gene, and applied co-accessibility analysis, identified a putative regulatory region, which corresponded to the human single nucleotide polymorphism rs11031006. The experimental validation that followed supports the value of co-accessibility analysis, available on the atlas web portal, for the identification of putative cis-regulatory domains for any genes of interest. This is the most

comprehensive atlas related with murine pituitary until now, and provides valuable information, which supports researchers to conduct future study on pituitary development, and identify clinically important new putative regulatory domains.

1.3. Lineage analysis reveals an endodermal contribution to the vertebrate pituitary

Fabian P, Tseng KC, Smeeton J, Lancman JJ, Dong PDS, Cerny R, Crump JG
Science. 2020 Oct 23;370(6515):463-467.
doi: 10.1126/science.aba4767. PMID: 33093109

These researchers used lineage tracing, combined time-lapse imaging with single cell RNA sequencing in zebrafish, and identified that endoderm actually also contributes to the formation of adenohypophysis primordium and resultant pituitary.

The pituitary gland is considered to be formed from an evagination of neural ectoderm that forms the posterior lobe and an invagination of oral ectoderm called Rathke's pouch, which gives rise to the anterior and intermediate lobes. Furthermore, endoderm can generate a rudimentary adenohypophysis-like structure in the near absence of ectodermal contributions. It would be interesting to see whether the endoderm also participates in the pituitary formation in other vertebrates, and if there is any extensive interchange-ability of ectodermal and endodermal epithelia in generating diverse cranial organs.

1.4. Rathke's cleft-like cysts arise from *Isl1* deletion in murine pituitary progenitors

Brinkmeier ML, Bando H, Camarano AC, Fujio S, Yoshimoto K, de Souza FS, Camper SA
J Clin Invest. 2020 Aug 3;130(8):4501–4515.
doi: 10.1172/JCI136745. PMID: 32453714.

This study used mouse models to investigate the role of LIM homeodomain transcription factor *Isl1* in pituitary development. It reveals that *Isl1* has multiple, critical roles in pituitary gland development. Pituitary-specific *Isl1* deletion caused hypopituitarism with increased stem cell apoptosis, reduced both the differentiation of thyrotropes and gonadotropes and body size, and even promoted the development of multiple Rathke's cleft-like cysts.

These studies support a model whereby expression of *Isl1* in pituitary progenitors drives differentiation into thyrotropes and gonadotropes and without it, activation of *Foxa1* and *Foxj1* permits the development of an oral epithelial cell fate with mucinous cysts. This comprehensive mechanistic study, focusing on a specific gene, provides a basis for disease prediction and diagnosis.

1.5. Cellular and molecular properties of neural progenitors in the developing mammalian hypothalamus

Zhou X, Zhong S, Peng H, Liu J, Ding W, Sun L, Ma Q, Liu Z, Chen R, Wu Q, Wang X
Nat Commun. 2020 Aug 13;11(1):4063.
doi: 10.1038/s41467-020-17890-2. PMID: 32792525.

These researchers proposed the heterogeneity of hypothalamic neural progenitor cells. Other than traditional hypothalamic radial glial (hRG) cells, which are predominantly located in the ventricular zone, there are additional basal progenitors in the mantle zone, which are named as hypothalamic mantle zone radial glial (hmRG) cells. The researchers utilized lineage tracing to reveal the movement and division properties of hRG

and hmRG cells. Meanwhile, they applied single cell RNA sequencing to investigate the diversity of hypothalamic neural progenitors, and found that *HMGA2*, a newly discovered neural progenitor marker, is involved in *E2F1* pathway, regulating the proliferation of progenitor cells by targeting the downstream *MYBL2*.

This study provides the cellular and molecular features of different types of hypothalamic neural progenitors, which are essential for addressing the mechanism of hypothalamic neurogenesis.

1.6. The histone H3-lysine 4-methyltransferase Mll4 regulates the development of growth hormone-releasing hormone-producing neurons in the mouse hypothalamus

Huisman C, Kim YA, Jeon S, Shin B, Choi J, Lim SJ, Youn SM, Park Y, K C M, Kim S, Lee SK, Lee S, Lee JW
Nat Commun. 2021 Jan 11;12(1):256.
doi: 10.1038/s41467-020-20511-7. PMID: 33431871.

These authors report two *Mll4* mutant mouse models that exhibited dwarfism and altered development of GHRH－neurons.

Inactivating mutations in *KDM6A* (aka *UTX*) or *KMT2D* (aka *MLL4*) genes result in Kabuki syndrome (KS), whose hallmarks include facial features, intellectual disability of variable degree, skeletal abnormalities, and postnatal growth retardation. *MLL4* encodes a histone H3－lysine 4－methyltransferase, and together MLL4 and UTX form the MLL4－complex, which controls gene expression. This study identifies the transcription factor Nrf1, that interacts with Mll4, to be mediator of Mll4 actions. By integrating Mll4 and Nrf1 ChIP－seq datasets with single cell RNA－seq analyses of E15 ARC neurons, the authors pinpointed 83 genes most specifically enriched in the developing GHRH neurons relative to other ARC neuronal types. Treatment of the *Mll4* mutant mice with the histone deacetylase inhibitor, AR－42, rescued the histone mark signature and restored GHRH－neuronal production.

The authors conclude that developmental dysregulation of Mll4－directed epigenetic control of transcription plays a role in the development of GHRH－neurons and dwarfism phenotype in mice.

1.7. Activating mutations in BRAF disrupt the hypothalamo-pituitary axis leading to hypopituitarism in mice and humans

Gualtieri A, Kyprianou N, Gregory LC, Vignola ML, Nicholson JG, Tan R, Inoue SI, Scagliotti V, Casado P, Blackburn J, Abollo-Jimenez F, Marinelli E, Besser REJ, Högler W, Karen Temple I, Davies JH, Gagunashvili A, Robinson ICAF, Camper SA, Davis SW, Cutillas PR, Gevers EF, Aoki Y, Dattani MT, Gaston-Massuet C
Nat Commun. 2021 Apr 1;12(1):2028.
doi: 10.1038/s41467-021-21712-4. PMID: 33795686.

The authors describe 5 patients with Cardio-Facio-Cutaneous (CFC) syndrome with features of septo－optic dysplasia (SOD), and GH/IGF－1 deficiency of variable degree. All were identified to carry a gain－of－function mutation in *BRAF*.

RASopathies encompass Noonan syndrome, Cardio－Facio－Cutaneous (CFC) syndrome, and neurofibromatosis type 1. They are a class of developmental syndromes that result from germline mutations in the genes encoding the components of the ERK/MAPK pathway. One of these genes is *BRAF*, in which mutations have been described to cause various RASopathies.

The authors found that *BRAF* is expressed along the developing human hypothalamus－pituitary axis. Activation of the ERK/MAPK pathway in mouse pituitary progenitors (*Prop1:Cre;BrafV600E/+*) resulted in the absence of terminally differentiated somatotroph, thyrotroph, and gonadotroph cells at E17.5. Murine knock－in of a CFC－causing human *BRAF* mutation (Q241R) also led to deficient differentiation of pituitary

hormone−producing cells. Finally, activation of the ERK/MAPK pathway by expressing both the *Braf*V600E and the *Braf*Q241R alleles reduced Pit1−dependent terminal differentiation of the somatotrophs (GH) and thyrotrophs (TSH), while increasing the number of ACTH+ve and PRL+ve cells.

The authors conclude that these findings show a critical role of BRAF in hypothalamo−pituitary−axis development both in mouse and human. For the clinician, it is important to look for pituitary hormone deficiencies in patients with RASopathies.

Genetics

1.8. Comprehensive identification of pathogenic gene variants in patients with neuroendocrine disorders.

Vishnopolska SA, Mercogliano MF, Camilletti MA, Mortensen AH, Braslavsky D, Keselman A, Bergadá I, Olivieri F, Miranda L, Marino R, Ramírez P, Pérez GN, Patiño Mejia H, Ciaccio M, Di Palma MI, Belgorosky A, Martí Marcelo A, Kitzman JO, Camper SA, Pérez-Millán MI

J Clin Endocrinol Metab. 2021 Mar 17:dgab177.
doi: 10.1210/clinem/dgab177. PMID: 33729509.

Vishnopolska *et al.* conducted a comprehensive evaluation of 67 hypopituitarism candidate genes in 170 congenital hypopituitarism (CH) patients from Argentina by using small molecule molecular inversion probes followed by sequencing (smMIPS) panel. 57 patients had isolated growth hormone deficiency and 113 patients had combined pituitary hormone deficiency (CPHD). Plausible disease associated variants were identified in 26 cases (15.3%), including 13 cases with unique variants meeting the American College of Medical Genetics criteria for pathogenicity or likely pathogenicity. Variants were identified in previously reported CH candidate genes, including: *LHX3*, *LHX4*, *GLI2*, *OTX2*, *HESX1*, *FOXA2*, *BMP4*, *FGFR1*, *PROKR2*, and *PNPLA6*. In addition, this study added 4 new genes to the list of CH candidate genes: *BMP2*, *HMGA2*, *HNF1A*, and *NKX2-1*.

1.9. Requirement of FAT and DCHS protocadherins during hypothalamic-pituitary development

Lodge EJ, Xekouki P, Silva TS, Kochi C, Longui CA, Faucz FR, Santambrogio A, Mills JL, Pankratz N, Lane J, Sosnowska D, Hodgson T, Patist AL, Francis-West P, Helmbacher F, Stratakis C, Andoniadou CL

JCI Insight. 2020 Oct 27;5(23):e134310.
doi: 10.1172/jci.insight.134310. PMID: 33108146.

Lodge *et al.* screened 28 patients with pituitary stalk interruption syndrome (PSIS) for mutations in the FAT/DCHS (FAT atypical cadherin/ Dachsous cadherin-related) family of protocadherins. *FAT2* and *DCHS2* putative damaging variants were found in 6/28 patients with ectopic posterior pituitary (EPP) and PSIS. The affected patients had either multiple pituitary hormone deficiency (MPHD) or isolated growth hormone deficiency.

New players participating in the development of the hypothalamus and the pituitary gland are found regularly. The authors focused on these ligand-receptor pairs because of previous reports supporting their possible involvement in hypothalamic-pituitary axis development or function. They also showed that *FAT2* and *DCHS* are expressed in the mesenchyme of developing human pituitary. When knocked out in a mouse model, the phenotype includes infundibular abnormalities and disturbance of the anterior pituitary morphogenesis. Nevertheless, all anterior pituitary cell types could be detected which, suggests that the etiology of MPHD caused by mutations is not defective cell differentiation.

All in all, we should keep *FAT2 and DCHS2* gene defects in the differential diagnostic list of EPP and PSIS.

1.10. MITOL dysfunction causes dwarfism with anterior pituitary hypoplasia

Matsuno K, Nagashima S, Shiiba I, Taniwaka K, Takeda K, Tokuyama T, Ito N, Matsushita N, Fukuda T, Ishido S, Inatome R, Yanagi S

J Biochem. 2020 Sep 1;168(3):305-312.
doi: 10.1093/jb/mvaa050. PMID: 32302394.

This study shows that mice mitochondrial regulatory gene *Mitol*-deficient mice display reduced growth in combination with anterior pituitary hypoplasia and reduced growth hormone levels.

MITOL encodes a ubiquitin ligase. Ablation of *Mitol* in nestin-expressing cells led to reduced expression of *Gh*, *Prl*, *Ghrhr*, and *Pit1*, suggesting dysregulation of pituitary transcription factor Pit1 by a yet unidentified mechanism. MITOL expression was significantly reduced in the posterior pituitary of the *Mitol*-deficient mice. Interestingly, the anterior pituitary MITOL expression was not remarkably changed, and, given that the nestin-positive anterior pituitary cell population in mice is very small, the authors hypothesized that it represented somatotroph progenitors that fail to differentiate into GH-secreting cells.

Many patients with mitochondrial diseases present with growth failure. These findings suggest that mitochondrial dysfunction may possibly underlie cases with unexplained hypopituitarism.

1.11. Pituitary stalk interruption syndrome broadens the clinical spectrum of the TTC26 ciliopathy

David O, Eskin-Schwartz M, Ling G, Dolgin V, Kristal E, Benkowitz E, Osyntsov L, Gradstein L, Birk OS, Loewenthal N, Yerushalmi B

Clin Genet. 2020 Sep;98(3):303-307.
doi: 10.1111/cge.13805. PMID: 32617964.

In this case series, David et al. describe clinical features of 4 patients in 2 unrelated consanguineous families with *TTC26* ciliopathy due to a homozygous c.695A > G p.Asn232Ser mutation. Three of the patients had MRI findings consistent with pituitary stalk interruption syndrome (PSIS), a congenital anomaly of the pituitary gland. Additionally, 2 of 3 patients with PSIS had multiple anterior pituitary hormone deficiency. Three patients also had ocular manifestations, including isolated bilateral esotropia, unilateral retinal coloboma, microphthalmia, corneal dystrophy, and optic disc edema, suggesting that ocular abnormalities are a part of the phenotype associated with defective *TTC26*.

Ciliopathies are a group of disorders characterized by impaired ciliary function and structure, and manifest in varying phenotypes. Recessively inherited mutations in *TTC26* are known to cause severe biliary ciliopathy, and also involve other organ systems such as the brain, kidneys, and hearth. Although *TTC26* is highly expressed in the pituitary and its deficiency dysregulates sonic hedgehog (SHH) signaling, the presence of pituitary gland involvement in ciliopathy caused by mutations in *TTC26* has not been described before.

Altogether, this study describes a novel PSIS phenotype in patients with ciliopathy caused by mutations in *TTC26*. The findings support the view that cilia and SHH signaling are important in pituitary and ocular embryonic development.

Clinical/Translational

1.12. Pituitary incidentalomas in paediatric population: Incidence and characteristics

Shareef M, Nasrallah MP, AlArab N, Atweh LA, Zadeh C, Hourani R

Clin Endocrinol (Oxf). 2021 Feb;94(2):269–276.
doi: 10.1111/cen.14353. PMID: 33098093.

Every clinician encounters sometimes tricky incidentalomas, *i.e.*, non-symptom-related imaging findings that are a consequence of increased availability and resolution of radiologic imaging.

This study evaluated pediatric pituitary incidentalomas (PIs). Pituitary microadenomas, which are frequently encountered in adults, were rare in children. Any PI was detected in 22:1000 pediatric patients screened by any MRI protocol that including the sellar region, and the incidence was higher in older children. The most common pediatric PIs were Rathke's cleft cysts (67.7%), followed by cystic lesions (19.4%). Only 12.9% of pediatric PIs were microadenomas.

The only endocrine abnormality found in the microadenoma patients ($n=7$) was mild hyperprolactinemia (although endocrine investigations were available only in $n=4$), which suggests that the adult microadenoma investigation strategy is valid also for pediatric patients: hence, all patients should be screened for hyperprolactinemia, and additional ACTH and GH axis evaluation is encouraged, especially if clinical suspicion. The study does not give a direct answer for how pediatric patients with PIs should be followed up. However, the most frequent pediatric PIs, Rathke's cleft cysts and pituitary cystic lesions, rarely grow in size.

1.13. Anterior pituitary function in Rathke's cleft cysts versus nonfunctioning pituitary adenomas

Fujii M, Nakagawa A, Tachibana O, Iizuka H, Koya D
Endocr J. 2021 Apr 3.
doi: 10.1507/endocrj.EJ21-0050. PMID: 33814485.

Mizue Fujii and colleagues retrospectively evaluated the pituitary function in 67 and 111 adult patients with Rathke's cleft cyst (RCC) and non-functioning pituitary adenoma (NFA), respectively. The study population represented consecutive patients encountered in their institute. They found that RCCs were smaller than NFAs and that anterior pituitary hormonal deficiencies were more frequent in the latter. A positive correlation was found between cyst/tumor size and a number of impaired hormones. However, some patients with even small RCCs exhibited pituitary dysfunction. Interestingly, compared with similar-sized NFAs, small RCCs tended to present more pituitary hormone deficiencies, which suggests that RCCs may impair hormone secretion more easily than NFAs. The authors speculate that this results from the location of RCCs between the anterior and posterior lobes, where they can potentially block anterior pituitary blood flow more effectively.

1.14. Pituitary germinomas: a multi-institutional study analyzing patient demographics and management patterns

Bhimani AD, Barrington NM, Aguilar TM, Arnone GD, Mehta AI
Pituitary. 2020 Aug;23(4):381-388.
doi: 10.1007/s11102-020-01042-2. PMID: 32388804.

Bhimani and colleagues analysed the National Cancer Institute's register data of pediatric and adult patients to describe patient demographics and management of intracranial germinomas, reporting the largest case series ($n=92$) to date.

In their cohort, pediatric patients were more likely to undergo chemotherapy as part of treatment, while adults were more likely to undergo surgical resection. Increased overall survival was associated with: younger age, radiation as a treatment component, and chemotherapy, but not surgery. In line with previous reports, young adult males predominated this pituitary germinoma population.

Intracranial germinomas are very rare tumors found in the suprasellar and pineal regions. These findings underline the need for clinicians to be particularly careful to exclude pituitary germinoma in this age group when imaging is inconclusive. This report is of high clinical importance, as the diagnosis of intracranial germinoma is frequently delayed due to insidious symptoms and inconclusive MRI findings.

1.15. Clinical outcomes and complications of pituitary blastoma

Liu APY, Kelsey MM, Sabbaghian N, Park SH, Deal CL, Esbenshade AJ, Ploner O, Peet A, Traunecker H, Ahmed YHE, Zacharin M, Tiulpakov A, Lapshina AM, Walter AW, Dutta P, Rai A, Korbonits M, de Kock L, Nichols KE, Foulkes WD, Priest JR

J Clin Endocrinol Metab. 2021 Jan 23;106(2):351–363.
doi: 10.1210/clinem/dgaa857. PMID: 33236116.

Here, the authors report the long−term outcomes of all 17 known, well−investigated cases of pituitary blastoma. The median age at diagnosis was 11 months, and the most frequent presentations were Cushing syndrome ($n=10$), cranial nerve palsies including ophthalmoplegia ($n=7$), reduced visual acuity ($n=4$), developmental delay ($n=4$), and symptoms of increased intracranial pressure ($n=3$). At a median follow−up of 6.7 years, 9 patients were alive. Chronic complications included neuroendocrine ($n=8$), visual ($n=4$), and neurodevelopmental ($n=3$) deficits. 16/17 cases were attributed to DICER1 abnormalities.

Post−transcriptional silencing of genes is mediated through several small RNA types, which are formed by processing precursor RNAs by the enzyme DICER1. Rare loss−of−function mutations in DICER1 gene give rise to DICER1 tumor predisposition syndrome. Pituitary blastoma, in turn, is a rare, potentially lethal tumor of the pituitary gland that occurs primarily in early childhood, and is highly suggestive of DICER1 syndrome.

These findings confirm that pituitary blastoma is a locally destructive tumor associated with high mortality.

Pituitary Function And Viral Diseases

1.16 COVID-19 and the pituitary

Frara S, Allora A, Castellino L, di Filippo L, Loli P, Giustina A

Pituitary. 2021 Jun;24(3):465–481.
doi: 10.1007/s11102-021-01148-1. PMID: 33939057.

Frara *et al*. review the main endocrine manifestations of COVID-19 with its implications for pituitary diseases. The authors report on the possible direct and indirect involvement of the pituitary gland in COVID-19 infection, and also the impact of COVID-19 on the management of pituitary diseases. Patients with comorbidities associated with hypopituitarism e.g. type 2 diabetes, hypertension, obesity and adrenal insufficiency, have an increased risk for developing a serious COVID-19 infection.

As many already know, coronavirus enters the host cells by the viral transmembrane spike glycoprotein binding to the metallopeptidase ACE2. ACE2 mRNA is expressed also in the hypothalamus, the pituitary gland, and beta cells, which makes them targets for COVID-19. Although no cases of hypopituitarism have been reported, recently several reports have been published of pituitary apoplexy associated with COVID-19 with or without a pre-existing macroadenoma.

The review reminds us that the clinical phenotype of COVID-19 comes close to endocrinology and our patients and warrants for data driven recommendations to guide management.

1.17. Clinical and biochemical features of hypopituitarism among brazilian children with Zika virus-induced microcephaly

Ferreira LL, Aguilar Ticona JP, Silveira-Mattos PS, Arriaga MB, Moscato TB, Conceição GC, Santos ACD, Costa F, Alves CAD, Antonini SR

JAMA Netw Open. 2021 May 3;4(5):e219878.
doi: 10.1001/jamanetworkopen.2021.987.

Ferreira *et al.* assessed postnatal growth and possible hypopituitarism at the age of 27 months in 65 children with microcephaly (head circumference, HC, $<$ -2 S.D.) associated with congenital Zika virus (CZV) infection. Despite short stature, marked microcephaly and severe brain abnormalities, no case of growth hormone or

thyrotropin deficiency was detected. However, central adrenal insufficiency and diabetes insipidus were diagnosed in a few patients.

Zika virus (ZV) was introduced to northeast Brazil between 2013-2015. It infects progenitor neuron cells, resulting in increased neuronal apoptosis and disturbed brain development during pregnancy. This study was based in Bahia, one of the Brazilian epicenters of the ZV epidemic. They evaluated clinical (micropenis, prolonged jaundice, neonatal hypoglycemia, poor growth, polyuria) and biochemical parameters (electrolytes, morning free T4, TSH, cortisol, ACTH, prolactin, IGF1, and IGFBP3, but no growth hormone stimulation tests).

Although CSV children were born SGA, they did not show postnatal catch-up growth and were short at evaluation (44.0% had length $<$ -2 S.D.). 23 children had severe microcephaly (median HC -7.0 S.D.) and 29 children and mild/moderate microcephaly (HC -4.9 S.D.). Most of them (61/65) had severe brain abnormalities (e.g. midline brain defects, optic nerve atrophy or agenesis/ hypoplasia of corpus callosum).

Newly evolving viral diseases will present challenges for pediatric endocrinologists, but they also provide natural models to inform understanding of pituitary gland development and its vulnerability to various insults. Careful follow-up of CSK is warranted to show whether GH or other pituitary hormone deficiencies will develop.

2. Antenatal and Neonatal Endocrinology

Khalid Hussain

Neonatal Hypoglycaemia

2.1. Continuous glucose monitoring for the prevention of morbidity and mortality in preterm infants

Galderisi A, Bruschettini M, Russo C, Hall R, Trevisanuto D
Cochrane Database Syst Rev. 2020 Dec 21;12:CD013309.
doi:10.1002/14651858.CD013309.pub2. PMID: 33348448.

This Cochrane review aimed to assess the use of continuous glucose monitoring (CGM) in preterm infants. It finds limited evidence to support the use of CGM devices to improve mortality or morbidity in preterm infants.

Both hypoglycaemia and hyperglycaemia are risk factors for adverse neurodevelopmental outcome in preterm infants. Abnormalities in glucose homeostasis in preterm infants are associated with permanent brain injury, retinopathy, sepsis, intraventricular haemorrhage and death (1). The routine detection of both hypoglycaemia and hyperglycaemia requires point of care glucometers with regular and frequent blood sampling usually by painful heel pricks. The use of CGM devices which measure subcutaneous glucose continuously for the detection of hypoglycaemia and hyperglycaemia could potentially reduce the frequency of blood sampling and help control blood glucose levels in preterm infants. However, the risks, benefits and accuracy of CGM are not known in preterm infants.

In this review, only 4 trials fulfilled the inclusion criteria – none reported neurodevelopmental outcomes for the preterm infants, and the patient numbers were small for trials with data on survival. Thus, the strength of evidence is limited in supporting the use of CGM devices to improve mortality or morbidity in preterm infants and the authors recommend that more research is needed on this topic.

Reference

1. Hays SP, Smith EO, Sunehag AL. Hyperglycemia is a risk factor for early death and morbidity in extremely low birthweight infants. *Pediatrics*. 2006 Nov; 118(5):1811–8.

2.2. Accuracy of continuous glucose monitoring in preterm infants: a systematic review and meta-analysis.

Nava C, Modiano Hedenmalm A, Borys F, Hooft L, Bruschettini M, Jenniskens K
BMJ Open. 2020 Dec 24;10(12):e045335.
doi:10.1136/bmjopen-2020-045335. PMID: 33361084

This BMJ review aimed to assess the accuracy of CGM devices to detect hypoglycaemia and hyperglycaemia in preterm infants. The key take home messages from the BMJ review were that the sensitivity for CGM devices to diagnose and detect hypoglycaemia in preterm infants was poor but the specificity was high. On the other hand, the sensitivity and specificity for CGM devices to diagnose and detect hyperglycaemia were high.

Thus, CGM devices will not correctly detect all episodes of hypoglycaemia and should therefore be supplemented with additional measures for glucose monitoring. CGM devices can be used to monitor trends of blood glucose in these preterm infants. Several technological issues (1) will need to be addressed before CGM devices can be recommended for routine glucose monitoring in neonatal intensive care units.

Reference
1. McKinlay CJD, Chase JG, Dickson J, Harris DL, Alsweiler JM, Harding JE. Continuous glucose monitoring in neonates: a review. *Maternal Health Neonatol Perinatol*. 2017 Oct 17;3:18.

2.3. Neonatal hyperglycaemia is associated with worse neurodevelopmental outcomes in extremely preterm infants.

Zamir I, Stoltz Sjöström E, Ahlsson F, Hansen-Pupp I, Serenius F, Domellöf M
Arch Dis Child Fetal Neonatal Ed. 2021 Apr 16. Epub ahead of print.
doi: 10.1136/archdischild-2020-319926. PMID: 33863775.

This observational study highlights the potential detrimental long-term effects of neonatal hyperglycaemia on neurodevelopmental outcomes in extremely preterm infants, while the benefit of insulin treatment remains unclear.

Hyperglycaemia is common in the extremely preterm infant and has been associated with neurodevelopmental disability. Insulin has been used to treatment the hyperglycaemia in extreme preterm infants with the aim of improving the neurodevelopmental outcome but the results are conflicting. A retrospective study (1) in preterm infants did not observe an association between insulin treatment and neurodevelopmental outcomes at 1 year of age. Another retrospective study (2) observed that infants treated with insulin for neonatal hyperglycaemia had a higher incidence of abnormal neurological development at 2 years of age compared with infants not exposed to hyperglycaemia.

This current study is one of the largest studies conducted in extreme preterm infants investigating the associations between neonatal hyperglycaemia and neurodevelopmental outcomes in childhood. Neonatal hyperglycaemia (defined as blood glucose >8 mmol/l) and longer duration of hyperglycaemia were associated with lower intelligence scores and worse motor outcomes at 6.5 years of age. Insulin treatment in hyperglycaemic infants was not associated with neurodevelopmental outcomes at 6.5 years of age. The mechanisms behind the possible effect of hyperglycaemia on neurocognitive functions are unclear. In rats subjected to hyperglycaemia there is lower brain weight and increased apoptosis especially in the hippocampus region and neonatal hyperglycaemia was found to induce oxidative stress and an increase in inflammatory cytokines, with ensuing microglial activation and astrocytosis (3). Further studies, including adequately powered randomised controlled trials, are needed to better define neonatal hyperglycaemia in preterm infants, to guide its treatment and to clarify its consequences.

Reference
1. van der Lugt NM, Smits-Wintjens VE, van Zwieten PH. Walther FJ. Short- and long-term outcome of neonatal hyperglycemia in very preterm infants: a retrospective follow-up study. *BMC Pediatr* **10,** 52 (2010). https://doi.org/10.1186/1471-2431-10-52.
2. Morgan C. The potential risks and benefits of insulin treatment in hyperglycaemic preterm neonates. *Early Hum Dev*. 2015;**91**:655–9.
3. Tayman C, Yis U, Hirfanoglu I, Oztekin O, Göktaş G, Bilgin BC. Effects of hyperglycemia on the developing brain in newborns. *Pediatr Neurol*. 2014 Aug;51(2):239–45.

2.4. Glucose Profiles in Healthy Term Infants in the First 5 Days: The Glucose in Well Babies (GLOW) Study.

Harris DL, Weston PJ, Gamble GD, Harding JE
J Pediatr. 2020 Aug;223:34–41.e4.
doi:10.1016/j.jpeds.2020.02.079. PMID: 32381469.

In this study, the plasma and interstitial (GCMS) glucose levels were measured in term healthy newborns for a period of 5 days to understand the changes in the glucose levels after birth. The findings suggest that in normal

term healthy newborns if hypoglycaemia persists after the 4th day of life, then further investigations are needed to rule out other causes of persistent hypoglycaemia.

Immediately after birth, blood glucose concentrations drop in the newborn due to the abrupt cessation of glucose delivery from the placenta. This drop in blood glucose triggers a counter regulatory responses to restore the blood glucose to normal values. These counter regulatory responses include reduction in beta cell insulin release, increase in alpha cell glucagon, increase in cortisol and growth hormone secretion, increases in adrenaline and noradrenaline, activation of glycogenolysis, gluconeogenesis, lipolysis and ketogenesis. This phase of transition from birth to the time when blood glucose levels stabilize is called transitional neonatal hypoglycaemia.

Based on the findings of this GLOW study, the period of transitional neonatal hypoglycaemia is around 4 days in normal healthy mostly breast-fed infants after which the blood glucose levels stabilize to normal values. Even in these normal healthy term infants the patterns of glucose concentrations varied widely (with many babies having glucose levels below the accepted thresholds for treatment of hypoglycemia) during the transition phase. These observations suggest that in normal term healthy newborns, if hypoglycaemia persists after the 4th day of life then they will need further investigations to rule out other causes of persistent hypoglycaemia. However, it is important to note that these observations are based only on term healthy newborns who are not at risk of any persistent hypoglycaemia condition. Newborns at risk of any persistent hypoglycaemia condition may require early intervention and management of the hypoglycaemia and not wait until day 4 of life.

2.5. SUR1-mutant iPS cell-derived islets recapitulate the pathophysiology of congenital hyperinsulinism.

Lithovius V, Saarimäki-Vire J, Balboa D, Ibrahim H, Montaser H, Barsby T, Otonkoski T
Diabetologia. 2021 Mar;64(3):630-640.
doi:10.1007/s00125-020-05346-7. PMID: 33404684.

The derivation of iPSCs and their subsequent conversion to islet like clusters from a patient with diffuse CHI due to a homozygous mutation in the *ABCC8* provided these authors a unique opportunity to study the molecular basis of CHI and to develop potential novel treatment options by screening for new drug targets in-vitro. The engineered iPSCs and the islet like clusters and their implantation into NOD-SCID gamma mice recapitulates the phenotype of human CHI extremely well. The mice have typical biochemical features of hyperinsulinaemic hypoglycaemia and histologically the islet like clusters in the mice show increased beta-cell mass and proliferation as well as increased nuclear size.

Congenital hyperinsulinism (CHI) leads to unregulated insulin secretion and severe hypoglycaemia especially in the newborn period. The most common cause of medically unresponsive CHI is mutations in the genes *ABCC8* and *KCNJ11* encoding the 2 subunits (SUR1 and KIR6.2 respectively) of the pancreatic beta-cell K_{ATP} channel. Patients with CHI due to recessive or dominant *ABCC8/KCNJ11* mutations typically have diffuse disease and require a near total pancreatectomy to alleviate the hypoglycaemia. However, the near total pancreatectomy in the long-term will lead to lifelong diabetes mellitus and pancreatic exocrine insufficiency. Rodent models of CHI do not recapitulate the typical clinical and biochemical features of CHI as observed in humans. Current treatment options are limited for patients with diffuse CHI who do not respond to conventional treatment options (like diazoxide). Thus, this novel model of iPSC derived CHI will be extremely valuable for studying new treatment options for diffuse forms of CHI.

2.6. Biphasic dynamics of beta cell mass in a mouse model of congenital hyperinsulinism: implications for type 2 diabetes.

Tornovsky-Babeay S, Weinberg-Corem N, Ben-Haroush Schyr R, Avrahami D, Lavi J, Feleke E, Kaestner KH, Dor Y, Glaser B
Diabetologia. 2021 May;64(5):1133-1143.
doi:10.1007/s00125-021-05390-x. PMID: 33558985.

In order to gain some insight into the potential mechanism/s of diminished beta cell function over time, this mouse model of CHI was developed with an activating GCK (Glucokinase) mutation. In the short term, the mice

developed mild fasting hypoglycaemia (this was very mild with fasting blood glucose 3.6mmol/L) associated with increased beta-cell proliferation and mass. However, in the long-term, impaired glucose tolerance developed associated with decreased beta cell mass but with preserved beta cell function. At a cellular level there was evidence of beta-cell DNA damage.

Hyperglycaemia (as in type 2 diabetes) elicits both short term and long-term changes in beta-cell function and mass. In the short-term beta-cells respond to hyperglycaemia by increasing insulin secretion thus lowering the blood glucose and maintaining normoglycaemia. However, long-term exposure to hyperglycaemia is thought to lead to exhaustion of beta-cells with diminished insulin secretion leading to persistence of the hyperglycaemia. Similarly, some forms of congenital hyperinsulinism (CHI) also improve over time and can develop diabetes in the long-term. The underlying mechanisms that potentially lead to beta-cell exhaustion are not clear.

The key question addressed in this manuscript was whether increased workload of the beta cell per se is responsible for the beta cell toxicity. GCK is a key enzyme regulating beta-cell flux; it converts glucose into glucose-6-phosphate and is termed the beta-cell glucose sensor. Activating GCK mutations lead to a rare form CHI and can be thought of as a model of increased beta cell workload. Thus, these observations suggest that increased beta cell workload induces a reduction in beta cell mass in the long-term. Whether this also applies to the situation with type 2 diabetes will need further investigations.

2.7. Possible new strategies for the treatment of congenital hyperinsulinism

Sikimic J, Hoffmeister T, Gresch A, Kaiser J, Barthlen W, Wolke C, Wieland I, Lendeckel U, Krippeit-Drews P, Düfer M, Drews G *Front Endocrinol (Lausanne)*. 2020 Oct 27;11:545638. doi:10.3389/fendo.2020.545638. PMID: 33193079.

Using human islets from CHI patients and islets from *ABCC8* (SUR1) knockout mice, the authors tested several novel compounds to inhibit insulin over-secretion. These novel compounds targeted K_{ATP} channels as well as K_{ATP} independent channels (such as Ca^{2+}-activated K^+ channels of intermediate conductance ($K_{Ca}3.1$) and L-type Ca^{2+} channels).

Congenital hyperinsulinism (CHI) leads to severe hypoglycaemia in the neonatal and infancy periods. Currently mutations in 14 different genes lead to CHI, most commonly mutations in the genes *ABCC8/KCNJ11* which encode the SUR1/KIR6.2 subunits of the pancreatic beta-cell K_{ATP} channel. Patients with recessive (or in some cases dominant) mutations in *ABCC8/KCNJ11* typically do not respond to conventional medical treatments such as diazoxide (which works by opening the K_{ATP} channel) and need near-total pancreatectomy to reduce the severity of hypoglycaemia, but with long-term side-effects of lifelong diabetes mellitus and pancreatic exocrine insufficiency. Thus, there is an urgent need to develop new medical treatment options for these patients.

Compound NN415 is similar to diazoxide but more potent and more selective for the SUR1 protein. This compound altered calcium homeostasis in human CHI islets but not in the mouse SUR1 islets (suggesting it only works if there are some K_{ATP} channels). Two other compounds (VU0071063 and DCEBIO which are also known to act on the K_{ATP} channels) altered calcium homeostasis in both human and mouse SUR1 islets. Other known drugs (such as dextromethorphan and simvastatin) which are used for treating other medical conditions were also tested and diminished calcium levels in both human and mouse SUR1 islets. Dextromethorphan inhibited L-type calcium channels and lowered the intracellular calcium levels in depolarized beta-cells thus inhibiting insulin secretion. Simvastatin also lowered insulin secretion by diminishing calcium entry via the L-type calcium channels.

These observations suggest potential novel treatments for CHI patients with diffuse disease and these compounds and existing drugs will need to be tested in clinical trials to assess efficacy and safety.

2.8. Long-term Follow-up of Glycemic and Neurological Outcomes in an International Series of Patients With Sulfonylurea-Treated *ABCC8* Permanent Neonatal Diabetes.

Bowman P, Mathews F, Barbetti F, Shepherd MH, Sanchez J, Piccini B, Beltrand J, Letourneau-Freiberg LR, Polak M, Greeley SAW, Rawlins E, Babiker T, Thomas NJ, De Franco E, Ellard S, Flanagan SE, Hattersley AT, Neonatal Diabetes International Collaborative Group

Diabetes Care. 2021 Jan;44(1):35–42.
doi:10.2337/dc20-1520. PMID: 33184150.

The key findings from this cohort of patients with *ABCC8* neonatal diabetes mellitus (NDM) are: A) good glycaemic control is maintained over the long-term without any serious adverse events (including severe hypoglycaemia) despite high doses of sulphonylurea, B) some patients show improvements in neurological manifestations (and the frequency of neurological manifestations is similar to NDM patients with *KCJN11* mutations), C) the diagnosis of NDM should be made as early as possible so that sulphonylurea treatment can be started promptly, D) the dose of sulphonylurea should be regularly adjusted to maintain good glycaemic control.

Mutations (heterozygous and homozygous) in the *ABCC8/KCNJ11* gene are a known cause of both transient and permanent NDM. In addition to NDM, patients may also show neurological manifestations, including developmental delay, epilepsy and mild neuropsychological impairments, which were thought to be more common in patients with *KCNJ11* mutations (both *KCNJ11* and *ABCC8* are also expressed in the brain). About 90% of patients with *ABCC8/KCNJ11* NDM can be successfully switched from insulin injections to oral sulphonylurea treatment. Long-term data on follow up with regard to the glycaemic control and the neurological manifestations has not been reported before specifically in patients with *ABCC8* gene mutations. It is interesting that not all patients showed an improvement in the neurological manifestations and the reasons for this are still unclear.

2.9. Differences between transient neonatal diabetes mellitus subtypes can guide diagnosis and therapy.

Bonfanti R, Iafusco D, Rabbone I, Diedenhofen G, Bizzarri C, Patera PI, Reinstadler P, Costantino F, Calcaterra V, Iughetti L, Savastio S, Favia A, Cardella F, Lo Presti D, Girtler Y, Rabbiosi S, D'Annunzio G, Zanfardino A, Piscopo A, Casaburo F, Pintomalli L, Russo L, Grasso V, Minuto N, Mucciolo M, Novelli A, Marucci A, Piccini B, Toni S, Silvestri F, Carrera P, Rigamonti A, Frontino G, Trada M, Tinti D, Delvecchio M, Rapini N, Schiaffini R, Mammì C, Barbetti F, Diabetes Study Group of ISPED

Eur J Endocrinol. 2021 Apr;184(4):575–585.
doi:10.1530/EJE-20-1030. PMID: 33606663.

These authors examined the likelihood of remission of diabetes without pharmacological therapy in a retrospective analysis of 34 Italian patients with Transient neonatal diabetes (TNDM).

TNDM is a type of neonatal diabetes that remits within the first a few months of life. It is most commonly due to either mutations in the *ABCC81/KCNJ11* genes or abnormalities in chromosome 6q24. The underlying mechanisms of hyperglycaemia differ by the cause. Mutations in *ABCC81/KCNJ11* lead to dysfunction of the pancreatic K_{ATP} channel which regulates the beta-cell membrane permeability and thus insulin secretion. In contrast, the mechanism of defective insulin secretion due to abnormalities in chromosome 6q24 is not known but might involve changes in the expression of imprinted genes (such as *PLAGL1* and *HYMA1*) leading to alterations in islet organogenesis. Patients with TNDM due to *ABCC81/KCNJ11* mutations and abnormalities in chromosome 6q24 have distinct clinical and biochemical features. Those with chromosome 6q24 abnormalities have lower birth weights, present early, are less likely to have diabetic ketoacidosis, and diabetes will spontaneously remit but then relapse again around adolescence. Important clinical clues in infants with TNDM due to 6q24 abnormalities are umbilical hernia and macroglossia. More importantly from the

therapeutic perspective, infants with *ABCC81/KCNJ11* mutations must be diagnosed as early as possible as they will respond to oral sulphonylureas and insulin injections can be stopped. Thus, patients with TNDM should be screened as early as possible for *ABCC81/KCNJ11* mutations (except those with obvious macroglossia and umbilical hernia who should be tested first for 6q24) as this might have important treatment implications.

2.10. NKX2-2 mutation causes congenital diabetes and infantile obesity with paradoxical glucose-induced ghrelin secretion.

Auerbach A, Cohen A, Ofek Shlomai N, Weinberg-Shukron A, Gulsuner S, King MC, Hemi R, Levy-Lahad E, Abulibdeh A, Zangen D

J Clin Endocrinol Metab. 2020 Nov 1;105(11):dgaa563.
doi:10.1210/clinem/dgaa563. PMID: 32818257.

This manuscript describes an unusual case of a baby born with a homozygous Nkx2.2 mutation who developed severe neonatal diabetes mellitus and then on follow up went onto develop severe obesity characterized by marked hyperphagia.

Nkx2.2 is an important transcription factor involved in the development of the pancreas and the central nervous system. Mice lacking Nkx2.2 develop severe neonatal diabetes mellitus and die shortly after birth. In the pancreas of these mice the islets on gross morphology look normal but there is a loss of beta-cells and a reduction in the number of alpha-cells and pancreatic polypeptide cells (the delta-cells are unaffected). These cells are replaced with an increase in the ghrelin producing cells (called Epsilon cells). Only a few rare cases of human Nkx2.2 neonatal diabetes have been reported so far (1) all without obesity on follow up.

In the case described here, biochemical investigations for obesity showed high baseline and glucose stimulated ghrelin levels which might explain the hyperphagia. In healthy and obese subjects, plasma ghrelin is increased during fasting and is suppressed by the ingestion of glucose, so this case demonstrates an unusual ghrelin profile. It would be interesting to measure glucagon and pancreatic polypeptide in patients with Nkx2.2 mutations as these should also be low/undetectable given the role of this transcription factor in the developmental biology of the pancreas. Patients with Nkx2.2 neonatal diabetes should be followed up and monitored for obesity. If the hyperphagia is due to the high ghrelin levels, then developing treatment options that target ghrelin production should be explored. It is unclear why the absence of Nkx2.2 reprograms the cell fate in the pancreas, especially in favour of ghrelin producing cells. The expansion of ghrelin-producing cells is not specific to Nkx2.2, but has also been observed in Pax4 mutant mice (2).

Reference

1. Flanagan SE, De Franco E, Lango Allen H, Zerah M, Abdul-Rasoul MM, Edge JA, Stewart H, Alamiri E, Hussain K, Wallis S, de Vries L, Rubio-Cabezas O, Houghton JA, Edghill EL, Patch AM, Ellard S, Hattersley AT. Analysis of transcription factors key for mouse pancreatic development establishes NKX2-2 and MNX1 mutations as causes of neonatal diabetes in man. *Cell Metab*. 2014;19(1):146–154.
2. Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L. Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proc Natl Acad Sci U S A*. 2004 Mar 2;101(9):2924–9.

2.11. YIPF5 mutations cause neonatal diabetes and microcephaly through endoplasmic reticulum stress

De Franco E, Lytrivi M, Ibrahim H, Montaser H, Wakeling MN, Fantuzzi F, Patel K, Demarez C, Cai Y, Igoillo-Esteve M, Cosentino C, Lithovius V, Vihinen H, Jokitalo E, Laver TW, Johnson MB, Sawatani T, Shakeri H, Pachera N, Haliloglu B, Ozbek MN, Unal E, Yıldırım R, Godbole T, Yildiz M, Aydin B, Bilheu A, Suzuki I, Flanagan SE, Vanderhaeghen P, Senée V, Julier C, Marchetti P, Eizirik DL, Ellard S, Saarimäki-Vire J, Otonkoski T, Cnop M, Hattersley AT

J Clin Invest. 2020 Dec 1;130(12):6338–6353.
doi:10.1172/JCI141455. PMID: 33164986.

This manuscript describes a novel disorder due to homozygous mutations in the YIPF5 gene which cause a complex syndrome of neonatal/early onset diabetes mellitus, epilepsy and microcephaly. The underlying mechanism of the diabetes involves the accumulation of proinsulin (unable to be transported to the Golgi) in the beta-cells with increased ER stress markers and a tendency to apoptosis.

An important site for protein maturation and folding is the endoplasmic recticulum (ER) before they are stored in the Golgi apparatus. Under normal physiological conditions, protein synthesis and folding are matched. In some cases, the accumulation of misfolded proteins or defects in the transport of misfolded proteins from the ER to the Golgi leads to ER stress and thus cellular damage. YIPF5 (Yip Doman Family Member 5) is a five-span transmembrane protein localized in the Golgi apparatus and the ER. YIPF5 recycles between the ER and the Golgi apparatus and is involved in the maintenance of the Golgi structure. The loss of YIPF5 seems to sensitize the beta-cells to ER stressed induced apoptosis. The ER stress response was found to be specific for the beta-cells, as the alpha-cells did not show any evidence of ER stress.

These observations highlight an important and previously unknown role of YIPF5 in beta-cell physiology and diabetes. Mutations in at least 8 known genes that are involved in the regulation of the ER stress response have now been found to cause either neonatal/early onset, adolescent or adult onset diabetes associated with neurological features (reviewed in 1).

Reference

1. Cnop M, Toivonen S, Igoillo-Esteve M, Salpea P. Endoplasmic reticulum stress and eIF2α phosphorylation: The Achilles heel of pancreatic β cells. *Mol Metab*. 2017;6(9):1024–1039.

2.12. Neonatal diabetes mutations disrupt a chromatin pioneering function that activates the human insulin gene

Akerman I, Maestro MA, De Franco E, Grau V, Flanagan S, García-Hurtado J, Mittler G, Ravassard P, Piemonti L, Ellard S, Hattersley AT, Ferrer J

Cell Rep. 2021 Apr 13;35(2):108981.
doi:10.1016/j.celrep.2021.108981. PMID: 33852861.

Mutations in the promotor region of the insulin gene are associated with a subtype of neonatal diabetes mellitus (NDM). These mutations lead to abnormal transcription of the insulin gene and do so by deleting the C1 and E1 cis regulatory elements, or three different single base-pair substitutions in a CC dinucleotide sequence located between E1 and A1 elements of the gene. The promoter mutations are highly informative because they provide human genetic evidence that discrete insulin gene cis regulatory elements are essential. In cultured cells, the activity of these cis regulatory elements seems important but it is not known if each of these cis elements is required *in-vivo* to activate insulin gene transcription. So, to address this question, the authors inserted the human insulin gene regulatory region into a non-coding region of the mouse insulin gene and used this to study how the common promotor mutation (c.-331C > G, CC nucleotide) in the insulin gene leads to NDM.

The study has two important findings. First, this mutation leads to disruption of active chromatin formation during the development of the pancreas so that transcription is repressed. Secondly, an important transcription factor (called GLIS3, which also causes NDM when disrupted) was required to activate insulin gene chromatin and the insulin gene transcription *in-vivo*. The CC element in the promotor region of the insulin genes acts as an essential seeding site to allow chromatin opening and transcriptional activation of the insulin promotor during beta-cell development. The c.-331C > G mutation prevented GLIS3 activation of both chromatin and insulin gene transcription.

2.13. Predictors of neonatal adiposity and associations by fetal sex in women with gestational diabetes mellitus and normal glucose-tolerant women

Benhalima K, De Landtsheer A, Van Crombrugge P, Moyson C, Verhaeghe J, Verlaenen H, Vercammen C, Maes T, Dufraimont E, De Block C, Jacquemyn Y, Laenen A, Devlieger R, Minschart C, Mathieu C
Acta Diabetol. 2021 Mar;58(3):341–354.
doi:10.1007/s00592-020-01619-0. PMID: 33216207.

The key findings of this multi-centre prospective cohort study were that neonates born to mothers treated for Gestational diabetes mellitus (GDM) (by lifestyle or medication, e.g. insulin or metformin) had high rates of macrosomia but similar adiposity to those born of mothers with normal glucose tolerance. In contrast, neonates born to untreated mothers who were overweight and had a normal glucose tolerance had increased adiposity. Increased gestational weight gain was associated with increased neonatal adiposity in boys. Maternal BMI, fasting blood glucose, triglycerides and gestational weight gain were independent predictors for neonatal adiposity.

Adiposity in the newborn period has been linked to obesity in childhood and adolescence. Understanding the risk factors that contribute to neonatal adiposity might help to understand the mechanisms of obesity in childhood and adolescence and thus help in developing any preventative measures. GDM, maternal obesity, hyperglycaemia and excessive gestational weight gain and are all associated with increased newborn adiposity. In addition, cord blood C-peptide, as well as amino acids and fatty acids, are also important mediators of newborn adiposity. A previous systematic review and meta-analysis found that infants born to mothers with GDM had higher fat mass and that there was a sex difference (more in boys than in girls (1)).

The findings here suggest that implementing measures to limiting gestational weight gain and treating GDM (by lifestyle or medication) might be important especially in the male fetus to reduce the risk of neonatal adiposity and the long-term risk of obesity.

Reference

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2.14. Early diagnosed gestational diabetes mellitus is associated with adverse pregnancy outcomes: A prospective cohort study

Liu B, Cai J, Xu Y, Long Y, Deng L, Lin S, Zhang J, Yang J, Zhong L, Luo Y, Zhou Y, Zhang Y, Li Z, Chen H, Wang Z
J Clin Endocrinol Metab. 2020 Dec 1;105(12):dgaa633.
doi:10.1210/clinem/dgaa633. PMID: 32898218.

In this study, low risk pregnant women had an ‘early’ OGTT at 18-20 weeks of gestation and these results were correlated with the standard OGTT at 24-28 weeks. Pregnant women with Gestational diabetes mellitus (GDM) who had early OGTT still had a higher risk of delivering macrosomic infants with fetal hyperinsulinaemia.

GDM leads to maternal, neonatal and childhood complications. Mothers with GDM have a higher risk of developing type 2 diabetes later in life. In the newborn, GDM leads to macrosomia, polycythemia and hypoglycaemia. Infants of mothers with GDM have a higher risk of obesity and diabetes later in life. The diagnostic criteria for GDM and the optical timing of testing for GDM are debated. Most guidelines recommend undertaking an oral glucose tolerance test (OGTT) in low risk pregnancies between 24-28 weeks of gestation. The authors hypothesized that early diagnosis of GDM might help in early intervention and treatment to reduce the complications.

Their findings suggest that GDM diagnosed in low risk pregnant women, even as early as 18-20 week’s gestation, is still associated with adverse outcomes. Thus, early diagnosis and management of GDM in low risk pregnant women could help improve outcomes. As this was a prospective study, further randomized control

trials comparing early and standard screening for GDM are required to understand if early intervention could have diagnostic and therapeutic benefits.

2.15. Gestational diabetes Is uniquely associated with altered early seeding of the infant gut microbiota

Soderborg TK, Carpenter CM, Janssen RC, Weir TL, Robertson CE, Ir D, Young BE, Krebs NF, Hernandez TL, Barbour LA, Frank DN, Kroehl M, Friedman JE

Front Endocrinol (Lausanne). 2020 Nov 27;11:603021.
doi:10.3389/fendo.2020.603021. PMID: 33329403.

This and previous studies have shown that Gestational diabetes mellitus (GDM) alone and maternal obesity/overweight are associated with alterations in the newborn gut microbiota. In this study, there were significant alterations in the newborn gut microbiota species at 2 weeks of age.

The gut microbiome in the newborn affects metabolism, maturation of the gastrointestinal tract, function of the immune system and brain development. Initial seeding of the neonatal microbiota occurs through maternal and environmental contact. Nutrients regulate the initial perinatal microbial colonization and nutritional stresses can alter both the initial colonizing bacteria and the development of signaling pathways controlled by microbial mediators. These stresses fine-tune the immune system and metabolic homeostasis in early life, potentially setting the stage for long-term metabolic and immune related health conditions (such as adiposity, type 2 diabetes and non-alcoholic fatty liver). GDM and maternal obesity/overweight are known risk factors for the later development of obesity, type 2 diabetes and non-alcoholic fatty liver disease.

It would be interesting to follow these newborns in the long-term for risks of obesity, type 2 diabetes as well as non-alcoholic fatty liver disease, while monitoring the changes in the gut microbiota. Dynamic changes in the gut microbiota have already been reported in infants at high risk of developing type 1 diabetes, prior to disease onset (1). Thus, understanding how changes in gut microbiota interact with the host components to drive changes in the infant's immune system will provide insights into the disease pathways involving GDM and obesity as well as type 2 diabetes in the offspring.

Reference

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2.16. Probiotics for preventing gestational diabetes

Davidson SJ, Barrett HL, Price SA, Callaway LK, Dekker Nitert M

Cochrane Database Syst Rev. 2021 Apr 19;4:CD009951.
doi:10.1002/14651858.CD009951.pub3. PMID: 33870484.

In summary, this review did not find certain evidence to support the use of probiotics to reduce the risk of GDM. Only 2 studies suggested a possible reduction in the risk of GDM with probiotics. More importantly, and of concern, the review found a high degree of certainty that the use of probiotics increases the risk of developing pre-eclampsia. This observation suggests that great care needs to be taken in future studies involving the use of probiotics in pregnancy.

As gestational diabetes mellitus (GDM) is associated with adverse outcomes for both the mother and the child it is important to identify intervention strategies. During normal pregnancy there are increases in body fat content and a reduction in insulin sensitivity and this seems to be linked to changes in the gut microbiome. The gut microbiome is altered in women with GDM as compared to normal pregnancy and may resemble that of non-pregnant women with type 2 diabetes mellitus (1). As the gut microbiome plays an important role in host glucose

and lipid metabolism, probiotics have been suggested as a possible intervention for improving glucose control in diabetes and GDM by helping to restore the imbalance in the species of microbiome in the gut. Previously published studies have shown mixed results in terms of using probiotics to prevent GDM.

Therefore, this systematic Cochrane review was conducted to collate and synthesize all the available evidence (comparing probiotics with placebo) for and against the use of probiotics for preventing GDM in pregnancy. Further studies need to be conducted to understand the physiological and biochemical mechanisms of the pre-eclampsia associated with the use of probiotics.

Reference

1. Crusell MKW, Hansen TH, Nielsen T, Allin KH, Rühlemann MC, Damm P, Vestergaard H, Rørbye C, Jørgensen NR, Christiansen OB, Heinsen FA, Franke A, Hansen T, Lauenborg J, Pedersen O. Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum. *Microbiome*. 2018 May 15;6(1):89.

2.17. Altered pancreas remodeling following glucose intolerance in pregnancy in mice

Szlapinski SK, Botros AA, Donegan S, King RT, Retta G, Strutt BJ, Hill DJ
J Endocrinol. 2020 May;245(2):315–326.
doi:10.1530/JOE-20-0012. PMID: 32171178.

As it is not possible to study the histology of the pancreas post-partum in humans, this study used a mouse model of mild glucose intolerance to assess the changes in pancreatic islets after post-partum and assess the impact of pro-inflammatory cytokines. Mice with glucose intolerance during pregnancy continued to have glucose intolerance after parturition for one month due to ongoing impairments in endocrine pancreas compensation and glucose levels normalized only after 3 months.

Pregnancy is a state characterized by insulin resistance and proinflammation. In a normal pregnancy to combat insulin resistance and prevent hyperglycaemia pancreatic beta-cells undergo hypertrophy and increase in mass leading to increased insulin production. After pregnancy this compensation discontinues so that beta-cell mass is returned to normal. In GDM pregnancies despite the increase in beta-cell mass and increased insulin secretion hyperglycaemia persists. It is known that GDM can lead to type 2 diabetes in the mother later in life but the mechanisms of this are unclear. Progressive beta-cell dysfunction is the most likely underlying mechanism.

In this study, histologically islet quantification demonstrated that the number of small, medium, and large-sized islets varied with time after parturition. At one week of age, there were lower beta-cell and surprisingly alpha-cell fractional areas in the pancreas (beta-cell and alpha-cell mass were not measured). Then at three months of age, there was compensatory increase in the number of small islets with increased insulin to glucagon ratio thus accounting for the normalization of the blood glucose levels. These histological changes were associated with an increase in pro-inflammatory cytokines. The changes in alpha-cell ontogeny observed are novel and suggest that GDM may also lead to possible changes in alpha-cell mass and possible function.

These observations in mice suggest that GDM leads to histological changes in pancreatic beta and alpha-cells which persist after birth and ares associated with an increase in pro-inflammatory cytokines.

Maternal Obesity and Long-term Infant Consequences

2.18. Maternal obesity interrupts the coordination of the unfolded protein response and heat shock response in the postnatal developing hypothalamus of male offspring in mice

Chen N, Zhang Y, Wang M, Lin X, Li J, Li J, Xiao X
Mol Cell Endocrinol. 2021 May 1;527:111218.
doi:10.1016/j.mce.2021.111218. PMID: 33636254

This study used a high fat and high sucrose diet to generate obese mice. The offspring of these mice showed activation of the unfolding protein response and the heat-shock response in the hypothalamus as early as weaning. This was associated with malformed paraventricular nucleus axonal projections and defective leptin signaling. Intriguingly early inhibition of hypothalamic ER stress in the offspring failed to improve the metabolic outcome but worsened it. A key molecule (called heat shock protein 70 (HSP70)) was altered in the early postnatal developing hypothalamus which might lead to permanent unfolded protein response activation later in life.

Maternal overnutrition and obesity are risk factors for later development of childhood obesity and type 2 diabetes mellitus. The underlying mechanisms involved in the development of childhood obesity following maternal obesity are unknown but might involve changes in the feeding center of the hypothalamus. In the hypothalamus, there are two key neuronal subtypes (anorexigenic and orexigenic) which impinge on the paraventricular nucleus which regulates feeding behavior. Leptin plays a key role in regulating this whole circuit. In mice models of obesity, endoplasmic recticulum (ER) stress is noted in the hypothalamus early in the neonatal period with defective changes in the paraventricular neuronal projections. ER stress is controlled by the unfolding protein response and the heat-shock response which is a cellular protective mechanism activated by different stressors where chaperon proteins prevent cellular stress response by protein folding and re-folding and by activating autophagy.

These observations suggest that developmental exposure to a maternal obesogenic environment may lead to an imbalance in the unfolded protein and heat shock responses in the postnatal developing hypothalamus. This imbalance might lead to defects in obesity programming of the offspring.

2.19. Differences of DNA methylation patterns in the placenta of large for gestational age infant

Shen Z, Tang Y, Song Y, Shen W, Zou C
Medicine (Baltimore). 2020 Sep 25;99(39):e22389.
doi:10.1097/MD.0000000000022389. PMID: 32991460.

In this relatively small study (6 placenta) the placentas from mothers of infants born large for gestational age (LGA) were compared to placentas of appropriate gestational age (AGA) infants for changes in genome wide DNA methylation. There were significant differences in the specific methylation patterns between the two groups of placenta. The changes included hypo and hypermethylation of a large number of genes with different patterns of methylation on different chromosomes. The differentially methylated genes were involved in diverse biological functions such as ion binding, protein binding and cell-to-cell signaling, adhesion, transport, as well as system development and function. Network analysis of these genes identified pathways linked to possible risk of metabolic disease.

LGA infants are born to mothers with gestational diabetes, maternal obesity and sometimes to normal weight pregnant women. LGA infants have an adverse cardio-metabolic profile during childhood which gets worse during adolescence. The underlying mechanisms for the adverse cardio-metabolic profile are unknown but might involve defects in placental function. As environment and lifestyle are known to impact an individual's DNA methylation pattern, the authors of this study hypothesized that LGA might correlate with changes in the placental DNA methylation and allow the expression of genes which confer the risk of developing metabolic disease. There are limited studies on placental methylation changes in LGA infants with studies mostly focusing on small for gestational age infants.

The current findings suggest that the intrauterine environment seems to alter the methylation status of some placental genes in LGA infants. However, how this then translates to obesity and metabolic syndrome later in life needs further study.

2.20. Maternal obesity influences placental nutrient transport, inflammatory status, and morphology in human term placenta

Nogues P, Dos Santos E, Couturier-Tarrade A, Berveiller P, Arnould L, Lamy E, Grassin-Delyle S, Vialard F, Dieudonne MN
J Clin Endocrinol Metab. 2021 Mar 25;106(4):e1880–e1896.
doi:10.1210/clinem/dgaa660. PMID: 32936881.

By studying placentas from normal weight mothers and obese (non-diabetic) mothers, the authors found that maternal obesity was associated with lower expression of nutrient transporters (such as for glucose and amino acids), surprisingly fewer immune cells, and compromised endocrine function.

Maternal health and fetal development are closely linked via the placenta. Normal pregnancy is characterized by a proinflammatory state and this is exacerbated in pregnancies associated with maternal obesity. Maternal obesity may alter placental morphology and placental function by affecting the transport of nutrients such as glucose and amino acids. Previous studies have highlighted the impact of maternal obesity on the development and exchange functions of the placenta but the results are often conflicting and debatable. So, the aim of the present study was to evaluate the impact of maternal obesity on the inflammatory status, the morphology and levels of nutrient transporter expression and activity of the placenta.

The current findings were in contrast to the original hypothesis that there would be increased inflammatory markers (immune cells) in the placenta. The birth weight of the newborn was not affected. This suggests that the placenta has the capacity or plasticity to adapt to an obesogenic environment during pregnancy so that the fetus is protected. It is possible that the immune and inflammatory changes are dampened and nutrient exchange are differentially regulated in maternal obesity in the placenta as a protective mechanism for the fetus. This adaptive capacity or plasticity of the placenta is probably compromised in pregnancies where there is gestational diabetes.

Fetal and Neonatal Cortisol and Growth Hormone Physiology

2.21. Evaluating the low-dose ACTH stimulation test in neonates: ideal times for cortisol measurement.

LeDrew R, Bariciak E, Webster R, Barrowman N, Ahmet A
J Clin Endocrinol Metab. 2020 Dec 1;105(12):dgaa635.
doi:10.1210/clinem/dgaa635. PMID: 32901267.

The authors performed a retrospective analysis of low dose synacthen tests performed in 49 neonates from January 1, 2009 to September 30, 2017 in a tertiary-care pediatric center. Of samples measured at 15, 30, and 60 minutes, the majority of neonates showed a peak cortisol level at 60 minutes.

ACTH stimulation testing is used to assess adrenal insufficiency. There are 2 types of ACTH stimulation tests, low dose and high dose. The low dose is used in cases of secondary adrenal insufficiency (typically involves giving 1 μg/kg of corticotropin) and the high dose is used in cases of primary adrenal insufficiency. In neonates there is uncertainty whether the 30-minute cortisol sample is the optimal time for a single measurement and it is unknown what is the value of additional cortisol measurements for the low dose synacthen test.

The current findings contrast to previous evidence (1) on the timings of the peak cortisol on the low dose versus standard dose ACTH stimulation tests. Of cortisol values at 30, 40, 60 and 120 minutes: on the low dose test 9/10 neonates had a peak cortisol at 30 or 40 minutes; on the standard-dose test most peaks occurred at 60 or 120 minutes. Another study (2) reported that 94.9% of neonates had peak cortisol levels at 40 minutes (when tested at 0, 20, 30, and 40 minutes), with a clear rise in cortisol values across the time points. However, no sampling was undertaken after 40 minutes leaving the possibility that cortisol values might continue to rise after 40 minutes.

Thus, it is suggested that cortisol should be measured at 30 and 60 minutes when undertaking the low dose synacthen test in neonates with the 60-minute sample probably being more important. The 15-minute cortisol measurement does not add any additional value.

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2.22. The utility of a random growth hormone level in determining neonatal growth hormone sufficiency

Mamilly L, Pyle-Eilola AL, Chaudhari M, Henry RK
Clin Endocrinol (Oxf). 2021 Mar;94(3):392–398.
doi:10.1111/cen.14364. PMID: 33140844.

In this retrospective study, random growth hormone (GH) levels were compared between newborns (mean age 9.07 ± 6.6 days) with or without GH deficiency. A cut off of 4.5 ng/ml was established as the GH value to diagnose congenital GH deficiency. This value had a 100% sensitivity and 85% specificity for diagnosing congenital GH deficiency.

Growth hormone (GH) is the key hormone that regulates linear growth. However, GH has other important metabolic effects such as regulating glucose, carbohydrate and fat metabolism. In the newborn congenital GH deficiency may present with hypoglycemia, jaundice and micropenis. GH levels tend to be elevated in the newborn period indicating a state of GH resistance. Establishing a diagnosis of GH deficiency in the newborn period can be challenging as provocation testing cannot be done in this age group. So, the idea of measuring a random GH level in the newborn period as an indicator of GH deficiency is appealing.

The current findings support the value of random GH measurement in newborns. However, a previous study (1) indicated a GH cut off level of 7ng/ml and another study (2) indicated a cut off level of <5 ng/ml as indicative of GH deficiency in the newborn period. So, the optimal level of a random GH to diagnose congenital GH deficiency has yet to be established. It is important to note that GH levels will vary with the specific assay method and that these assays have varying performance characteristics so this should be kept in mind when using specific cut off values.

Reference
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2. 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(6):361–397.

Miscellaneous

2.23. Maternal organophosphate flame-retardant exposure alters offspring energy and glucose homeostasis in a sexually dimorphic manner in mice

Walley SN, Krumm EA, Yasrebi A, Kwiecinski J, Wright V, Baker C, Roepke TA
J Appl Toxicol. 2021 Apr;41(4):572–586.
doi:10.1002/jat.4066. PMID: 329695

In this study, pregnant dams were exposed to different types of organophosphate flame retardants (OPFRs) at a sensitive development period (from gestation to lactation). The offspring were fed either a high or a low-fat diet

for 17 weeks and then underwent a battery of metabolic assessments. OPFR exposure did not alter the weight of the offspring but there were sex dependent changes in metabolic rate, glucose clearance, insulin tolerance, haemodynamics and liver gene expression, potentially via interactions involving steroid and nuclear receptor expression.

Endocrine disrupting compounds (EDC) are exogenous chemicals that interfere with hormone actions and have been implicated in the etiology of metabolic disease with effects on glucose homeostasis, hormone production, haemodynamics and oxygen consumption. One such group of EDCs are flame retardants, including polybrominated diphenyl ethers (PBDEs) and organophosphate flame retardants (OPFRs). PBDEs have been phased out but OPFRs are increasingly being used in household products. OPFRs interact with nuclear and steroid receptors. There is limited information on the effects of maternal OPFR exposure on offspring energy homeostasis in mammalian rodent models. The key steroid hormone receptor in the liver identified in this study was the estrogen receptor (ERα). EDC are known to affect the expression of nuclear receptors (1) so it is likely that exposure to OPFRs affects the activity and expression of ERα and nuclear receptor thus leading to toxicity and providing an explanation for the sex specific changes observed here.

Reference

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3. Thyroid

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Preface

Over the last twelve months important clinical and basic research in the field of thyroidology was published. Here a rapid overview on important topics in the field: A better understanding of carcinogenesis through ionizing radiation came from a large genomic study on papillary thyroid cancer samples of patients exposed to radioactive iodine as children in 1986 in Chernobyl. A remarkable step in regeneration of thyroid function in thyroidectomized patients was achieved by patient-derived adult human thyroid cells cultured as organoids. The European Reference Network (ERN) initiated revision of the ESPE and EJE consensus guidelines on congenital hypothyroidism updated the current state of care of these patients throughout life. A randomized controlled trial comparing block and replace *vs* dose titration of anti-thyroid drugs in children with Graves' disease showed no advantage of block and replace strategy on long-term stability. Further significant and innovative publications are summarized in the following chapter.

Mechanism of the Year

3.1. Radiation-related genomic profile of papillary thyroid carcinoma after the Chernobyl accident

Morton LM, Karyadi DM, Stewart C, Bogdanova TI, Dawson ET, Steinberg MK, Dai J, Hartley SW, Schonfeld SJ, Sampson JN, Maruvka YE, Kapoor V, Ramsden DA, Carvajal-Garcia J, Perou CM, Parker JS, Krznaric M, Yeager M, Boland JF, Hutchinson A, Hicks BD, Dagnall CL, Gastier-Foster JM, Bowen J, Lee O, Machiela MJ, Cahoon EK, Brenner AV, Mabuchi K, Drozdovitch V, Masiuk S, Chepurny M, Zurnadzhy LY, Hatch M, Berrington de Gonzalez A, Thomas GA, Tronko MD, Getz G, Chanock SJ
Science. 2021;372(6543):eabg2538.
doi:10.1126/science.abg2538.

Radioactive iodine (^{131}I)-exposed children in the region of Chernobyl (Ukraine) showed an increased incidence of papillary thyroid carcinoma (PTC) after the 1986 nuclear plant accident [1]. This so far the largest and most comprehensive study to characterize the genomic landscape of radiation induced PTC in a large number of tissues from the Chernobyl Tissue Bank originating from a precisely phenotyped cohort of ^{131}I exposed patients. The findings reveal DNA damage as early driver of carcinogenesis and lack of a unique molecular signature for radiation induced PTC.

To investigate the dose-dependent contribution of ^{131}I-related radiation on carcinogenesis of PTC, Morton *et al*. performed a genomic, transcriptomic, and epigenomic analysis of PTC and normal thyroid tissue in 359 radiation exposed *vs.* 81 non-radiation exposed patients with PTC. Mean estimated radiation dose to the thyroid was 250 mGy (range 11-8800). The authors describe three key observations: First, they observed a linear radiation dose-dependent increase in DNA double strand breaks as an early event during carcinogenesis. Second, in $>90\%$ of PTC, they found a known tumor driver in the mitogen-activated protein kinase (MAPK) pathway, such as mutations in *BRAF*, *RAS*, and fusions with *RET* in accordance with current knowledge on sporadic PTC [2]. Third, gene expression and methylation patterns were not associated with radiation dose but correlated with the affected driver gene pathway. Thus, no molecular pattern of PTC after radiation exposure could be identified.

Reference
1. Tronko M, Brenner AV, Bogdanova T, Shpak V, Oliynyk V, Cahoon EK, Drozdovitch V, Little MP, Tereshchenko V, Zamotayeva G, Terekhova G, Zurnadzhi L, Hatch M, Mabuchi K. Thyroid neoplasia risk is increased nearly 30 years after the Chernobyl accident. *Int J Cancer*. 2017;141:1585–1588. doi: 10.1002/ijc.30857.
2. Cancer Genome Atlas Research Network Integrated genomic characterization of papillary thyroid carcinoma. *Cell*. 2014;159:676–90. doi: 10.1016/j.cell.2014.09.050.

Thyroid Hormone Action

3.2. A coregulator shift, rather than the canonical switch, underlies thyroid hormone action in the liver

Shabtai Y, Nagaraj NK, Batmanov K, Cho YW, Guan Y, Jiang C, Remsberg J, Forrest D, Lazar MA
Genes Dev. 2021;35(5–6):367–378.
doi:10.1101/gad.345686.120.

The longstanding concept of thyroid hormone (TH) action is summarized as the canonical switch model. This study adds important aspects of TH action to our current understanding, modifying this longstanding switch model to a "shift" model.

According to the current concept, TH dependent gene expression is upregulated upon binding of TH to nuclear thyroid hormone receptors (TR) which are bound to DNA at TR binding sites (TRBS). TH binding to TRs at TRBS induces a switch between repressed and activated states by corepressor release and coactivator recruitment resulting in activated gene expression [1,2]. A recent study presented in the 2020 Yearbook chapter suggested that this model may explain only the upregulation of a subset of TH regulated genes [3].
Shabtai *et al*. add more evidence to this more differentiated view of thyroid hormone action studying thyroid hormone action in the murine liver. To overcome technical limitations of earlier studies due to the low physiological expression level of TRs, they developed a specific *in vivo* mouse model with epitope-tagged TRbeta1 protein to allow efficient and specific chromatin immunoprecipitation experiments under hypothyroid and hyperthyroid conditions. First, the authors showed for the first time, that not only transcriptional activation of TH target genes but also transcriptional repression was dependent on direct binding of TH to TRBS. Second, the authors quantified corepressor and coactivator levels at enhancers of genes upregulated or downregulated by TH. They observed a shift of corepressors and coactivators upon TH action rather than a complete switch from repressive to activating state.

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Thyroid Development

3.3. Generation and differentiation of adult tissue-derived human thyroid organoids

Ogundipe VML, Groen AH, Hosper N, Nagle PWK, Hess J, Faber H, Jellema AL, Baanstra M, Links TP, Unger K, Plukker JTM, Coppes RP
Stem Cell Reports. 2021;16:913–925.
doi:10.1016/j.stemcr.2021.02.011.

In analogy to historical data [1], the authors provide important proof of principle that adult thyroid tissues cultivated *in vitro* show self-renewal and differentiation capacities and might ultimately be of use for regenerative autologous transplantation back into the donor patient affected by hypothyroidism.

Over the last decade several pluripotent murine and human stem cell (SC) approaches have been established to produce differentiated thyroid follicular cells using thyroid transcription factor overexpression, directed differentiation, or combination of both (reviewed in detail by Posabella *et al*. [2]). However, the use of such pluripotent SC-derived thyroid follicular cells is limited by ethical and practical aspects.

Recently, the existence of adult thyroid SC in the murine and human thyroid gland have been shown opening potential avenues for autologous thyroid regeneration techniques [3,4]. Based on these results, and as proof of principle, Ogundipe *et al*. aimed at developing a patient derived thyroid organoid model with proliferative and differentiation capacity *in vitro.*

The authors established organoid protocols for murine and human adult thyroid tissue organoids, which showed proliferation, as well as structural and functional differentiation shown by follicle formation, expression of the key proteins of the thyroid hormone synthetic machinery, finally culminating in some thyroxin synthetic capacity after (xeno-) transplantation into mice. Even after one year, organoids did not dediffentiate or develop tumours.

Although representing an important step towards thyroid regeneration by overcoming biological and ethical hurdles of pluripotent SC approaches, the presented organoid model is so far limited in cell number. Further optimization of the model will be necessary.

Reference

1. Martin A, Valentine M, Unger P, Lichtenstein C, Schwartz AE, Friedman EW, Shultz LD, Davies TF. Preservation of functioning human thyroid organoids in the scid mouse: 1. System characterization. *J Clin Endocrinol Metab*. 1993;77:305–10. doi: 10.1210/jcem.77.2.8345031.
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3.4. Single-cell transcriptome analysis reveals thyrocyte diversity in the zebrafish thyroid gland

Gillotay P, Shankar M, Haerlingen B, Sema Elif E, Pozo-Morales M, Garteizgogeascoa I, Reinhardt S, Kränkel A, Bläsche J, Petzold A, Ninov N, Kesavan G, Lange C, Brand M, Lefort A, Libert F, Detours V, Costagliola S, Sumeet Pal S

EMBO Rep. 2020;21:e50612.
doi:10.15252/embr.202050612.

This zebrafish study identified and molecularly characterized adult transcriptionally different thyrocyte subpopulations even within the same follicle.

It is well established that within a thyroid gland, follicles are heterogenous concerning functional activity. More active follicles are characterized by a high columnar epithelium in contrast to a flat epithelium in inactive follicles [1,2]. Here, Gillotay *et al*. aimed to characterize the thyrocyte gene expression pattern between follicles and thyrocytes within the same follicle by single cell transcriptome analysis in zebrafish. For this, they established a *pax2a* gene knock-in reporter line. Further, pax2a expression is the first step of thyroid development in the zebrafish, determining thyrocyte precursors in the zebrafish endoderm [3].

The key findings, first, confirmed thyrocyte heterogeneity in the adult zebrafish thyroid in accordance with earlier reports in human and murine thyroid. Second, they could detect transcriptionally different thyrocytes with low *vs*. high expression of *pax2a* not only in different follicles but within the same follicle. Thyrocytes with high *pax2a* expression showed functional differentiation with expression of the thyroid hormone synthetic machinery (*tpo, tg, slc5a5*). In contrast, these differentiation markers were not expressed (or only at low levels)

in thyrocytes with low *pax2a* expression. Based on these results, the authors suggest a functional and a resting thyrocyte population within the same follicle. Interestingly, the *pax2a* low thyrocyte population also showed lower *nkx2.4b* expression, thus resembling rather a determined thyrocyte progenitor with hypothetically higher proliferative capacity than the *pax2a* high thyrocyte population.

This knowledge from the zebrafish model provides possible molecular insights, how patient derived adult thyrocyte subpopulations might contribute to tissue regeneration as described in the previous paper 3.3.

Reference

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Drug Induced Thyroid Disease

3.5. Genetic variation associated with thyroid autoimmunity shapes the systemic immune response to PD-1 checkpoint blockade

Khan Z, Hammer C, Carroll J, Di Nucci F, Acosta SL, Maiya V, Bhangale T, Hunkapiller J, Mellman I, Albert ML, McCarthy MI, Chandler GS

Nat Commun. 2021 Jun 7;12(1):3355.
doi:10.1038/s41467-021-23661-4.

This study describes the interaction of individual genetic variation for autoimmune thyroid disease with risk of thyroid immune related adverse events (irAE) during or after immune checkpoint inhibitor (ICI) treatment for advanced cancer.

ICIs are monoclonal antibodies blocking T-cell exhaustion and resulting in a T-cell antitumor response. However, ICIs are associated with irAE. Reversible destructive thyroiditis, hyperthyroidism and hypothyroidism (thyroid irAE) are the most frequent endocrine side effects of anti-programmed cell death-1 antibodies (PD-1-AB), one frequently used ICI. Further endocrine irAE are type 1 diabetes, adrenal insufficiency and hypophysitis [1]. Anti-thyroid antibodies before treatment seem critical for development of PD-1-AB induced thyroid irAE mediated by cytotoxic CD4(+) T cells [2,3].

In this large analysis of data from 7 PD-1 blockade trials, the authors found that hypothyroidism was more frequent than hyperthyroidism in the context of thyroid irAE, and was associated with longer survival of the treated cancer patients. To investigate the role of genetic variation for thyroid irAE, the authors developed a polygenic risk score for hypothyroidism using GWAS. They observed that patients with high lifetime genetic susceptibility for autoimmune thyroid disease (indicated by high polygenic risk score) also had higher risk for PD-1 blockade induced thyroid irAE.

This study extends current evidence that not only manifest biochemical or radiological signs of Hashimoto thyroiditis [1,3] but even genetic susceptibility for autoimmune thyroid disease/hypothyroidism predicts risk for thyroid irAE. Both, genetic and clinical information allow personalized decision making in cancer patients.

Reference

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Nagino M, Kodera Y, Fujishiro M, Hibi H, Sone M, Kiyoi H, Gotoh M, Ando Y, Akiyama M, Hasegawa Y, Arima H. Anti-thyroid antibodies and thyroid echo pattern at baseline as risk factors for thyroid dysfunction induced by anti-programmed cell death-1 antibodies: a prospective study. *Br J Cancer*. 2020 Mar;122(6):771–777. doi: 10.1038/s41416-020-0736-7.

Thyroid and Pregnancy

3.6. Maternal health, *in-utero*, and perinatal exposures and risk of thyroid cancer in offspring: a Nordic population-based nested case-control study

Kitahara CM, Slettebø Daltveit D, Ekbom A, Engeland A, Gissler M, Glimelius I, Grotmol T, Trolle Lagerros Y, Madanat-Harjuoja L, Männistö T, Sørensen HT, Troisi R, Bjørge T
Lancet Diabetes Endocrinol. 2021;9:94–105.
doi:10.1016/S2213-8587(20)30399-5.

This population based nested case-control study integrated registry data from Denmark, Norway, Sweden and Finland over 40 years to investigate the association of maternal, *in-utero*, and postnatal factors with thyroid cancer risk in offspring. Each patient with thyroid cancer (cases $n = 2437$) was matched with 10 controls without thyroid cancer ($n = 24'362$).

As expected, there was a predominance of papillary thyroid carcinoma (81% of cases) and female sex (77% of cases). Mean age at diagnosis was 27.5 years. Although only available from the Danish registry, maternal thyroid disease diagnosed before or during pregnancy were associated with highest odds ratio (OR) for offspring thyroid cancer: maternal goitre OR 67, benign thyroid neoplasm OR 22, hypothyroidism OR 18, hyperthyroidism OR 12, thyroiditis OR 3. Thyroid-independent maternal conditions associated with increased offspring thyroid cancer risk were: diabetes OR 1.7, and postpartum haemorrhage OR 1.3. Interestingly, congenital hypothyroidism was associated with a relevant OR of 4.5 although occurring in only 5 patients with thyroid cancer vs. 11 controls.

In summary, this extensive case-control study identified maternal and neonatal thyroid disorders associated with increased thyroid cancer risk. The underlying mechanisms remain unknown. Nevertheless, these results might be helpful for clinical decision making in paediatric and young adult patients with thyroid nodules.

Follow-up Paper From the 2018 Yearbook

3.7. A Novel homozygous mutation in the solute carrier family 26 member 7 gene causes thyroid dyshormonogenesis in a girl with congenital hypothyroidism

Hermanns P, Claßen C, Pohlenz J
Thyroid. 2020;30:1831–1833.
doi:10.1089/thy.2020.0293.

Every year, we report on new genes that have been associated with congenital hypothyroidism. But it is the first time in all these years that, following the first description, other groups from different continents confirm the first reports in independent cohorts in such a short time after publication.

This is the case for mutations in the *SLC26A7* gene, first described by Cangul *et al*. in 2018 in six independent families from Pakistan, Turkey and Finland [1] and presented in the 2018 Yearbook chapter. The typical phenotype is congenital hypothyroidism with goiter, however goiter is not present in all patients. In this short period of time, four further publications described new cases in patients from Saudi-Arabia, Japan, Sudan [2-4] and Germany (the selected most recent publication).

This rapid progress may be explained by technical advances for mutational screening by next generation screening (NGS) over the last years, or by publication bias, but on the other hand, it might also suggest a relevant new genetic cause of disease, as Ishi *et al*. provided further mechanistic and functional data on the role of the

SLC26A7 transporter as a new iodine transporter at the apical membrane of the thyrocytes [3]. Systematic genetic analyses by complete NGS panels for all known genes associated with congenital hypothyroidism integrating *SLC26A7* will give precise incidence rates in different cohorts in the future. Until then, *SLC26A7* is a strong new candidate gene in patients with congenital hypothyroidism due to thyroid dyshormonogenesis.

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Congenital Hypothyroidism

3.8. Congenital hypothyroidism: A 2020-2021 consensus guidelines update-An ENDO-European Reference Network initiative endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology

van Trotsenburg P, Stoupa A, Léger J, Rohrer T, Peters C, Fugazzola L, Cassio A, Heinrichs C, Beauloye V, Pohlenz J, Rodien P, Coutant R, Szinnai G, Murray P, Bartés B, Luton D, Salerno M, de Sanctis L, Vigone M, Krude H, Persani L, Polak M
Thyroid. 2021:387–419.
doi:10.1089/thy.2020.0333.

These updated ENDO-European Reference Network (ENDO-ERN), European Society for Paediatric Endocrinology (ESPE) and European Society for Endocrinology (ESE) guidelines for congenital hypothyroidism will serve as comprehensive review of the literature providing recommendations to all aspects of the disease.

The first Consensus Guidelines for congenital hypothyroidism of ESPE were published in 2014 [1]. This revised version of the guidelines was realized by a panel of paediatric and adult endocrinologists, obstetricians, and a representative of patient organisations. They give an update on all recommendations in the light of new evidence and knowledge from the literature between 2013 and 2020 according to the GRADE (grading of recommendations, assessment, development, and evaluation) system. The evidence section for each recommendation provide detailed discussion of the current available literature and questions that remain and need further clinical research. The main chapters comprise neonatal screening, diagnostic criteria, substitutive treatment, outcome, genetics, and antenatal diagnosis and cover all aspects of primary congenital hypothyroidism. Major innovations are the integration of recommendations for central congenital hypothyroidism and a comprehensive update on known and new genetic forms of congenital hypothyroidism.

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3.9. Newborn screening TSH values less than 15 mIU/L are not associated with long-term hypothyroidism or cognitive impairment

West R, Hong J, Derraik JGB, Webster D, Heather NL, Hofman PL
J Clin Endocrinol Metab. 2020;105:dgaa415.
doi:10.1210/clinem/dgaa415.

The optimal cut-off for neonatal screening has long been a matter of debate. The optimal balance between optimal detection of cases and increase of false positive patients is difficult to define. Also, in the most recent guidelines for congenital hypothyroidism (see previous paper in this chapter 3.7), no precise cut-off was recommended, as screening approaches differ considerably [1].

West *et al*. addressed this open question by a retrospective study. They analysed the long-term neurodevelopmental outcome of 96 healthy individuals who passed routine neonatal screening according to the 15 mU/l TSH cut-off. They had a neonatal screening TSH of 8–14 mU/l. At age 6–12 years, these individuals and 76 siblings were investigated by Wechsler Intelligence Scale for Children. The study was powered to detect differences of 5 IQ points or more between cases and siblings.
Lower mid-childhood IQ showed a mild correlation with increasing TSH levels <15 mU/l. However, if IQ was compared between cases and siblings, no difference was detected over the whole TSH-range of 8–14 mU/l. The authors conclude that there was no clinically relevant long-term negative effect when screening cut-off is 15 mU/l. They argue against lowering the neonatal screening cut-off to below 15 mU/l.

This study is of importance in a field where long-term data are scarce. The study was well designed and limitations discussed in detail. As also discussed by the authors, it is noteworthy that 80% (53/67) of cases with available siblings included had a TSH of 8–11 mU/l, while only 20% (13/67) of cases with siblings had a TSH value between 12–14 mU/l rendering the data more robust in the lower range of TSH values below ≤ 11 mU/l, where 12-14 case-sibling pairs could be compared for each additional unit of TSH. Nevertheless, these data add information on a TSH range, where also previous studies included only few cases. [2]. Ultimately, only prospective studies of treated *versus* untreated patients will provide robust evidence for the optimal TSH screening cut-off.

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3.10. Cognitive and motor outcome in patients with early-detected central congenital hypothyroidism compared with siblings

Naafs JC, Marchal JP, Fliers E, Verkerk PH, Luijten MAJ, Boelen A, van Trotsenburg ASP, Zwaveling-Soonawala N
J Clin Endocrinol Metab. 2021;106:e1231–e1239.
doi:10.1210/clinem/dgaa901.

The results of this study are a robust argument to implement neonatal screening for central congenital hypothyroidism (CCH) worldwide. Only few countries, such as the Netherlands, screen for CCH. Neurodevelopmental outcome data are scarce for CCH compared to primary congenital hypothyroidism (PCH) not only because of the lack of widely established screening but also due to lower incidence (CCH 1:16,000 *vs.* PCH 1:2500-1:3000) [1].

Naafs *et al*. present a large cohort of well phenotyped patients with CCH, all detected by neonatal screening and treated early [2]. They compared full scale intelligence quotient (FSIQ) in patients with isolated CCH (iCCH, $n=35$), CCH in the context of multiple pituitary hormone deficiencies (MPHD, $n=52$) and patient

siblings ($n = 52$). They found no significant difference in FSIQ between iCCH patients and siblings. However, patients with CCH in the context of MPHD, compared to iCCH and siblings, showed a significantly lower mean FSIQ (-7.5, and -7.9 points, respectively), most pronounced in the subgroup of performance IQ.

So far, data on neurodevelopmental outcome in clinically detected and late treated CCH and MPHD patients are lacking for comparison. But the cost-effectiveness of neonatal screening for CCH has already been shown more than a decade ago also in the Netherlands [1].

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New Genes

3.11. Human type 1 Iodothyronine deiodinase (DIO1) mutations cause abnormal thyroid hormone metabolism

França MM, German A, Fernandes GW, Liao XH, Bianco AC, Refetoff S, Dumitrescu AM
Thyroid. 2021;31:202–207.
doi:10.1089/thy.2020.0253.

This study describes a new genetic thyroid disease that might be unnoticeable in individuals with normal thyroid synthetic capacity, but may cause harm in all patients who are dependent on levothyroxine substitution such as congenital hypothyroidism, acquired hypothyroidism, or post-thyroidectomy.

Three iodothyronine deiodinases (D1, D2, D3) are known. They modulate the availability of thyroid hormones. D1 is encoded by the *DIO1* gene. D1 regulates thyroid hormone metabolism in two ways: first, it regulates serum levels of T3 by T4 outer ring deiodination and second, deiodinates the inactive reverse T3 (rT3, a product of T4 deiodination by D3) to inactive T2. Thus, the production of active T3 or inactive rT3 from T4 depends on the balance of D1 + D2 and D3 activities, a regulation level of serum T3 already active *in utero* protecting the fetus from too high maternal T3 [1].

Franca *et al*. report a novel genetic thyroid disorder associated with heterozygous mutations in the Iodothyronine Deiodinase Type 1 (*DIO1*) gene. The index patients presented with slightly elevated TSH values and elevated rT3 levels but were otherwise healthy in the context of further family members with the same biochemical constellation. Functional studies *in vitro* and study of *dio1* knock-out mice confirmed the human phenoytpe.

Of much more relevance than these otherwise healthy patients was a *DIO1* mutation in siblings with thyroid dyshormonogenesis due to *TPO* mutations in a more recent publication of the same authors. Two of three siblings affected by *TPO* mutations had an additional heterozygous *DIO1* mutation. These two siblings showed significantly worse neurological outcomes than the sibling without the additional *DIO1* mutation, suggesting a phenotype modifier role of the *DIO1* mutation. On a biochemical level, the additional *DIO1* mutation resulted in reduced enzyme activity causing decreased T3 production from the substituted levothyroxine. Consecutively, more levothyroxine was inactivated by physiological D3 enzyme activity to rT3, resulting in unrecognizable underdosing of patients, if T4 but not rT3 levels are measured.

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Clinical Studies

3.12. Randomised trial of block and replace vs. dose titration thionamide in young people with thyrotoxicosis

Wood CL, Cole M, Donaldson M, Dunger DB, Wood R, Morrison N, Matthews JNS, Pearce SHS, Cheetham TD
Eur J Endocrinol. 2020;183:637–645.
doi:10.1530/EJE-20-0617.

This study provides for the first time clear evidence that the block and replace (BR) strategy (combination of carbimazole plus levothyroxine) is not superior to the carbimazole dose titration (DT) strategy for treatment of paediatric Graves' disease.

Wood *et al*. present the first randomized controlled multicentre trial of patients with Graves' diseases in the paediatric age group comparing treatment stability of DT *vs.* BR. Each patient ($n = 82$) was randomized shortly after diagnosis and treated for three years. The authors found no difference between DT and BR in percentage of TSH or FT4 levels in or outside the reference range during the study period. However, 3/40 patients on BR *vs.* 0/41 on DT developed neutropenia.

These data are important, providing for the first-time clear evidence that BR therapy has no advantage on long-term biochemical stability compared to DT, although BR is still widely used by paediatric endocrinologists [1]. These data strengthen the current guidelines of the American Thyroid Association and European Thyroid Association (ATA and ETA) [2,3] which do not recommend BR because of its higher carbimazole dose and therefore increased risk of drug-related side effects, as shown by a meta-analysis in adult patients [4].

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3.13. Disease characteristics of MCT8 deficiency: an international, retrospective, multicentre cohort study

Groeneweg S, van Geest FS, Abacı A, Alcantud A, Ambegaonkar GP, Armour CM, Bakhtiani P, Barca D, Bertini ES, van Beynum IM, Brunetti-Pierri N, Bugiani M, Cappa M, Cappuccio G, Castellotti B, Castiglioni C, Chatterjee K, de Coo IFM, Coutant R, Craiu D, Crock P, DeGoede C, Demir K, Dica A, Dimitri P, Dolcetta-Capuzzo A, Dremmen MHG, Dubey R, Enderli A, Fairchild J, Gallichan J, George B, Gevers EF, Hackenberg A, Halász Z, Heinrich B, Huynh T, Kłosowska A, van der Knaap MS, van der Knoop MM, Konrad D, Koolen DA, Krude H, Lawson-Yuen A, Lebl J, Linder-Lucht M, Lorea CF, Lourenço CM, Lunsing RJ, Lyons G, Malikova J, Mancilla EE, McGowan A, Mericq V, Lora FM, Moran C, Müller KE, Oliver-Petit I, Paone L, Paul PG, Polak M, Porta F, Poswar FO, Reinauer C, Rozenkova K, Menevse TS, Simm P, Simon A, Singh Y, Spada M, van der Spek J, Stals MAM, Stoupa A, Subramanian GM, Tonduti D, Turan S, den Uil CA, Vanderniet J, van der Walt A, Wémeau JL, Wierzba J, de Wit MY, Wolf NI, Wurm M, Zibordi F, Zung A, Zwaveling-Soonawala N, Visser WE
Lancet Diabetes Endocrinol. 2020;8:594–605.
doi:10.1016/S2213-8587(20)30153-4.

Groenenweg *et al*. present the so far largest retrospective multicentre cohort study on 151 patients with 73 different MCT8 (*SLC16A2*) mutations to compare and describe in detail the phenotypic spectrum and the

disease course of MCT8 deficiency. The careful description of presenting clinical features, and age at onset of signs and diagnosis provides a good basis for clinical suspicion of MCT8 deficiency in clinical practice.

Detailed auxiological, and clinical parameters are presented in an age-dependent manner highlighting the important problems of resting tachycardia becoming more obvious beyond 10 years of age, while low weight developing in the first five years of life. They also summarize the neurodevelopmental and neuroorthopedic problems as well as secondary organ problems as consequence of MTC8 deficiency (e.g. low bone mineral density, gastroesophageal reflux, recurrent pulmonary infections, elevated systolic blood pressure). Finally, overall survival, and causes of the high mortality rate in the paediatric age group give a precise prognostic view on MCT8 deficiency.

This publication is of importance for diagnosis and care of affected patients in a multidisciplinary setting and widely extends current knowledge on this rare disease. The same authors published outcomes after specific pharmacological treatment in 2019, supporting the importance of collaborative networks in care of rare diseases.

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3.14. Identification of resistance to exogenous thyroxine in humans

Lacámara N, Lecumberri B, Barquiel B, Escribano A, González-Casado I, Álvarez-Escolá C, Aleixandre-Blanquer F, Morales F, Alfayate R, Bernal-Soriano MC, Miralles R, Yildirim Simsir I, Özgen AG, Bernal J, Berbel P, Moreno JC
Thyroid. 2020;30:1732–1744.
doi:10.1089/thy.2019.0825.

This study is of importance, revealing a relevant clinical problem in patients under long-term levothyroxine (LT4) substitution and challenging the concept that all hypothyroid patients can be well controlled with LT4 monotherapy.

Lacámara *et al*. present a small but elegant clinical study describing a so far unrecognized resistance to exogenous thyroxine (RETH) in 18 paediatric and adult patients under LT4 substitution for congenital and acquired hypothyroidism. These patients presented with elevated TSH values despite increased FT4 values due to supraphysiological substitution with LT4, suggesting hypothalamic and pituitary non-responsiveness to the exogenous LT4. Such a phenotype has been described earlier in iodothyronine deiodinase type 2 (*dio2)* knockout mice [1]. Dio2 encodes D2, that mediates T4 to T3 deiodination, as D1, but with tissue specificity in the hypothalamus and pituitary gland.

To characterize the phenotype in affected patients more in detail, the authors combined detailed laboratory investigations to provide insight into the deiodination process in these patients by measuring not only TSH and FT4, but also T4, T3 and rT3 and their ratios under different doses of LT4. The results suggested reduced T4 to T3 deiodination and increased T4 to inactive rT3 conversion, resulting in decreased negative feed-back loop activation. Further, they excluded mutations in thyroid hormone receptor-beta (*THRB)*, *DIO2* and selenocysteine binding protein 2 (*SECISBP2*), all genes possibly explaining the biochemical phenotype if mutated. Finally, they showed in one patient, that a combination of T4 and T3 for substitution, with reduced T4 dose, normalized their elevated TSH levels with normal FT4 and FT3 levels.

Whether T4 + T3 combination therapy is the key in this context remains open and needs to be analysed by randomized controlled trial, as suggested by a recent consensus statement from the American, British, and European Thyroid Associations (ATA/BTA/ETA) [2].

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Thyroid Cancer

3.15. Lymph node metastasis prediction of papillary thyroid carcinoma based on transfer learning radiomics

Yu J, Deng Y, Liu T, Zhou J, Jia X, Xiao T, Zhou S, Li J, Guo Y, Wang Y, Zhou J, Chang C
Nat Commun. 2020;11:4807.
doi:10.1038/s41467-020-18497-3.

This interesting paper illustrates the potential of machine learning to improve the sensitivity and specificity of routine techniques in clinical practice if large cohorts for training and validation of models are used. Yu *et al*. present a transfer learning model (machine learning approach to solve research problems by reusing information from previously learned tasks for the learning of new tasks) in radiology of lymph node metastasis in papillary thyroid cancer patients.

Lymph node metastasis (LNM) are a major concern in patients with papillary thyroid carcinoma (PTC). While preoperative radiologic detection rate by ultrasound of lateral cervical LNM is reliable, identification rate of LNM in the central cervical area is as low as 30%. These authors studied a main cohort of 1013 PTC cases with unifocal lesions and a second dataset of 368 PTC patients with multifocal lesions from the same hospital. Finally, a third independent data set came from two further hospitals with 513 PTC patients with unifocal lesions. Within the datasets, the authors used different ultrasound machines and the results of three independent radiologists. They compared sensitivity by ROC curves and probability threshold curves and showed high average area under the curve (AUC) of 0.90 in the main cohort, and 0.93 in the two validation cohorts using their transfer learning model for LNM detection, showing clear advantage over routinely used methods (clinical statistical model, traditional radiological model, non-transfer learning model).

Over the last year, such radiomics approaches have also been developed for thyroid nodule diagnostics in adult as well as very recently for paediatric patients [1, 2].

Reference

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Reviews

3.16. New therapeutic horizons for Graves' hyperthyroidism

Lane LC, Cheetham TD, Perros P, Pearce SHS
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Over the last decades, the treatment of Graves' disease has been limited to antithyroid drugs, radioactive iodine ablation or surgery. Especially in the paediatric age group, all three therapeutic options have their advantages and side effects.

New therapeutic options focus on the immune-pathophysiology of the disease by targeting either B-lymphocyte function or the TSH-receptor directly. Monoclonal antibodies may cause B-cell depletion, attenuated B-cell activation, inhibition of IgG recycling, or reduced B-cell proliferation and survival and all are under investigation in Phase 2 trials for treatment of Graves' disease. TSH-receptor directed therapies aiming at directly inhibiting TSHR signalling (small molecule TSHR antagonist or TSHR blocking antibody) or TSHR specific immunotherapy are promising approaches under development in preclinical or Phase 1 trials.

Lane *et al*. provide a comprehensive overview of all new pharmacological developments for Graves' disease treatment that promise completely new, innovative and hopefully efficient and safe treatment modalities not only for adults but also children and adolescents.

4. Growth and Growth Factors

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Preface

The selection of papers in this chapter focused on clinical research, as major advancements in the pathophysiology, diagnostic work-up, management and treatment of growth disorders have been reported in 2020–21. The new cut-off values of blood GH concentrations for diagnosis of GH deficiency in the newborn, the insights into the phenotype and therapeutic response to rhGH in patients with *NPR2* mutations, the individualisation of the optimal timing for GH retesting, and the evidence-based assessment of the beneficial effects of rhGH therapy in children with Prader–Willi syndrome, will likely have an impact on clinical practice. A bunch of papers deal with the still controversial issue of the long-term safety of rhGH therapy. The observation of growth impairment in patients with anorexia nervosa, the natural history of patients with *NPR2* mutations, and the elucidation of the genetic architecture underlying familial short stature and SGA open new perspectives for research and management of these conditions. Finally, the genetic approach to the prediction of individual responses to rhGH therapy and the discovery of collagen pathogenic gene variants underlying some cases of familial short stature represent new paradigms in the field.

Important for Clinical Practice

4.1. Diagnosis of severe growth hormone deficiency in the newborn

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Clinical Endocrinology. 2020;93:305–311.
doi:10.1111/cen.14264. PMID: 32521075

This study analyzed retrospectively GH content in newborn screening cards of 20 children with clinical features suggestive of GH deficiency (such as recurrent hypoglycemia) compared to screening cards from 281 healthy newborns, and determined 7 ng/ml to be the optimal a cut off value for the diagnosis of GH deficiency (GHD).

Congenital hypopituitarism in neonates is a rare disease (estimated incidence $<$ 1:20 000 newborns) and may be associated with developmental defects such as ocular, midline and genital abnormalities or be initially asymptomatic but at risk of developing multiple pituitary hormone deficiencies over time. Mutations in genes involved in pituitary development underlie congenital hypopituitarism, which is characterized by the deficiency of one or more pituitary hormones (1). Early diagnosis of GHD is crucial for preventing recurrent severe hypoglycemia in newborns and infants. GH stimulation tests are not feasible in neonatal age and IGF1 measurement is not helpful for diagnosis due to the physiological low blood concentrations at this age.

In this study, according to the clinical, endocrine and brain MRI characteristics, the newborns were classified as having severe GHD (recurrent hypoglycemia with either a significant cerebral MRI morphology or two additional pituitary hormone deficiencies), and without severe GHD (no recurrent hypoglycemia or recurrent

hypoglycemia but no morphological or additional functional defects of the pituitary gland). A detailed questionnaire was sent to the physicians of each newborn with the tentative diagnosis of severe GHD, asking for clinical, endocrine, biochemical and radiological data of the patients. GH levels from 20 term newborns with severe GHD were compared with 441 term and 151 preterm healthy newborns. ROC plot analysis revealed 7 ng/ml as the cut-off value with the best predictive value (90% sensitivity and 98.7% specificity).

The conclusion was that a single measurement of GH concentrations in screening cards may confirm the clinical diagnosis of neonatal GHD. It is noteworthy that GH immunoreactivity in newborn screening cards shows a progressive time-dependent decay when stored at 6 °C indicating the need of adjusting the measured GH content by the storage time. In addition, GH levels vary with the assay method. For instance, another recent study has reported a cut-off value of 4.5 ng/ml having a 100% sensitivity and 85% specificity for diagnosing neonatal GH deficiency (3). Though the potential clinical impact of this study is beyond question, the retrospective design, the small number of patients with GHD and the dependence of diagnosis on the questionnaire responses may limit its value. A thorough clinical evaluation remains the basis for the suspicion of GHD which can be then confirmed by neuroradiology and GH measurement.

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4.2. Clinical characteristics of short-stature patients with an *NPR2* mutation and the therapeutic response to rhGH

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This study aimed to describe the clinical characteristics of 6 patients with *NPR2* gene mutations and the response to rhGH treatment in 2 of them.

The natriuretic peptide receptor 2 gene (*NPR2*) is a paracrine factor involved in the regulation of cell proliferation and differentiation as well as in the synthesis of extracellular matrix in the growth plate (1). Compound heterozygous or homozygous mutations are responsible for severe skeletal dysplasia, whereas heterozygous mutations are associated with idiopathic short stature (ISS) (2). Data on rhGH treatment response in these disorders are still scarce (3, 4).

Here, the 6 patients (3 girls and 3 boys) underwent routine examinations for short stature. The average height SDS at presentation was -3.3 ± 1.9 (range: – 6.3 to – 1.4 SDS). Suspicion of genetic defects in osteochondral dysplasia–related genes (*FGFR3*, *ACAN*, *SHOX*, *NPR2*) was based on clinical evidence of skeletal abnormalities (shortened metacarpals/metatarsal, mesomelic limb shortening, scoliosis) and facial dysmorphic features (midface hypoplasia, flat nasal bridge, high-arched palate). Two of the 6 patients received rhGH treatment; they showed height gains of 3 and 2 cm on rhGH therapy, which was discontinued after 1.5 months and 1 month, respectively.

The authors also performed a literature review of *NPR2* mutations, finding 270 *NPR2* mutation cases, of which 85 cases with homozygous or compound heterozygous mutations and 185 cases with heterozygous mutations. Average height SDS at diagnosis was -6.6 ± 2.2 in patients with homozygous or compound heterozygous mutations, and -2.7 ± 0.9 in those with heterozygous mutations. 21 patients were treated with rhGH, starting at

a mean age of 8.2 ± 3.5 years and with a mean treatment duration of 3.8 ± 2.6 years. A significant increase in height SDS after rhGH treatment was detected. The height gain was more pronounced in girls than in boys, and the height increase was negatively associated with age at start of treatment. Due to the short-term rhGH therapy in all patients reported to date, it is impossible to draw any conclusion yet about the efficacy of such treatment in patients with *NPR2* mutations.

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4.3. Growth hormone retesting during puberty: a cohort study

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These authors enrolled 80 prepubertal patients (46 boys; 34 girls) with idiopathic isolated GHD and normal brain MRI, who were treated with rhGH for at least two years. The data show that *GH therapy could be safely stopped in the 55% who showed normal* GH peak values when retesting was performed *at the intermediate stage of puberty.*

The last international consensus statement recommended re-testing for GH secretion in young adults with childhood-onset GHD (COGHD) and to continue GH replacement therapy, without the need for GH re-testing, in patients with more than three pituitary hormone deficits or with isolated GHD associated with an identified genetic mutation (1). The majority of patients with COGHD show normal GH secretion when re-evaluated at the end of growth (2). If transient prepubertal GH deficiency is postulated, it would be reasonable to retest GH secretion during puberty rather than at the end of growth.

In this study, clinical, auxological and biochemical parameters at five time points were analyzed in all patients: at diagnosis; after the first year of therapy; at the intermediate stage of puberty; at retesting and at the near-adult height. Treatment was discontinued when patients reached the intermediate stage of puberty. After 12 weeks from cessation of therapy, GH secretion was retested using the arginine stimulation test. GH was definitively discontinued after retesting GH in 44 children (55%) who showed a normal GH peak ($\geqq 8$ μg/L) on retesting, and was restarted in 36 (45%) with persistent GHD. No significant differences were found in adult height or the delta height between genetic target and adult height between the two groups. IGF-1 SDS levels < -0.45 were indicative of persistent GHD (84% sensitivity, 63% specificity).

The authors conclude that in children with idiopathic isolated GHD, GH secretion should be retested at mid-puberty, as more than half of these patients show a normalization of GH secretion and GH therapy can be safely discontinued. The results of this study are consistent with previous reports on early retesting showing normalization of GH secretion during the transition phase in children with isolated idiopathic GHD (2).

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4.4. Growth hormone treatment in Prader-Willi syndrome patients: systematic review and meta-analysis

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This systematic review and meta-analysis provides an up to date report on rhGH in patients with Prader–Willi syndrome (PWS). The review included 463 papers published until September 2019. 16 randomized (RCTs) and 20 not randomized (NRCTs) trials were selected for the meta-analysis. The findings support the efficacy and safety of rhGH in patients with PWS.

PWS is the most frequent genetic cause of obesity, occurring in approximately 1:10,000–1:30,000 live births (1). PWS is a complex multisystem disorder, characterized by neonatal hypotonia, feeding difficulties in early infancy, short stature, behavioral disturbances, cognitive impairment, psychiatric illness, dysmorphic features, multiple endocrine abnormalities, early development of hyperphagia and progressive development of severe obesity (2). Reduced GH response has been documented in 58%–100% of PWS children and the efficacy of rhGH treatment on growth, body composition, resting energy expenditure, motor development, muscle strength, exercise tolerance, bone health, and lipid profiles has been reported in several studies. rhGH was approved for treatment of PWS children, without need of GH stimulation tests in 2000 (1,3,4). It has been suggested that rhGH therapy in PWS patients would also improve their cognitive function (5). This review followed the recommended Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

The key findngs were: rhGH treatment improves height (by +1.67 SD scores (SDS) in RCTs and +1.52 SDS in NRCTs); body mass index *z*-scores (−0.67 SDS only in RCTs) and fat mass proportion (−6.5% SDS in RCTs and –7.0% in NRCTs). RCTs also found improvements in lean mass (+2.0%) and head circumference (+0.55 SDS). It was not possible to draw a conclusion on the impact of rhGH therapy on cognitive function and behavior. The number of reported adverse events (mainly sleep apnea) was negligible. Twelve deaths were reported across all these studies.

This is the first systematic review and meta-analysis on rhGH therapy in children with PWS. The findings support the use such therapy in children with PWS and provide reassurance on the potential risk of obstructive sleep apnea syndrome associated to therapy.

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4.5. Long-term safety of growth hormone treatment in childhood: two Large observational studies: nordiNet IOS and ANSWER

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This report gathered data from two large observational studies (NordiNet International Outcome Study and ANSWER Program) aimed at assessing the incidence of adverse drug reactions (ADRs), serious adverse events (SAEs), and their relation with rhGH dose. The whole study cohort included 37,702 subjects, with >130,000 patient-years follow-up. As in previous studies, subjects were stratified into different risk groups according to underlying diagnosis: low-risk (68.4%; comprising isolated GHD, SGA children, and ISS); intermediate-risk (27.5%; multiple pituitary hormone deficiencies, Turner and other syndromes), and high-risk (4.1%, malignancies and syndromes with high risk of malignancy).

As expected, the incidence of adverse events was lowest in the low-risk group. The most frequent ADRs and SAEs included "nervous system disorders" (such as headache) and "musculoskeletal and connective tissue disorders" (such as arthralgia and scoliosis). Among the low-risk group, the incidence of SAEs was significantly higher in SGA children. Perhaps counter-intuitively, GH dose was inversely associated with AE incidence rates. This finding is likely explained by the practice to prescribe lower GH doses to patients considered at higher risk of AEs. No case of cerebral hemorrhage and no increase in mortality risk was observed.

It has to be pointed out that this study reports with events occurring during GH treatment, whereas SAGhE (see paper 4.6) analyzed events occurring in young adults who had received GH treatment during childhood. The lack of long-term follow-up limits the potential of this study to capture the risk of developing non-communicable diseases (eg, diabetes, cardio-vascular morbidity, neoplasms or neurodegenerative diseases).

4.6. Long-term mortality after childhood growth hormone treatment: the SAGhE cohort study

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SAGhE is a large independent European consortium including eight different countries (Belgium, France, Germany, Italy, The Netherlands, Sweden, Switzerland, and the UK) which was set up to evaluate the long-term safety of rhGH in a large cohort (>24 000) of young adult patients treated during childhood (1). In this report, overall and cause-specific mortality was assessed by comparison to general population data to calculate standardized mortality ratios (SMR). Patients were subdivided into different risk groups: 1) low-risk, further subdivided in group 1a (isolated GHD, idiopathic short stature (ISS) or mild skeletal dysplasia) and group 1b (small for gestational age (SGA); 2) intermediate-risk, including multiple pituitary hormone deficiencies and specific syndromes (Turner, Noonan, Down, etc.), and 3), high-risk, history of malignancies or chronic renal failure. The follow-up was >400 000 patient-years (to age 25 years).

In group 1a, all-cause mortality was not significantly increased. In group 1b, mortality risk was significantly increased (SMR 1.5, 95%CI 1.1–1.9) although this result was mainly driven by the French sub-cohort. Mortality risk was also significantly increased in the intermediate and high-risk groups. Notably, mortality from diseases of the circulatory and hematological systems was increased in all risk groups.

This large long-term independent study confirms earlier data from post-marketing surveillance studies indicating no significant effect of childhood rhGH therapy on overall mortality in patients with isolated growth hormone deficiency or idiopathic short stature. Whether the increased mortality observed in subjects born SGA should be attributed to rhGH treatment during childhood is open to debate, as it may be due to their inherent cardiometabolic risk (2),(3) or to the genetic cause underlying small size at birth (4). Consistent with this, mortality was not associated with mean daily or cumulative doses of rhGH for any of the risk groups.

Overall, these data are reassuring, however prolonged long-term surveillance of patients treated with rhGH in childhood is still needed as mortality from cardiovascular and hematological diseases in all treated groups was higher than the general population.

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4.7. Association of childhood growth hormone treatment with long-term cardiovascular morbidity

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This nationwide population-based study assessed the long-term risk of cardiovascular events in patients who had received rhGH therapy during childhood and adolescence. The study cohort comprised 3.408 subjects treated under the GHD, SGA or ISS indications, and 50 036 age-, sex-, and region-based matched controls. Median follow-up was 14.9 years (to age 25 years) with a total of 795 125 person-years. Patients in each diagnostic group had a significant higher risk for cardiovascular events than controls (hazard ratio 1.69; 95% CI, 1.30–2.19), especially women (HR, 2.95; 95%CI, 1.31–3.20) and those treated for SGA (HR, 1.97, %CI, 1.28-3.04). The increased risk was found in all treated groups and was associated with longer therapy duration and cumulative rhGH dose. The most common severe cardiovascular events were ischemic heart disease, cardiomyopathy and stroke.

GH has pleiotropic effects on the cardiovascular system (1). Acromegaly, a pathological model of exposure to high GH concentrations, is characterized by an increased cardiovascular mortality (2). The results of this study are consistent with previous data from French SAGhE cohort showing higher cerebrovascular mortality (3) and a higher risk of hemorrhagic stroke in subjects receiving rhGH for GHD, SGA and ISS indications during childhood (4). The authors wondered whether the observed increased cardiovascular risk might have been caused by discontinuation of rhGH treatment in adulthood in GHD patients rather than treatment during childhood. However, a further analysis of data from patients who continued therapy after completion of growth confirmed the increased cardiovascular morbidity.

These results are consistent with those reported by the SAGHE consortium (see paper 4.6). It should be noted that neither of these study designs can separate effects of rhGH therapy from risks related to the underlying condition. However, they further suggest the need of a long-term close cardiovascular monitoring in children treated with rhGH, especially in women and those treated for the SGA indication.

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4.8. Longitudinal study on metabolic health in adults SGA during 5 years after GH with or without 2 years of GnRHa treatment

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The aim of this longitudinal study was to investigate the potential long-term adverse effects of combined GnRHa/GH treatment on metabolic and bone health in short SGA children. Reassuring data are found at 5 years after cessation of GH therapy.

Children born small for gestational age (SGA) have weight and/or length at birth less than -2 SDS for gestational age (1),(2). Approximately 10% of SGA children do not catch-up their growth retardation and have permanent short stature. Growth Hormone (GH) treatment is effective in improving the growth outcome of SGA children especially when therapy is started well before the onset of puberty. In children born SGA with predicted adult height less than -2.5 S.D., the combined therapy with GH plus 2-years gonadotropin releasing hormone analogues (GnRHa) has been suggested to postpone puberty and eventually increase adult height (3). Therapy with GnRH analogues may affect fat deposition, BMI and bone density as shown in retrospective studies conducted in children with central precocious puberty.

In this study, insulin sensitivity and β-cell function (by frequently sampled intravenous glucose tolerance tests, FSIGT), blood pressure, serum lipid levels, body composition and bone mineral density (by dual-energy x-ray absorptiometry (DXA) scans), were assessed during the 5 years after cessation of GH therapy in SGA young adults who were treated during childhood with the combination of GH + GnRHa ($n = 112$) compared to those treated with GH alone ($n = 251$) and 145 age-matched adults born appropriate for gestational age (AGA). In the GH treated group, a subgroup ($n = 95$) was randomly assigned to treatment with either GH 1 or 2 mg/m2/day ($\sim$ 0.033 or 0.067 mg/kg/d).

At 5 years after discontinuation of GH therapy, the three groups showed no significant differences in fat mass, FSIGT results, blood pressure, serum lipid levels and bone mineral density. Total fat mass and trunk fat were lower, and lean body mass was higher in those treated with 2 mg GH/m^2/day, compared to 1 mg GH/m^2/day, whereas FSIGT results, limb fat, blood pressure, serum lipid levels, and bone density were similar in both GH-dose groups.

This further elegant study from the Rotterdam team shows that combined GnRHa/GH therapy is not associated with long-term adverse effects on metabolism, body composition and bone mineralization markers. However, the efficacy of the combined GH/GnRHa therapy has to be tested in a larger group of short SGA children before being transferred to clinical practice. Moreover, it has to be pointed out that the available metabolic markers are a surrogate of the real cardiometabolic status. In this regard, two recent studies reporting an increased long-term cardiovascular morbidity (4) and mortality (5) in SGA young adults treated with GH during childhood raises concern about a potential, likely IGF1-mediated, adverse effect on endothelial function, whose detection may elude the standard metabolic assessment (6).

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New Perspectives

4.9. Prospective longitudinal assessment of linear growth and adult height in female adolescents with anorexia nervosa

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This prospective observational study on a cohort of 255 adolescents with anorexia nervosa (AN) aimed to investigate the effects of nutritional restriction on linear growth and adult height. Whereas premorbid height did not differ from reference standards, girls with AN showed a mild but significantly reduced height at admission, discharge and at the achievement of adult height that was lower than mid-parental target height, though still within the normal range. The observation that a younger age at admission was associated with a more unfavorable growth outcome, is likely explained by the stronger impact on growth of nutritional restriction occurring before or during pubertal growth spurt.

Anorexia nervosa (AN) is an increasingly common eating disorder whose age of onset is progressively decreasing. It affects approximately 0.2-1% of adolescents and young women in developed countries (1) and patients typically show multiple endocrine disorders (2,3), which represent mechanisms of adaptation to chronic starvation. Growth is impaired in patients with AN who show a state of acquired GH resistance, with higher GH and low IGF-I levels. As GH stimulates gluconeogenesis, its higher levels represent an adaptive mechanism favoring the maintenance of euglycemia in subjects with a reduced availability of energy substrates (3). Nutritional interventions are associated with an incomplete catch-up growth and genetic height potential is often not fulfilled.

This study confirms the importance of nutrition in the maintenance of normal growth trajectory and when nutritional restriction occurs during critical periods of growth, it may irreversibly affect adult height.

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4.10. Short stature is progressive in patients with heterozygous *NPR2* mutations

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This study describes the clinical characteristics of an extended family with novel *NPR2* mutations. The family was an Ashkenazi Jewish family with no history of consanguinity and included two sisters with compound heterozygous *NPR2* missense mutations causing acromesomelic dysplasia Maroteaux type (AMDM), 6 subjects with heterozygous *NPR2* mutations and short stature, and 6 relatives with wild-type *NRP2* sequences and normal stature. The study finds progressive abnormalities with age in the heterozygous carriers.

The application of next-generation sequencing has revealed the molecular basis of growth failure in many children with idiopathic short stature (ISS). *NPR2* encoding the transmembrane receptor atrial natriuretic peptide receptor 2 (ANPRB or NPR2) has recently emerged as a factor involved in the pathogenesis of ISS (1-3). ANPRB is a plasma membrane protein expressed in chondrocytes that produces the second messenger cyclic GMP (cGMP) upon binding with its ligand, the C-type natriuretic peptide (CNP) (4,5). This signaling pathway represents a critical regulator of longitudinal growth and skeletal development. Short stature associated with nonspecific skeletal anomalies in heterozygous carriers of *NPR2* mutations has been described (2,3,6,7).

In this study, whole-exome sequencing was used to detect *NPR2* mutations in parents and daughters with severe short stature. Sanger sequencing was used to confirm the mutations and functional analysis of mutations was carried out. The heterozygous relatives showed initial proportionate short stature and progressive decline of height *z*-scores leading to worsening of growth retardation and of adult height potential. Disproportionate short stature developed over time (reduced arm span to height ratio but normal sitting height to height ratio). Bone age was delayed in the younger children and progressively advanced over time. The phenotype characterized by progressive loss of adult height potential, advancement of bone age and appearance of disproportionate anthropometric features, represents a novel finding and a potential useful clinical flag to help clinicians identify these patients.

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4.11. Genetic architecture associated with familial short stature

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Genetic control of height has been widely explored using genome-wide association studies (GWAS) in multi-ethnic populations (1-4). Although familial short stature (FSS) is the most common type of short stature, its genetic profile and impact on bone metabolism remains to be investigated. This GWAS study identifies 10 novel common genetic variants associated with FSS in 1163 Han Chinese subjects with FSS.

The selection criteria for FSS was (a) height less than the 3rd percentile, (b) father and/or mother height less than the 3rd percentile, (c) bone age appropriate for chronologic age, (d) normal onset of puberty, (e) normal annual growth rate, and (f) normal results of clinical and biochemistry evaluation. The control group consisted of 4168

individuals from the Taiwan Biobank and type 2 diabetes cohorts. For both participants and control subjects, genotyping by GWAS method was performed and a genetic predisposition score was calculated.

Ten novel genetic single nucleotide polymorphisms (SNPs) were identified. These 10 novel SNPs within the 5 closest genes were rs202128628 and rs116988614 in *COL6A5*; rs2375843 in *LOC105374144* (a non-coding ribonucleic acid gene); rs525537 and rs367599822 in *UGT2B17*; rs7659854 in *IQCM*; and rs13183322, rs117002249, rs7033295, and rs199690933 in *PGM5P2*. Furthermore, based on SNPs related to adult height reported by previous GWAS studies (1-6), the associations between these known human height-related SNPs and FSS risk was evaluated and 9 reported GWAS human height-related SNPs were identified for FSS risk. A risk prediction model was performed by ROC curves based on the 10 novel SNPs analyzed. These 10 novel SNPs served as a polygenic risk predisposition score for FSS risk prediction (area under the curve: 0.940 in the testing group). There was no significant increase in the predictive value with the combined 10 novel and 9 reported SNPs when compared with the 10 novel SNPs alone. An association of the 10 novel and 9 reported genetic SNPs with height reduction in the general population emerged from the analysis.

Interestingly, two SNPs mapped to *COL65A,* which encodes a member of the supramolecular assembly of collagen VI (ColVI). ColVI is involved in the formation of the extracellular matrix of articular cartilage and fetal bone. ColVI is responsible for the survival and proliferation of chondrocytes and mutations of this gene have been described in different disorders of bone and cartilage. Another interesting gene among the 10 novel SNPs is *UGT2B17*, which encodes for the uridine 5'-diphosphoglucuronosyltransferase [UDP]-2B17 protein, involved in testosterone metabolism. Homozygous deletions of *UGT2B17* are associated with higher concentrations of total serum testosterone and estradiol. Given the known role of sex hormones in regulating bone and chondrocytes metabolism, *UGT2B17* may affect human height by modulating serum levels of sex hormones.

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4.12. DNA methylation profiling and genomic analysis in 20 children with short stature who were born small for gestational age

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This study aimed to identify potential (epi)genetic causes of short stature in 20 SGA children (13 boys; 7 girls) treated with rhGH. Exome sequencing, single-nucleotide polymorphism (SNP) array (both performed in the whole cohort) and genome-wide methylation analysis (performed in a random subset of 10 children) were applied. This extended genetic approach allowed to identify pathogenic/likely pathogenic variants in 4 children: two siblings harboring variants in *ELAC2* gene, a female with homozygous frameshift variant of *CEP57* gene, and a male with a likely pathogenic variant of *HNRHPH1* gene. SNP array analysis showed pathogenic copy number variants in 5q35.2q35.3 and 22q11.21q11.22 in two subjects. Finally, genome-wide methylation

analysis detected different patterns of methylation in specific regions regulating the GNAS gene. Notably, all but one (male patient who has 5q35.2q35.3 microduplication) with genetic variants or CNVs showed a clinical picture including other features beyond shortness, such as dysmorphic features, additional anomalies and neurodevelopmental delay. 5 of the 6 children with a genetic diagnosis responded well during the first year of GH treatment.

Approximately 2.3% of all newborns are born SGA (1). Maternal, placental, and/or fetal factors may account for the small size at birth (2). Low birth size can be either the only clinical feature or be associated with other features suggestive of a specific syndrome. Albeit SGA represents a worldwide accepted indication for rhGH treatment, the efficacy is extremely variable. This variability in the response to rhGH is due to the fact that SGA is not a single diagnosis, but a definition which includes a heterogeneous group of different underlying etiologies and clinical outcomes. The accurate identification of the underlying etiology may lead to a more appropriate clinical management and genetic counseling. Genetics and epigenetics account for about 30-50% of birth weight variability (3,4), and the use of array-CGH, genome-wide methylation analysis and whole exome sequencing may allow to identify a genetic abnormality in a high proportion of SGA subjects (4). The current study confirms a thorough advanced diagnostic approach based on (epi)genetic analyses is successful in revealing new pathways responsible for short stature and may allow future better prediction of individual height responses to rhGH therapy.

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New Paradigms

4.13 A Genome-wide pharmacogenetic study of growth hormone responsiveness

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The authors performed a large genome-wide association study (GWAS) to assess the role of common genetic variants in the response to GH therapy. A total of 614 children treated with GH were included: 276 with idiopathic GHD, 297 with ISS, and 41 born SGA. The findings implicate some novel mechanisms that may contribute to the wide individual variation in GH response.

Recombinant human GH (rhGH) is used in children for treatment of short stature of various etiologies, including disorders of the growth hormone–insulin like growth factor-1 (GH/IGF-1) axis, genetic syndromes such as Turner and Noonan, and failure to catch-up growth in children born small for gestational age (SGA) (1). The response to treatment varies not only across different disorders but also between individuals with the same cause of short stature, suggesting that individual factors may contribute to GH responsiveness (2). Clinical parameters, such as age and severity of growth retardation at start of treatment, peak GH levels on stimulation tests, GH dose, birth size and mid-parental height, predict rhGH response only partially (3). Individual genetic factors

could influence response to rhGH and explain part of this variability. Polymorphisms in genes within the GH/IGF-1 axis have been proposed as candidate determinants of rhGH response (4-7).

The aim of this study was to identify genetic variants that influence the height response to rhGH therapy, by evaluating the association between individual single nucleotide polymorphisms (SNPs) and the height gain in the first-year of rhGH. The primary analysis included all samples, regardless of diagnosis, and was adjusted for age, gender and principal components of genetic ancestry. The secondary analyses included all samples stratified by ancestry and diagnosis and was adjusted for the full set of clinical variables. Finally, a genetic polygene score was calculated in each individual using 697 known height-associated SNPs and the variance in GH response explained by this polygene score was evaluated by linear regression.

In the primary analysis, common variants near the *B4GALT4* and *TBCE* genes showed the strongest suggestive signals. *B4GALT4* is part of beta-1,4-galactosyltransferase (beta4GalT) gene family, composed by 7 genes. Despite mutations in *B4GALT4* have not yet been reported to be associated with any specific disease, mutations in another gene family member, *B4GALT7*, cause abnormal skeletal growth. In secondary analyses, several loci reached significance including variants near *ST3GAL6*. *ST3GAL6* encodes a sialyltransferase that acts in the same pathway as the beta4GalT family genes. The association of rhGH responses with SNPs related to glycosylation pathways is a novel finding and raises the hypothesis that variation in glycosylation may contribute to the individual variation in GH response.

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4.14. Familial short stature-A novel phenotype of growth plate collagenopathies

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This study aimed to assess the frequency of genetic collagenopathies in children with familial short stature (FSS), describing the phenotype and reporting the response to rhGH treatment after 1 and 3 years and at adult height.

Collagen types II, IX, X, and XI are synthesized by chondrocytes and represent the main collagen types in the extracellular matrix of the growth plate (1). Defects in collagen genes cause different disorders affecting growth

plate and are frequently associated with short stature (1,2). Phenotypes include syndromic and non-syndromic short stature with mild clinical signs (3,4).

The authors studied 87 children affected by FSS, defined as height $\leq$ –2 S.D. in both the patient and the shorter parent, and treated with rhGH. Next-generation sequencing (NGS) for variants in the *COL2A1*, *COL9A1*, *COL9A2*, *COL9A3*, *COL10A1*, *COL11A1,* and COL11A2 genes was used and results were confirmed by Sanger sequencing. For all children with pathogenic variants, response to rhGH treatment after 1 and 3 years was evaluated retrospectively. In 4 children, adult height was compared with pretreatment height SDS and height of the untreated parent with the same genetic variant.

A likely pathogenic variant was found in 10/87 children (11.5%). The pathogenic variants were in *COL2A1* (5 patients), *COL11A1* (4 patients) and *COL11A2* (1 patient). Eight of 10 children were born small for gestational age (SGA) with birth length more severely affected than birth weight. Clinical features included proportional short stature in 8/10 affected children; the remaining 2 had mildly shorter limbs. Four of 10 children had mild signs of bone dysplasia such as scoliosis, pronounced lumbar lordosis, genua valga or limited elbow extension. rhGH treatment increased growth velocity during the first year. Height increased from a median of –3.1 SDS (range: –2.4 to –4.3 SDS) at start of GH treatment to –2.2 SDS (–0.8 to –2.9 SDS) after 3 years of therapy. Adult height, reached in only 4 patients, did not show a significant improvement compared to the untreated parent with the same causative genetic variant.

This study shows that mildly symptomatic collagenopathies are relatively frequent in children with FSS. GH treatment seems to improve stature in the short-term.

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5. Bone, Growth Plate and Mineral Metabolism

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Preface

The skeletal research field continues to develop rapidly and has produced several seminal findings in the last year including advances in the treatment of rare skeletal disorders, and an even deeper understanding into the molecular mechanisms that control skeletal development, endochondral bone formation, mineralization of skeletal tissues and skeletal biology. The targeting of the C-type natriuretic peptide (CNP) pathway and options to directly antagonize the overactivity of the FGFR3 pathway in achondroplasia continues to be a subject of high interest and excitement and we here highlight the double-blind, randomized placebo-controlled phase 3 study of a CNP analogue (vosoritide) in children with achondroplasia. We also highlight the identification of a novel gene for autosomal dominant hypophosphatemic rickets, publication of new growth charts for X-linked hypophosphatemia and two large well-designed paediatric vitamin D trials for the prevention of tuberculosis and asthma exacerbation, respectively.

Translational highlights include an update on the current understanding and knowledge gaps in mineral metabolism and biomineralization, a Nature Medicine article presenting *in vivo* data suggesting that modification of the synovial microenvironment can allow endogenous skeletal stem cells to form hyalin cartilage and thereby heal articular cartilage injuries, as well as a study using gene targeting in zebra fish to reveal the pathogenic mechanism by which mutations in CRTAP and P3H1 causes osteogenesis imperfecta type VII and VIII, respectively.

In the section of skeletal biology, a study by McDonald *et al.*, published in Cell, challenges the current dogma on the origin and fate of osteoclasts as they show evidence that multinucleated osteoclasts can fission into daughter cells, a.k.a. osteomorphs, that subsequently are recycled into bone resorbing osteoclasts via a RANKL-dependent process. Additional articles in this section directly and indirectly highlight the critical role of loading and mechanical stress on the growing skeleton. The findings by Hendrickx *et al.* indicates that mechano-sensing of growth plate chondrocytes is critical to bone formation in the secondary spongiosa. These findings in addition to Xie *et al.*'s findings that growth plates and especially hypertrophic chondrocytes are critically sensitive to mechanical stress and their hypothesis that secondary ossification centers have evolved to protect the growth plates are exciting and have direct implications to the discussion on the type of sport activities that should be recommended to growing children.

In addition, the chapter highlights several other important findings, including the high risk of hypoparathyroidism after total thyroidectomy in children, insights into the molecular mechanism by which PTHrP signalling prevents growth plate chondrocyte hypertrophy, as well as the critical role of Sox9 to prevent epiphyseal fusion and articular cartilage degeneration.

5.1. Once-daily, subcutaneous vosoritide therapy in children with achondroplasia: a randomised, double-blind, phase 3, placebo-controlled, multicentre trial

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Abstract: https://pubmed.ncbi.nlm.nih.gov/32891212/

In brief: Activating mutations in FGFR3 inhibit endochondral ossification in achondroplasia resulting in disproportionate extreme short stature. In this randomised, double-blind, phase 3, placebo-controlled trial, once-daily subcutaneous treatment with vosoritide, a C-type natriuretic peptide-analogue, was found to increase the rate of linear growth in children with achondroplasia.

Comment: Achondroplasia is characterised by extreme, disproportionate short stature with macrocephaly and several complications including foramen magnum stenosis with cervico-medullary compression, hydrocephalus, obstructive sleep apnea. There is an increased risk of sudden death in infancy. Mortality is increased from birth to 4 years of age as well as in the fourth and fifth decades of life.

The condition is caused by an autosomal dominant mutation in the fibroblast growth factor receptor 3 gene (*FGFR3*) that constitutively activates the mitogen-activated protein kinase (MAPK)–extracellular signal-regulated kinase pathway in chondrocytes. C-type natriuretic peptide (CNP) acts on the natriuretic peptide receptor 2 (NPR2) and is a potent stimulator of endochondral ossification at the level of the growth plate. CNP stimulates growth, at least in part, by inhibiting FGFR3-mediated MAPK signalling. Vosoritide is a recombinant C-type natriuretic peptide analogue with an extended half-life.

A previous phase 2 and dose-finding study of daily subcutaneous vosoritide treatment in children (5 to 14 years of age) with achondroplasia demonstrated that the dose-dependent increase in growth rate appeared to level off at a positive effect of approximately 1.5 cm/year that was maintained up to 42 months of treatment.

In this phase 3, randomized, double-blind, placebo-controlled, 52-week trial (NCT03197766), the efficacy and safety of daily subcutaneous injections of vosoritide (15.0 μg/kg) was compared to placebo in 121 children (age range, 5 to <18 years) with achondroplasia. Two patients in the vosoritide group discontinued their participation after 2 and 6 days due to pain from injection and fear of needles, respectively. The remaining 119 participants completed the study and enrolled in the extension study. All 121 randomized patients were included in the efficacy analyses.

After 52 weeks of treatment, the annualized growth velocity was 1.57 cm/year higher in the vosoritide compared to the placebo group (95% CI 1.22–1.93; $P < 0.0001$) and slightly better at 1.71 cm/year (95% CI 1.40–2.01) if adjusted for baseline growth velocity. In addition, all subgroup analyses (sex, age, tanner stage, height z-score, baseline growth velocity) indicated a positive effect of vosoritide. This study demonstrates that vosoritide is efficacious in increasing the growth velocity in children with achondroplasia and supports previous studies indicating that is safe. Further studies with earlier start and longer duration of treatment are needed to determine the total amount of height gain that can be accomplished with this treatment and possible effects on other important complications of the condition.

5.2. Evaluation of FGFR inhibitor ASP5878 as a drug candidate for achondroplasia

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Sci Rep. 2020 Dec 1;10(1):20915
Abstract: https://pubmed.ncbi.nlm.nih.gov/33262386/

In brief: Inhibition of excessive FGFR3 signalling constitutes the key mechanism of pharmacological treatments in achondroplasia. In this pharmacokinetic and pharmacodynamic animal study, the FGFR inhibitor ASP5878 with potential oral application mode revealed beneficial effects on skeletal growth in achondroplasia mice.

Comment: Multiple strategies for treatment of achondroplasia by inhibition of excessive FGFR3 signalling are subject to clinical and basic research. So far, CNP analogues acting at the MAPK level downstream of FGFR3 have been shown the most promising results with positive phase II & III trial results and likely imminent approval of Vosoritide and on-going Phase II trials of TransCon-CNP as the most promising agents.

Pan-FGFR blockers were originally developed as oncological treatments of FGF receptor expressing tumours. Here, Ozaki *et al*. investigated the effects of the novel FGFR-blocker ASP5878 for pharmacodynamic studies in an achondroplasia model system. Comparative analysis with a vosoritide-like peptide treatment as "gold-standard" of FGFR3 inhibition has been performed. The study showed amelioration of bone length and growth plate thickness in male mice, although CNP analogues revealed superior results. Interestingly, the effect was weaker in female mice.

Several FGFR-inhibiting molecules with potential to effectively inhibit the overactive FGFR3 receptor in achondroplasia have been investigated in achondroplasia animal models (reviewed in 1). However, pan-FGFR inhibition commonly has adverse effects on other organ systems. The authors have therefore given special emphasis on off-target effects. Despite of a quite favourable risk profile in this animal study, adverse effects in ASP5878-treated adult carcinoma patients including hyperphosphatemia and retinal detachment raise concerns for potential safety issues in the use of ASP5878 in paediatric patients.

Other potential treatments with oral administration include the antihistamine meclizine as well as the tyrosine kinase inhibitor infigratinib. Infigratinib is currently under investigation in phase II studies, whereas meclizine has completed phase I study showing promising safety data (2).

In conclusion, this study extends the spectrum of promising pharmacological candidates for medical treatment to improve skeletal growth in achondroplasia. The potential of oral administration is very attractive. However, concerns of limited efficacy and adverse effects may limit its prospects for future development to a safe and efficacious medicine that can be used to augment growth in children with FGFR3-related skeletal dysplasias.

Reference

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Advances in Clinical Practice

5.3. Mutation of *SGK3*, a novel regulator of renal phosphate transport, causes autosomal dominant hypophosphatemic Rickets

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J Clin Endocrinol Metab. 2020 Jun 1;105(6):dgz260.
Abstract: https://pubmed.ncbi.nlm.nih.gov/31821448/

In brief: In large kindred including five hypophosphatemic rickets (HR) patients with a pattern of autosomal dominant inheritance, a novel c.979–96 T > A variant in the *SGK3* gene segregated perfectly with the phenotype, i.e. present in all 5 patients and in none of the healthy family members, providing strong support for its causation.

Comment: Hereditary hypophosphatemic rickets (HR) is a group of renal phosphate wasting disorders. X-linked HR, caused by genetic variant in the *PHEX* gene is the most common hereditary form of HR representing about 80% of all cases, while the rest are caused by variants in *FGF23, DMP1, ENPP1, CLCN5, SLC9A3R1, SLC34A1,* or *SLC34A3* genes.

The Index case was a 3-year-old female who was referred for short stature and bone deformities of the lower limbs. Mother, maternal grandmother, aunt, and a cousin were also affected with short stature and bone deformities. Biochemical and radiological investigations were confirmative of hypophosphatemic rickets with hypophosphatemia and reduced renal tubular phosphate reabsorption. Patients and their family members were negative for *PHEX* and *FGF23* mutations. Exome sequencing was subsequently performed to identify novel candidate genes. No copy number variation was observed in the genome using CytoScan HD array analysis. Also, no mutations were found in *DMP1, ENPP1, CLCN5, SLC9A3R1, SLC34A1,* or *SLC34A3* by exome sequencing.

A novel c.979–96 T > A variant in the *SGK3* gene inherited in an autosomal dominant pattern was found in all 5 affected individuals and was absent in all unaffected family members that were tested. This gene codes for SGK3 (serum and glucocorticoid-regulated kinase 3), a serine/threonine kinase closely related to Akt. It has been shown to regulate renal tubular phosphate transport and bone density. The mutation is located 1 bp downstream of a highly conserved adenosine branch point, resulted in exon 13 skipping and in-frame deletion of 29 amino acids and therefore disruption of the protein tertiary structure and part of the protein kinase domain and is thus likely a loss-of-function variant.

The mechanism by which mutations in SGK3 cause phosphate wasting and hypophosphatemia is not fully elucidated. SGK3 has been shown to regulate renal tubular phosphate transport by increasing NaPi-2a activity, thus stimulating renal tubular phosphate reabsorption and bone mineralization (1). Consistently, Sgk3 knockout mice show phosphate wasting and have decreased bone density, likely related to reduced NaPi-2a activity (2). In addition, both plasma FGF23 and calcitriol (1,25(OH)2D3) concentrations were reduced in the Sgk3 knockout mice and the authors therefore speculated that SGK3 may participate in the regulation of calcitriol synthesis. In the index case, treatment for 5 years with 50 mg/kg/day phosphate sodium tablets, gradually increased to 75 mg/kg/day divided in 6 equal daily doses, and calcitriol at 40 ng/kg/day led to resolution of leg deformities and improvement in biochemical features of hypophosphatemic rickets.

In summary, this finding of a novel splice variant in the SGK3 gene that co-segregates perfectly within a 3-generation family with HR adds SGK3 to the list of genes in which mutations may cause hypophosphatemic rickets with an autosomal dominant inheritance.

Reference

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2. Bhandaru, M., *et al*., Decreased bone density and increased phosphaturia in gene-targeted mice lacking functional serum- and glucocorticoid-inducible kinase 3. *Kidney Int*, 2011. 80(1): p. 61–7.

5.4. Children are at a high risk of hypocalcaemia and hypoparathyroidism after total thyroidectomy

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J Pediatr Surg. 2020 Jul;55(7):1260–1264

Abstract: https://pubmed.ncbi.nlm.nih.gov/31383578/

In brief: Hypoparathyroidism is a common complication after total thyroidectomy and has been reported to occur in 30% to 60% of adult patients. The current study of 106 pediatric patients undergoing total thyroidectomy between 1998–2018 reports that hypoparathyroidism was common, with hypocalcemia occurring during the first 24h in 63 (59%) children and that hypoparathyroidism persisted at 6 months post-surgery in 23 (22%) children.

Comment: The authors report the outcomes in calcium homeostasis in a retrospective chart review of 106 pediatric patients undergoing total thyroidectomy between 1998 and 2018. All children who underwent total thyroidectomy between 1998–2018 at either University College London Hospital or Great Ormond Street Hospital (both London, United Kingdom) and were $\leq$18 years of age at the time of surgery were included in the analysis. The indications for surgery were Graves' disease ($n=52$; 49%), Multiple Endocrine Neoplasia type-2 ($n=36$; 34%), multinodular goiter (n = 3; 3%) and follicular/papillary thyroid carcinoma ($n=15$; 14%). Neck dissection was performed in 23 children (19%) and autotransplantation in 14 children (13%). In 31 children (29%), $\geq$1 glands were found in the specimen.

Hypocalcaemia occurred within 24h of thyroidectomy in 63 children (59%) and 52 children (49.3%) were discharged on supplements. Hypoparathyroidism persisted at 6 months in 23 children (21.7%) and was more likely if $<$ 4 parathyroid glands remained in situ.

Hypoparathyroidism is a very common complication after total thyroidectomy in adults and is at least as prevalent in children. This study highlights the need for expert, high—volume surgeons and improved imaging techniques that can visualize the often well-hidden parathyroid glands intraoperatively (1). In addition, adequate recognition and management is crucial to minimize the morbidity of post-thyroidectomy hypoparathyroidism.

Reference

1. Benmiloud *et al*. Association of Autofluorescence-Based Detection of the Parathyroid Glands During Total Thyroidectomy With Postoperative Hypocalcemia Risk: Results of the PARAFLUO Multicenter Randomized Clinical Trial. *JAMA Surg*. 2020 Feb 1;155(2):106–112.

5.5. Growth curves for children with X-linked hypophosphatemia

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J Clin Endocrinol Metab. 2020 Oct 1;105(10):3243–3249
Abstract: https://pubmed.ncbi.nlm.nih.gov/32721016/

In brief: X-linked hypophosphatemia (XLH) is the most common hereditary form of rickets and osteomalacia. The study used retrospective, pre-burosumab growth data from four different studies and constructed growth charts that demonstrate that the growth rate of children with XLH clearly becomes subnormal at approximately 1 year of age.

Comment: To better characterize growth impairment in patients with XLH, retrospective growth data collected in the observational study UX023-CL002 and pre-treatment data from the 3 pediatric clinical trials UX023-CL201, -CL205, and -CL301 were used to construct growth curves for children with XLH, a large majority of whom were receiving conventional therapy with phosphate supplements and active vitamin D. The annual height increment in children with XLH deviated from that of healthy children starting at age 6 months. For boys, median height percentiles were 46% at 3 months, 37% at 6 months, 18% at 1 year, and 5% at 2 years of age. Similar declines were observed in girls: 52% at 3 months, 37% at 6 months, 18% at 1 year, and 7% at 2 years of age.

These findings confirm previously published smaller data sets assessing linear growth in children with XLH that have showed a distinct decline in height z-scores/percentiles after 1 year of age due to rapid growth deceleration that may occur as early as age 9 months. These findings are also consistent with previous studies suggesting that early start of treatment is important result in improved outcomes in terms of height and deformities (1). These disease-specific growth charts for boys and girls will be very useful tools in the monitoring general health as well as the effects of medical treatments in children with XLH.

Reference
1. Makitie, O., *et al*., Early treatment improves growth and biochemical and radiographic outcome in X-linked hypophosphatemic rickets. *J Clin Endocrinol Metab*, 2003. 88(8): p. 3591–7.

5.6. Vitamin D supplements for prevention of tuberculosis infection and disease

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N Engl J Med 2020; 383:359–368 DOI: 10.1056/NEJMoa1915176
Abstract: https://pubmed.ncbi.nlm.nih.gov/32706534/

In brief: Vitamin D metabolites support innate immune responses to Mycobacterium tuberculosis. In this phase 3, randomized, controlled trial of vitamin D supplementation to prevent tuberculosis infection, there was no reduction of risk of tuberculosis infection, tuberculosis disease, or acute respiratory infection than placebo among vitamin D-deficient schoolchildren in Mongolia.

Comment: Most cases of tuberculosis disease arise as a consequence of reactivation of asymptomatic latent Mycobacterium tuberculosis infection. However, primary infection is most commonly acquired in childhood; therefore, measures to prevent acquisition of latent tuberculosis infection in children will need to be implemented if desired reductions in tuberculosis incidence are to be achieved.

Previous studies have reported that (a) vitamin D deficiency is associated with susceptibility to latent tuberculosis infection in school children, (b) vitamin D supplementation boosts immunity to mycobacterial infection in persons in contact with others who have tuberculosis disease, (c) reduces the risk of conversion to a positive result on a tuberculin skin test in schoolchildren and (d) a meta-analysis of longitudinal studies has shown that vitamin D deficiency predicts the risk of tuberculosis disease in a concentration-dependent manner. Vitamin D supplementation has therefore been proposed as an intervention to reduce the risk of acquiring latent tuberculosis infection in populations in which deficiency is prevalent.

A total of 8851 children underwent randomization: 4418 were assigned to the vitamin D group who received weekly oral supplementation with 14 000 IU of vitamin D3 for 3 years, and 4433 to the placebo group; 95.6% of children had a baseline serum 25(OH)D level of less than 20 ng/ml. Among children with a valid tuberculosis assay (QFT) result (at the threshold of 0.35 IU/mL) at the end of the trial, a positive result was seen in 3.6% (147 of 4074 children) in the vitamin D group and 3.3% (134 of 4043) in the placebo group (adjusted risk ratio, 1.10; 95% confidence interval [CI], 0.87–1.38; $P=0.42$). The mean 25(OH)D level at the end of the trial was 31.0 ng/ml in the vitamin D group and 10.7 ng/ml in the placebo group (mean between-group difference, 20.3 ng/ml; 95% CI, 19.9–20.6). Tuberculosis disease was diagnosed in 21 children in the vitamin D group and in 25 children in the placebo group (adjusted risk ratio, 0.87; 95% CI, 0.49–1.55). Hospitalization for acute respiratory infection was seen in 29 children in the vitamin D group and 34 in the placebo group (adjusted risk ratio, 0.86; 95% CI, 0.52–1.40). The incidence of adverse events did not differ between groups.

As QFT conversion at the higher threshold of 4.0 IU/mL has recently been reported to be more sustained than at the threshold of 0.35 IU/mL on which primary outcome was based, the authors conducted a post hoc analysis using the QFT threshold 4.0 IU/mL. No significant effect was seen in the trial population as a whole, but in children with baseline 25(OH)D levels <10 ng/mL, the risk of QFT conversion >4.0 IU/mL was lower among children assigned to vitamin D than to placebo. The results of this post-hoc subgroup analysis should of course be interpreted with caution. However, it does provide some support for the presumed adverse effects of severe vitamin D deficiency on the immunological defence against mycobacterial infection.

In conclusion, vitamin D supplementation did not reduce the risk of tuberculosis infection, tuberculosis disease, or acute respiratory infection than placebo among vitamin D–deficient schoolchildren in Mongolia.

5.7. Effect of vitamin D3 supplementation on severe asthma exacerbations in children with asthma and low vitamin D levels: the VDKA randomized clinical trial

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JAMA. 2020 Aug 25;324(8):752–760.
doi: 10.1001/jama.2020.12384.
Abstract: https://pubmed.ncbi.nlm.nih.gov/32840597/

In brief: Several observational studies have linked low serum 25(OH)D levels to severe asthma exacerbations, lower lung function, and reduced response to corticosteroids. In this randomized controlled trial in children with persistent asthma and low vitamin D levels, vitamin D3 supplementation compared with placebo did not improve the time to a severe asthma exacerbation. The findings do not support the use of vitamin D3 supplementation to prevent severe asthma exacerbations in these children.

Comment: Vitamin D has immune-modulatory and anti-inflammatory effects. Vitamin D may also attenuate viral-induced asthma attacks by reducing rhinovirus replication in bronchial epithelium. A meta-analysis of clinical trials including participant-level data showed that vitamin D3 supplementation was associated with lower risk of asthma exacerbations, but the pooled estimate was not significant among children $<$ 16 years old. However, previous paediatric randomized trials have not focused on children with low vitamin D levels or who are at high risk for severe asthma exacerbations. Moreover, previous trials did not monitor vitamin D levels and did not show consistently higher levels after supplementation.

In this randomized trial, participants were randomized to vitamin D3, 4000 IU/d ($n=96$), or placebo ($n=96$) for 48 weeks, and maintained with fluticasone propionate, 176 μg/d (6–11 years old), or 220 μg/d (12–16 years old). Among 192 randomized participants (mean age, 9.8 years; 77 girls [40%]), 180 (93.8%) completed the trial. A total of 36 participants (37.5%) in the vitamin D3 group and 33 (34.4%) in the placebo group had 1 or more severe exacerbations. Compared with placebo, vitamin D3 supplementation did not improve the primary outcome, time to a severe exacerbation: mean 240 days on vitamin D3 vs 253 days on placebo (mean difference, −13.1 days [95% CI, −42.6–16.4]; adjusted hazard ratio, 1.13 [95% CI, 0.69–1.85]; $P=0.63$). Likewise Vitamin D3 supplementation did not improve secondary outcomes: time to a viral-induced severe exacerbation, the proportion of participants whose dose of inhaled corticosteroid was reduced, or the cumulative fluticasone dose during the trial.

This study has limitations as it was underpowered to, (a) assess if small difference in severe asthma exacerbation (37.5% in the vitamin D3 group and 34.4% in the placebo group) was statistically significant and, (b) to determine whether vitamin D3 supplementation reduces severe asthma exacerbations in children with vitamin D levels $<$20 ng/mL because only few participants had such levels. In addition, findings cannot be extrapolated to other age groups, including preschool children, or to settings with limited ability to monitor vitamin D levels.

To conclude, among children with persistent asthma and low vitamin D levels, this trial showed that vitamin D3 supplementation, compared with placebo, did not significantly improve the time to a severe asthma exacerbation. The findings do not support the use of vitamin D3 supplementation to prevent severe asthma exacerbations in such patients.

Translational Highlights

5.8. Hormonal regulation of biomineralization

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Nat Rev Endocrinol. 2021 May;17(5):261–275
Abstract: https://pubmed.ncbi.nlm.nih.gov/33727709/

In brief: This article systematically reviews the current advances in the understanding of mineral metabolism with focus on the regulation of mineralization in skeletal tissue and inhibition of mineralization in non-skeletal tissue. This is mandatory reading for any aspiring endocrinologist.

Comment: Mineralization of the skeleton and teeth are critically important to allow ambulation and feeding. Inhibition of this process is equally important to prevent ectopic mineralization of soft tissues and organs, which is disabling and potentially lethal. The review is well-structured as it presents the current understanding of the complex regulation of the physiological and pathological aspects of the mineralization process. The roles and regulation of calcium and inorganic phosphate, dietary intake of minerals as well as the balance between activators and inhibitors of mineralization are discussed. The major regulators of biomineralization include parathyroid hormone (PTH), the vitamin D system, vitamin K, fibroblast growth factor 23 (FGF23) and phosphatase enzymes, and their respective roles in the mineralization process are discussed in detail. In addition, alternative regulatory mechanisms that control mineral delivery, skeletal metabolism and biomineralization in the fetus, the neonate, and in the mother during pregnancy and lactation are also discussed.

5.9. Articular cartilage regeneration by activated skeletal stem cells

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Nat Med. 2020 Oct;26(10):1583–1592
Abstract: https://pubmed.ncbi.nlm.nih.gov/32807933/

In brief: Improved treatments for osteoarthritis and other degenerative joint diseases are urgently needed. This study demonstrates, for the first time, that the synovial microenvironment can be modified to allow resident skeletal stem cells to form hyaline articular cartilage and thereby regenerate injured articular cartilage.

Comment: Murphy *et al*. investigated the ability of resident skeletal stem cell (SSC) populations to promote cartilage regeneration in relation to age. Age likely contributes to osteoarthritis, a degenerative joint disease that results in progressive destruction of articular cartilage, caused by a combination of multiple factors. This paper demonstrates that a progressive depletion of resident stem-cell pool and diminished chondrogenesis are associated with aging in mice and human joints, by using different β*actin-Cre-ERT/Rainbow* mouse models assessing changes in clonal skeletogenesis. Results were reproduced by human SSCs in a preclinical xenograft model.

Microfracture (MF) surgery is widely used to attempt to regenerate cartilage in OA. A fibrous cartilage is formed, providing relief to patients despite having reduced mechanical properties. The authors used MF surgery in a mouse model of OA and observed a localized expansion of SSCs in adult osteoarthritic mice joints and increased cartilage formation. To promote articular rather than fibrous cartilage formation, the authors used BMP2 localized co-delivery in a hydrogel, together with soluble VEGFR1, a VEGF receptor antagonist, in a model of meniscectomy-induced OA, and found that it overcomes the MS-activated SSC formation of fibrous cartilage and promote the differentiation of MF-activated SSCs toward hyaline cartilage.

In summary, this is one of the first studies to show that specific activation of resident skeletal stem cell population could be used for stable articular cartilage regeneration for localized treatment of articular cartilage injuries and osteoarthritis.

5.10. Single cell transcriptomic analysis of human pluripotent stem cell chondrogenesis

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Nat Commun. 2021 Jan 13;12(1):362
Abstract: https://pubmed.ncbi.nlm.nih.gov/33441552/

In brief: Heterogenous differentiation patterns and low yields limit the use of human induced pluripotent stem cells (hiPSCs) for the generation of cartilage. In the present work, bulk- and single cell RNA sequencing during chondrogenic differentiation were used to identify regulatory networks responsible for these unintended effects. An improved protocol for highly specific chondrocyte differentiation was established based on these data.

Comment: The discovery of human induced pluripotent stem cells has revolutionized the field of regenerative therapies. While major progress has been made in conditions such as macular degeneration or type I diabetes, generation of chondrocytes and functional cartilage remains a major challenge. Low cellular yield, hypertrophy and non-cartilaginous populations limit advances in hiPSC-based applications for cartilage regeneration.

Wu *et al*. systematically analyzed hiPSC cultures under chondrogenic differentiation and identified neural- and melanocyte-like cells as major off-target populations under current standard protocols. Interestingly, specific WNT signaling at major differentiation branchpoints was shown to be causative for both off-target differentiation as well as for unintended effects such as chondrocyte hypertrophy. By application of a WNT-inhibitor during hiPSC pellet cultures, improved homogeneity and GAG production could be achieved. In addition, the purity of chondrocyte cultures allowed the identification of novel hub genes associated with chondrogenesis such as *C1QL1*.

In summary, the presented work tackled several limitations of hiPSC-based chondrocyte generation in a targeted experimental setup based on state-of-the art methodology including single cell transcriptomics. In addition to the achieved progress in cartilage regeneration research, they describe a prototype study for analytic optimization of suboptimal results in the use of hiPSCs that may further progress in other fields of stem cell research.

5.11. Crtap and p3h1 knock out zebrafish support defective collagen chaperoning as the cause of their osteogenesis imperfecta phenotype

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Matrix Biol. 2020 Aug;90:40–60
Abstract: https://pubmed.ncbi.nlm.nih.gov/32173581/

In brief: Mutations in 3-hydroxylation complex genes *CRTAP* and *P3H1* cause osteogenesis imperfecta type VII and VIII, respectively. However, the pathogenic mechanism by which these mutations cause disease remains unclear. This study points to a defective chaperone role of the 3-hydroxylation complex as the main cause of the skeletal phenotypes.

Comment: Collagen type I in fibrillar collagens undergoes 3-hydroxylation of proline residues, a rare post translational modification with still unknown role. The endoplasmic reticulum (ER) complex composed of cartilage associated protein (CRTAP), prolyl 3-hydroxylase 1 (P3H1) and prolyl *cis/trans* isomerase B are responsible for 3-hydroxylationof proline in collagen type I, and has both an enzymatic and chaperon function. Its mutation cause moderate to lethal recessive forms of osteogenesis imperfecta (OI) with impaired levels of α1(I)3Hyp986, namely OI type VII and VIII. However, what causes the bone phenotype in these OI patients when 3-hydroxylation complex is altered is still unclear.

As collagen type I with 3-hydroxylated proline is absent in wild type zebrafish, the authors used this model to study the role of the chaperone function of the ER complex. CRISPR/Cas9 was used to generate zebrafish knock

outs for *crtap* and *p3h1* and extensive molecular, morphological, biochemical and histomorphometric analyses demonstrated that mutant fish exhibit reduced size, body disproportion, delayed mineralization, as well as altered vertebral bone morphology and skeletal deformities, resembling features in human OI patients. Mutant fish also showed impaired bone properties such as reduced size, thickness and bone volume. At intracellular level collagen type I was overmodified and retained in ER, inducing an ER enlargement, whereas it created disorganized fibers with altered diameter in the surrounding extracellular matrix. Taken together, these findings suggest that the complex has an important chaperone role in collagen folding and that disruption of this function is the main pathogenic mechanisms by which mutation in *CRTAP* and *P3H1* causes phenotypes of OI type VII and VIII, respectively.

Advances in Skeletal Biology

5.12. Osteoclasts recycle via osteomorphs during RANKL-stimulated bone resorption

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Cell. 2021 Mar 4;184(5):1330–1347.e13

Abstract: https://pubmed.ncbi.nlm.nih.gov/33636130/

In brief: This paper reports an alternative cell fate for multinucleated, bone-resorbing osteoclasts and shows that they may undergo fission into smaller osteomorphs. The findings challenge the current dogma that osteoclasts primarily differentiate from hematopoietic progenitor cells and are terminally differentiated cells that are destined to undergo apoptosis after resorption is completed.

Comment: Fusion of monocyte/macrophage-derived precursor cells leads to the formation of osteoclasts, which are specialized bone-resorbing cells. These cells are thought to undergo apoptosis once bone resorption is complete. However, their life cycle *in vivo* is still elusive. Moreover, tracking osteoclast formation and fate in their native environment is challenging.

Here, McDonald *et al*. show, by intravital imaging of *in vivo* osteoclast dynamics, an alternative cell fate for RANKL-stimulated osteoclasts, which underwent fission into daughter cells called osteomorphs. They also found that this osteoclast recycling is regulated by RANKL signaling, as RANKL inhibition by decoy receptor led to accumulation of osteomorphs, which retain the ability to fuse into resorbing osteoclasts. Single-cell RNA sequencing of osteomorphs revealed that they are transcriptionally distinct from osteoclasts and macrophage precursors. Osteomorph upregulated genes control bone structure and function and may be implicated in the pathogenesis of rare monogenic skeletal dysplasias and common polygenic bone diseases.

In summary, this paper reports that osteoclasts recycle via osteomorphs, which are involved in regulation of bone resorption. These findings fundamentally challenge the current thinking of the fate and behavior of osteoclasts. They point towards novel mechanisms for the regulation of bone resorption and may lead to new therapeutic approaches for osteoporosis and other bone fragility disorders.

5.13. SOX9 keeps growth plates and articular cartilage healthy by inhibiting chondrocyte dedifferentiation/osteoblastic redifferentiation

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Proc Natl Acad Sci USA 2021 Feb 23;118(8):e2019152118.
Abstract: https://pubmed.ncbi.nlm.nih.gov/33597301/

In brief: Sox9 is the key transcription factor and master regulator of chondrocyte differentiation during skeletal development. This paper demonstrates that SOX9 also has a key role during postnatal life to maintain open growth plates and healthy articular cartilage by preventing dedifferentiation of chondrocytes into skeletal progenitors and their subsequent differentiation into osteoblasts.

Comment: Haseeb *et al*. investigated the essential role of the transcription factor SOX9 in growth plate and articular cartilage. During development chondrocytes undergo differentiation programs to form growth plate cartilage that drives skeletal growth, but also to form permanent articular cartilage at the joint surfaces. It is well-established that SOX9 is essential during embryonic chondrogenesis, but its role during postnatal life is still elusive.

The authors used conditional knock-out mice and high-throughput sequencing analyses and showed that SOX9 prevents growth plate closures at postnatal level as well as deterioration of articular cartilage in an osteoarthritic microenvironment. In absence of SOX9, growth plates are rapidly fused as chondrocytes differentiate to hypertrophic chondrocytes or revert to a dedifferentiated progenitor state, followed by upregulation of osteogenic specific genes and differentiation toward osteoblast cells. Pathway analysis revealed that SOX9 controls TGFβ and BMP signaling during this cell lineage transition. SOX9 deficiency did not alter articular cartilage differentiation, except in the load-bearing regions, in which the chondrocyte-to-osteoblast transition occurred.

In summary, the findings reveal that SOX9 is crucial to maintain cells in the chondrocytic state, prevent hypertrophy and also restrain plasticity towards dedifferentiation to the progenitor state, as well as toward osteoblast differentiation. This highlights the essential role of SOX9 in maintaining healthy growth plate and articular cartilage, improving our understanding of the functional mechanisms underlying cartilage skeletal disorders and points towards a potential target for novel therapeutic approaches for growth regulation as well as for the prevention of osteoarthritis.

5.14. Piezo1 inactivation in chondrocytes impairs trabecular bone formation

Gretl Hendrickx, Verena Fischer, Astrid Liedert, Simon von Kroge, Melanie Haffner-Luntzer, Laura Brylka, Eva Pawlus, Michaela Schweizer, Timur Yorgan, Anke Baranowsky, Tim Rolvien, Mona Neven, Udo Schumacher, David J Beech, Michael Amling, Anita Ignatius, Thorsten Schinke
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J Bone Miner Res. 2021 Feb;36(2):369–384
Abstract: https://pubmed.ncbi.nlm.nih.gov/33180356/

In brief: Chondrocyte-specific ablation of the mechano-sensory Piezo1 results in substantially impaired formation of secondary spongiosa during endochondral bone formation. The study explores this unexpected finding and show that mechano-sensing in growth plate chondrocytes directly regulates the formation of bone trabeculae in the secondary spongiosa.

Comment: Osteocytes are considered to be the main mechano-sensors in bone. Nevertheless, the distinct mechanisms of mechanical sensing and the role of other skeletal cells and tissues is still subject to research. Recently, the mechano-sensing ion channel Piezo1 was shown to activate Wnt1 signal pathway as well as to induce Akt phosphorylation to increase bone formation (1).

Here, Hendrickx *et al*. investigated the role of Piezo1 and Piezo2 in different skeletal cells from conditional knock-out mice. Unexpectedly, knockout in Runx2 expressing cells (intended to target osteoprogenitors and early osteoblasts) showed a more severe phenotype than osteocyte-specific knockout. The authors further

observed pronounced structural impairments in the trabecular compartment adjacent to the growth plate. Pursuing these findings, they found that Runx2-expressing growth plate chondrocytes, not the targeted osteoblast progenitors, were responsible for the bone phenotype.

The unexpected and novel finding is that growth plate chondrocytes are mechano-sensory cells that regulate formation of trabecular bone. This opens a new perspective on the role of the transient growth plate cartilage in bone formation. The bone trabeculae formed during endochondral bone formation at the growth plates fuse with cortical bone and thereby make substantial contributions to the strength of the newly formed bone (2). It therefore makes sense that mechano-sensing in growth plate chondrocytes can regulate the formation of the secondary spongiosa. This study, taken together with the study of Xie *et al*. (see paper 5.16), point to the importance of hypertrophic chondrocytes as a potential key cell to transfer mechanical stress cues to the newly formed bone during endochondral bone formation.

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5.15. PTHrP targets salt-inducible kinases, HDAC4 and HDAC5, to repress chondrocyte hypertrophy in the growth plate

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Bone. 2021 Jan;142:115709
Abstract: https://pubmed.ncbi.nlm.nih.gov/33148508/

In brief: In the developing bone, PTHrP signaling inhibits hypertrophic differentiation, the key step in the coupling of chondrogenesis and osteogenesis during endochondral bone formation. The current paper reviews findings of previous knockout-studies on PTHrP-induced signaling including HDAC4/5, salt-inducible kinases and transcription factors MEf2 and Runx2 and, for the first time, outlines the molecular mechanisms by which PTHrP controls chondrocyte hypertrophy.

Comment: The feedback loop between PTHrP and Indian hedgehog IHH represents a crucial regulating mechanism of growth plate chondrocyte differentiation *in vivo*. The essential role of PTHrP is exerted by inhibition of chondrocyte hypertrophy while IHH, synthesized by postproliferational chondrocytes, is essential for the synthesis of PTHrP in early differentiation stages. Thus, the balance between the two factors controls the pace of growth plate chondrocyte differentiation.

While the physiologic role of PTHrP has been investigated in several knockout models, the precise mechanism of inhibition of chondrocyte hypertrophy remained incompletely understood. As reported in the ESPE Yearbook 2020, Nishimori *et al*. identified both salt-inducible kinases as well as their substrates HDAC4 and HDAC5 as key components of PTHrP signaling in growth plate chondrocytes. Here, the authors merge findings and conclusions of their group as well as others on PTHrP induced regulatory cascades. The resulting hypothesis postulates a key role of PTHrP-induced HDAC4 nuclear translocation by direct inhibition of Sik3 kinase activity. Nuclear HDAC4 then blocks specific transcription factors MEf2 and Runx2, resulting in a halt of differentiation specific genes and inhibition of chondrocyte hypertrophy.

Since PTHrP is a master regulator in growth plate physiology, the deepened understanding of its major function reviewed here represents a milestone in the understanding of endochondral bone formation.

5.16. Secondary ossification center induces and protects growth plate structure

Meng Xie, Anna Nele Herdina, Jordi Estefa, Ekaterina V Medvedeva, Lei Li, Phillip T Newton, Svetlana Kotova, Boris Shavkuta, Aditya Saxena, Lauren T Shumate, Brian D Metscher, Karl Großschmidt, Shigeki Nishimori, Anastasia Akovantseva, Anna P Usanova, Anastasiia D Kurenkova, Anoop Kumar, Irene Linares Arregui, Paul Tafforeau, Kaj Fried, Mattias Carlström, András Simon, Christian Gasser, Henry M Kronenberg, Murat Bastepe, Kimberly L Cooper, Peter Timashev, Sophie Sanchez, Igor Adameyko, Anders Eriksson, Andrei S Chagin

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Elife. 2020 Oct 16;9:e55212

Abstract: https://pubmed.ncbi.nlm.nih.gov/33063669/

In brief: In some species, growth plate and articular cartilage are not separated by secondary ossification centers. Here, the authors used mathematical modeling, *ex vivo* models and biophysical tests and indirectly demonstrate that secondary ossification centers evolved in order to protect the growth plate, and especially hypertrophic chondrocytes, from mechanical stress.

Comment: During development, the secondary ossification center (SOC) physically separates the initially contiguous growth plate and articular cartilage. However, the reason why these structures exist as separate entities is unclear. The evolutionary analysis used by Xie *et al*. demonstrated that SOC appeared during evolution in amniotes, when animals relocated their entire lifecycle to terrestrial environments, and this development often correlates with mechanical loads, as exemplified by the ossification pattern analysis in mammals with specialized extremities, such as whales, bats and jerboa, a species of bipedal hopping rodents.

The authors used mathematical modeling to investigate the influence of SOC on stress distribution within cartilage and found that SOC enhances the stiffness of epiphysis and reduces mechanical stress within the growth plates, preventing distortion and instability during locomotion. Using a combination of *ex vivo* models and biophysical tests, the authors show evidence suggesting that hypertrophic chondrocytes are the most sensitive cells to mechanical stress within the growth plate. Moreover, pharmacological and genetic manipulation of the SOC suggested that hypertrophic chondrocytes are protected by the SOC from apoptosis induced by extensive mechanical stress. Using atomic force microscopy, authors find that hypertrophic chondrocytes exhibit low stiffness, which may make them more sensitive to mechanical stress.

In summary, the authors used a combination of approaches and elegantly argue that the primary reason for the evolution of SOCs was the need to protect hypertrophic chondrocytes from mechanical stress and consequently that SOCs are critical to protect growth plates in growing children. The findings indicate that growth plate chondrocytes are critically sensitive to loading and that overloading may impair growth. The findings have direct implications for growing children's participation in gymnastics and other sports that may cause extreme loading of growth plates.

6. Differences of Sexual Development (DSD) and Gender Dysphoria (GD)

Martine Cools, Anna Nordenström, Christa E. Flück

Basic and Genetic Research of DSD

6.1. Ovotesticular disorders of sex development in FGF9 mouse models of human synostosis syndromes

Bird AD, Croft BM, Harada M, Tang L, Zhao L, Ming Z, Bagheri-Fam S, Koopman P, Wang Z, Akita K, Harley VR
Hum Mol Genet. 2020 Aug 3;29(13):2148–2161.
doi:10.1093/hmg/ddaa100.
https://www.ncbi.nlm.nih.gov/pubmed/32452519

This study explores the hitherto unknown role of *FGF9* in human testis development. The authors use two mouse models that phenocopy the skeletal defects of dominant *FGF9* mutations that cause skeletal synostosis syndromes in humans. The results show partially reversible disrupted testis development in male mice.

The loss of *FGF9* signalling in $Fgf9^{-/-}$ mice leads to disrupted Sertoli cell differentiation and up-regulation of ovary-promoting genes such as *Wnt4* and *Foxl2* with subsequent male-to-female sex reversal. Although loss-of-function mutations in its receptor *FGFR2* have been associated with disturbed gonadal development in 46,XY individuals, pathogenic *FGF9* mutations have never been identified in series of patients who have 46,XY DSD. $FGF9^{S99N}$ and $FGF9^{R62G}$ cause human synostosis syndromes in a dominant fashion. However, the gonadal phenotype in these syndromes has not been described. Here, the $Fgf9^{S99N}$ and $Fgf9^{N143T}$ mouse models, that phenocopy the skeletal defects seen with the respective human mutations are used to study the impact of these variants on mouse testis development. The paper offers a series of comprehensive experiments that are presented and analysed in detail. Paradoxically, the mutants exert gain-of function effects in skeletal tissue and loss-of-function effects in the gonad, and the experiments offer mechanistic insight into these intriguing differential effects. In bone, Fgf9 and its ligands are often expressed in non-overlapping regions, suggesting that diffusion of Fgf9 is required before it can exert its activity. The mutants that cause synostosis have been suggested to act through hyperdiffusion, as they have been shown to disrupt Fgf9 dimerisation. In the testis, a loss-of-function effect is observed, due to disrupted binding of the Fgf9 mutant to its receptor.

Overall, the experiments demonstrate a hypomorphic and transient delay in testis development in mice due to reduced Sertoli cell proliferation, and highlight at the same time the importance of the genetic background. Parallels and differences with the human situation are clearly outlined. In conclusion, this basic research paper elegantly illustrates how mouse models can further progress our understanding of human sex development even in the absence of informative human case reports.

6.2. Testis formation in XX individuals resulting from novel pathogenic variants in Wilms' tumor 1 (WT1) gene

Eozenou C, Gonen N, Touzon MS, Jorgensen A, Yatsenko SA, Fusee L, Kamel AK, Gellen B, Guercio G, Singh P, Witchel S, Berman AJ, Mainpal R, Totonchi M, Mohseni Meybodi A, Askari M, Merel-Chali T, Bignon-Topalovic J, Migale R, Costanzo M, Marino R, Ramirez P, et al.
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doi:10.1073/pnas.1921676117. PMID: 32493750

On the search for a genetic cause for 46,XX virilization due to testicular (TDSD) or ovotesticular DSD (OTDSD), 78 individuals were studied by whole exome sequencing. In 7 cases, heterozygous *de novo* variants were found in the 4th zinc finger (ZF4) of the *Wilms tumor 1* gene (*WT1*). Modelling of these variants in human granulosa cells and mice recapitulated the phenotype.

While many individuals with a non-syndromic form of OTDSD/TDSD carry the *SRY* gene, the underlying genetic cause in others remains unsolved. *WT1* is a transcription factor with 4 zinc finger motives for DNA binding, which comes in 4 isoforms. It is important for normal development of the urogenital organs, heart and diaphragma. Together with steroidogenic factor 1, WT1 is crucial for mammalian gonadogenesis. So far, haploinsufficiency of *WT1* was identified in the WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, retardation), while specific variants were found in autosomal dominant Frasier, Denys-Drash and Meacham syndromes. 46,XY individuals with *WT1* variants manifest with ambiguous or female typical external genitalia at birth, while affected 46,XX individuals so far were noted to have either normal ovaries, primary ovarian insufficiency or streak gonads in combination with nephrotic syndrome. For an overview see the OMIM synopsis: https://www.omim.org/clinicalSynopsis/table?mimNumber=194080,136680,608978,156240,256370,194070.

The paper by Eozenou et al. now shows that with specific heterozygous variants located in the ZF4 of *WT1,* yet another phenotype is possible. It is suggested that these variants bind and thereby sequester the essential pro-ovary factor β-cathenin, which promotes testis formation and inhibits ovary development. This illustrates nicely, how genetic aberrations of one gene can result in many different phenotypes.

6.3. The FKBP4 gene, encoding a regulator of the androgen receptor signaling pathway, is a novel candidate gene for androgen insensitivity syndrome

Ilaslan E, Markosyan R, Sproll P, Stevenson BJ, Sajek M, Sajek MP, Hayrapetyan H, Sarkisian T, Livshits L, Nef S, Jaruzelska J, Kusz-Zamelczyk K
Int J Mol Sci. 2020 Nov 9;21(21):8403.
doi:10.3390/ijms21218403. PMID: 33182400

This case report describes a patient with clinically diagnosed partial androgen insensitivity syndrome (PAIS). However, no mutation in the androgen receptor gene was identified. Instead, whole genome sequencing revealed a heterozygous point mutation inherited from the mother in the *FKBP4* gene. This gene is a positive modulator of *AR* gene transcription. The mutation was located in one of the three repeats (TPR) responsible for the interaction with several proteins essential for upregulation of AR transcription and thus activity.

A corresponding gene in mice has been shown to cause genital anomalies in homozygous but not in heterozygous loss-of-function mutations. The authors speculate that the gene may be subject to imprinting in humans, as an explanation for the discrepancy. It has been postulated since some time that deficiencies related to androgen receptor (AR) cofactors or translation of the *AR* gene would be involved in AIS (1). This is the first time that a mutation in a regulatory gene of the androgen signalling pathway is identified in humans.

A recently published thorough review on androgen insensitivity describes the intricate molecular system and factors involved in the transportation and activation of the androgen receptor (1). Mutations in the *AR* gene are detected in $< 25\%$ of cases with a clinical PAIS phenotype and the phenotype/genotype correlation is especially variable in PAIS, even when a mutation is identified. The androgen-regulated endogenous AR-target gene in genital skin fibroblasts, *Apolipoprotein D (APOD)* has enabled the development of an assay using this gene as a biomarker of androgen signalling at the cellular level, even in absence of an *AR* mutation. This has been defined AIS type II and further stimulated the search for unidentified cofactors responsible for PAIS. (1). The paper by Ilaslan et.al. is the first one to identify such a possible genetic link, and the APOD assay as proposed by Hornig et al. could serve as an optimal *in vitro* model to test the functional effect of this and other newly identified genetic variants.

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6.4. COG6-CDG: Expanding the phenotype with emphasis on glycosylation defects involved in the causation of male disorders of sex development.

Mandel H, Cohen Kfir N, Fedida A, Shuster Biton E, Odeh M, Kalfon L, Ben- Harouch S, Fleischer Sheffer V, Hoffman Y, Goldberg Y, Dinwiddie A, Dumin E, Eran A, Apel-Sarid L, Tiosano D, Falik-Zaccai TC
Clin Genet. 2020 Oct;98(4):402–407.
doi:10.1111/cge.13816. PMID: 32683677.

This short report describes two 46,XY siblings of consanguineous parents manifesting a complex syndrome consisting of multiple dysmorphic features including growth and developmental retardation, gastrointestinal disorders, musculoskeletal and cardiac anomalies, as well as ambiguous genitalia (non-palpable testes, micropenis, underdeveloped scrotum). Whole exome sequencing revealed a homozygous novel splicing variant in the *COG6* gene important for glycosylation.

Although this case report provides only clinical and genetic data, it puts the findings nicely into the context of published knowledge on congenital disorders of glycosylation (CDG). Studying the reported cases in the literature, the authors recognize that so far the DSD phenotype of several CDGs was disregarded, although present in at least 13 CDGs identified by a recent review (1). Related to sex development, it is noted that FSH, LH and their receptors belong to the family of glycoprotein hormones, for which the glycans play important roles for proper dimerization, stability, secretion and signaling. However, the observed DSD phenotype in several CDGs suggests disruption of early events in sexual development that are independent of gonadotropin secretion and action. Thus, the exact targets of glycosylation in the pathogenesis of DSDs is currently unknown and this needs to be further explored, but genetic data clearly indicate but genetic data clearly indicate that pathogenic variants in CDG genes are associated with various DSD phenotypes.

With current methods, the genetic cause of about half of DSD cases can be solved. This study illustrates how powerful unbiased next generation sequencing (NGS) approaches are in discovering novel genetic causes and potential mechanisms of DSD. Whole exome sequencing has become the method of choice in routine genetic diagnostic of DSDs and is replacing candidate gene and panel analyses. I agree with the authors that variants in genes causing CDGs should be added to the list of syndromic forms of DSDs. In the same line, we learn from those CDG cases with a DSD phenotype that proper glycosylation is needed for normal human sexual development.

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6.5. Applying Single-Cell Analysis to Gonadogenesis and DSDs (Disorders/ Differences of Sex Development)

Estermann MA, Smith CA
Int J Mol Sci. 2020 Sep 10;21(18). pii: E6614.
doi:10.3390/ijms21186614. PMID: 32927658

This review first summarises current molecular knowledge of gonadal development and related DSDs, and then overviews methods of single-cell sequencing (sc-seq) technologies and their applications and findings for healthy gonads and disease states. Recent studies are listed in two tables and major findings for normal tissues, DSD, infertility and cancers are shortly described. The advantages, challenges, and limitations of sc-seq and multi-omics approaches for elucidating and understanding the complex biology of the gonads from conception to aging are discussed.

Sc-seq was the chosen 'Method of the year 2013' by Nature (1). Meanwhile its use in research to understand complex developmental biological processes and disease states and mechanisms expands, while its application in routine investigation is pending. Important for its successful wider application is the comprehensive collection of normal data of all organ systems in a spatio-temporal fashion. This task is currently ongoing in the Human Cell Atlas project (https://www.humancellatlas.org/) and not yet completed for the human gonads. Sc-seq comprises sc genomics, transcriptomics, and proteomics; combined to sc multi-omics it allows unique insights into genotype-phenotype correlation and developmental processes. So far, sc seq studies on normal human, primate and non-primate gonadal tissues have largely confirmed previous findings, but also revealed novel details that had been missed in previous tissue bulk analyses. For instance, it has confirmed that the mutually exclusive sexually dimorphic program of the female and male gonads must be actively maintained throughout life (2), otherwise trans-differentiation will occur (3-5). On the other hand, sc-seq studies of the adult human testis discovered pericytes as a novel cell population that seem to act as multi-functional cells of the microvasculature (6). Currently, sc-seq multi-omics studies on DSD gonads are still missing, but they bear the potential to provide deep insight into genotype-phenotype correlation and disease mechanisms. Once obstacles, such as tissue sample availability and preservation issues, costs, and lack of normal datasets for comparison have been overcome, sc-seq omics might become a powerful tool for clinical applications improving diagnostic and therapeutic possibilities.

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6.6. Gonad differentiation toward ovary

Lamothe S, Bernard V, Christin-Maitre S
Ann Endocrinol (Paris). 2020 Jun;81(2–3):83–88.
doi:10.1016/j.ando.2020.04.004. PMID: 32340851

This short review summarises the history and current knowledge of molecular genetic aspects of the human gonadal differentiation toward ovary.

Major scientific milestones on the topic are described. It is interesting to learn on what grounds it was common doctrine until the end of the last century that the female phenotype occurred 'by default'. First, Alfred Jost showed experimentally in 1947 that removing testes in rabbit embryos resulted in a female phenotype at birth. Second, in 1990, after identifying the SRY factor on the Y chromosome, experimental manipulation of XX mice by adding *Sry+* resulted in a male phenotype. Then, through studies of individuals with different forms of 46,XX gonadal DSDs, it became obvious that the formation of the ovary requires an active inhibition of factors promoting testicular development and a positive stimulation of factors promoting ovary development. Major pro-ovary factors include WNT4, RSPO1, WNT/b-catenin pathway and FOXL2. Human mutations of all these factors have been described in individuals with 46,XX DSD presenting with dysgenetic gonads or (ovo-)testis-like gonads and ambiguous or male typical sex organs.

Knowledge on ovary development still lacks behind the testis. A short search in PubMed revealed a significant increase in published articles on genetics of gonadal differentiation after 1990 with a focus on testicular

development. Years later, research data on ovarian differentiation start to appear in the literature but are still outnumbered by +50% data on testis development.

Patient Related Outcomes

6.7. Young voices: sexual health and transition care needs in adolescents with intersex/differences of sex development-A pilot study

Callens N, Kreukels BPC, van de Grift TC
J Pediatr Adolesc Gynecol. 2021 Apr;34(2):176–189.e2.
doi:10.1016/j.jpag.2020.11.001
https://www.ncbi.nlm.nih.gov/pubmed/33181339

This study explores the well-being and sexual health needs of contemporary youth with a DSD. It is a small scale, mixed methods study by specialized DSD psychologists-sexologists in a sample of well-educated youth aged 16–21 who attend DSD clinics and/or support groups in the Netherlands.

Studies in adults show an increased risk for diminished sexual well-being and decreased sexual functioning related to having a variation in sexual development. Although many specialized DSD clinics offer mental and sexual health services nowadays to address these difficulties, clinical experience shows that these services are often poorly attended by clients. Thus, studies exploring how these services can be better tailored to clients' needs are important. This study focuses on the adolescent and transition period.

In this study, participants emphasize the importance of an open climate among all team members to discuss questions and uncertainties about romantic relationships, sexual experiences and dealing with difficulties surrounding feelings of otherness, however without overemphasizing negative aspects of the condition. Those who had not been involved in some of the decision-making steps or who felt they had not been well informed expressed regret about this. Emotional support, mainly from parents and sometimes from peers or from a psychologist was considered important. Importantly, youth who have a DSD highly value contact with peers who have the same or a similar condition as a way of dealing with aspects of their condition.

In summary, this study offers comprehensive information for all multidisciplinary team members about what contemporary youth who have a DSD want to know and learn about their condition during consultations and in their transition phase to adult clinics. As few guidelines exist for psychosocial counselling of DSD youth, this study provides novel and important data.

6.8. Do parents really know best? Informed consent to sex assigning and 'normalising' treatment of minors with variations of sex characteristics

Cannoot P
Cult Health Sex. 2021 Apr;23(4):564–578.
doi:10.1080/13691058.2020.1785012.
https://www.ncbi.nlm.nih.gov/pubmed/32876546

This paper, by a human rights researcher, explores how European law systems, by their conceptualisation of sex as a binary construct, may favour medical interventions in children with variations in sex characteristics and reflects on the tension between the legal obligation for parents to represent their minor child and their child's personal right for autonomy and self-determination.

The merit of the paper is that it helps medical professionals understand why modifications to the legal framework in which they operate are needed. Parental views, inspired by their own background and history, do not necessarily align with the future views of their children, especially not when it comes to identity and personality. The question if parents are optimally placed to make decisions with life-long and far-reaching

implications for their child merits attention. Medical professionals, when seeking informed consent for any form of treatment, should be more aware of the pitfalls related to this ethical dilemma and should seek thorough assessment of the fully informed child's opinions and wishes, prior to any intervention. Law should include procedures that guide caregivers when potential conflicts may occur between parental and minor's opinions on the proposed interventions, especially when there is no medical emergency. Unfortunately, throughout the text, medical practices and the potential psychological problems related to living with variant sex characteristics are oversimplified or sometimes neglected, and little attention is paid to differences among individual children's and their families' needs.

Interdisciplinary interaction and exchange of knowledge that allows mutual understanding is crucial to evolve towards a situation where the physical and mental well-being of every individual child is protected, in accordance with each family's personal values, and within their sociocultural and spiritual context. A generally applicable law will not suffice. Instead, a law that secures the child's autonomy and right for self-determination as an overarching fundament, and at the same time allows for variations in medical approach inspired by the child's individual psychological, medical, intra-familial and societal context, with the best interest of the child as the aim in all circumstances, is how progress can be made.

6.9. Early Genital Surgery in Disorders/Differences of Sex Development: Patients' Perspectives

Bennecke E, Bernstein S, Lee P, van de Grift TC, Nordenskjöld A, Rapp M, Simmonds M, Streuli JC, Thyen U, Wiesemann C; dsd-LIFE Group

Arch Sex Behav. 2021 Apr;50(3):913–923.

doi:10.1007/s10508-021-01953-6. PMID: 33712989

This paper describes a patient cohort study of 459 individuals with various DSD diagnoses, as part of the dsd-LIFE study. Patients were included at 14 different sites in 6 European countries.

Genital surgery has been increasingly questioned in the past decade. Both the timing and age at first surgery have been debated, due to less than satisfactory outcomes reported historically but also from a human rights perspective. Patient advocacy groups have demanded a moratorium on early surgery. United Nations and European Union commissions have made statements related to this issue from the perspective of the (age-related) possibility to obtain informed consent.

In this study, patients were asked about their own opinion concerning early genital surgery both from their personal perspective and from a general perspective, i.e. if they thought it would be best to postpone genital surgery to an age when the individual can participate in the decision on genital surgery. Studies that ask patients about their personal experiences of procedures they have undergone are prone to bias, as many individuals are positively inclined towards their own experiences. This is part of an individual's coping strategy and is generally good, but it does hamper the interpretation of qualitative studies. These authors are aware of this issue and they address it specifically.

This paper illustrates and discusses the complexity of the questions concerning surgery of the genitalia, including the ethical and human rights aspects. Data are presented on surgery, vaginoplasty and clitoris reduction, in girls with CAH and other diagnoses with 46,XY karyotype and androgen effects. The patients' opinions on timing of hypospadias surgery in boys/men, a topic which has attracted considerably less attention, is addressed as well.

6.10. Participant- and Clinician-Reported Long-Term Outcomes After Surgery in Individuals with Complete Androgen Insensitivity Syndrome

Lise Duranteau, Marion Rapp, Tim C van de Grift, Angelica L Hirschberg, Agneta Nordenskjöld, dsd-LIFE group

J Pediatr Adolesc Gynecol. 2021 Apr;34(2):168–175.

doi:10.1016/j.jpag.2020.11.012. PMID: 33248216

The authors report the long-term outcomes of 71 patients with Complete Androgen Insensitivity Syndrome (CAIS) in the multicentre cross-sectional dsd-LIFE study. Patients were included at 14 different sites in 6

countries in Europe. This is one of the largest long-term studies on CAIS and is of interest to clinicians who manage girls and women with CAIS.

Data on treatment, surgery, and gynaecological assessment are presented. Importantly, the patients' personal views were extensively assessed by questionnaire. These focussed on psychological aspects, such as body image and the effects of surgery on quality of life, genital satisfaction after surgery, genital body image, sexual complaints, and overall sexual function.

Sixty-two (87%) had undergone gonadectomy, of whom 12 were before puberty, 21 (30%) had vaginoplasty and/or vaginal dilations, and 2 had breast enlargement surgery. Gynecological examination, performed in 61%, showed that vaginal length did not differ between those who had surgery or vaginal dilatation versus those who had no intervention. Strictures were seen only in the surgery group, in 3 individuals, and scarring in 50%. These are important aspects when making treatment decisions. Vaginal satisfaction was significantly associated with sexual satisfaction in general. Functional problems were commonly reported across the different nonsurgical and surgical groups. Current treatment with estrogen, or estrogen plus progesterone, was reported by 74%, including 2 with retained gonads, and 9 received testosterone treatment. Gonad histology was available in 19 cases; one patient showed a sertoli cell adenoma; one other patient showed bilateral gonadoblastomas.

Reviews with Clinical Impact for DSD Care

6.11. Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome: a comprehensive update

Herlin MK, Petersen MB, Brännström M
Orphanet J Rare Dis. 2020 Aug 20;15(1):214.
doi:10.1186/s13023-020-01491-9.
https://www.ncbi.nlm.nih.gov/pubmed/32819397

This is an excellent and detailed systematic review on all relevant aspects of the MRKH syndrome. This review is also very timely, due to the many recent genetic and therapeutic advancements and the last in-depth overview of the syndrome dates several years previously.

Apart from comprehensively summarizing the available scientific evidence on MRKH, the authors highlight the importance of a holistic approach, with an emphasis on psychosocial support and supportive care. The safety and advantages of vaginal dilation convincingly place this technique as the first line vaginal substitution therapy. Major recent advancements include a breakthrough in the genetic etiology of MRKH type II (MURCS syndrome), with the identification of autosomal dominant i*GREB1L* missense mutations in 5 unrelated families with MURCS. A therapeutic breakthrough was the first successful uterus transplantation procedures in 7 of 9 women with MRKH in 2013 and the first healthy livebirth after uterus transplantation in 2014. The subsequent implementation of this technique in specialist centres around the world and its future applications are discussed. In summary, this review will be the reference for all health professionals involved in the care for women with MRKH in the next few years.

6.12. Peptide hormone analysis in diagnosis and treatment of Differences of Sex Development: joint position paper of EU COST Action 'DSDnet' and European Reference Network on Rare Endocrine Conditions

Johannsen TH, Andersson AM, Ahmed SF, de Rijke YB, Greaves RF, Hartmann MF, Hiort O, Holterhus PM, Krone NP, Kulle A, Ljubicic ML, Mastorakos G, McNeilly J, Pereira AM, Saba A, Wudy SA, Main KM, Juul A
Eur J Endocrinol. 2020 Jun;182(6):P1–P15.
doi:10.1530/EJE-19-0831. PMID: 32268295.

This paper describes the concerted efforts of the EU COST Action DSDNet and the European network for rare endocrine diseases Endo-ERN. A total of 33 laboratories were involved and they describe state of the art

assessments of the peptide hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH), anti-Müllerian hormone (AMH), and Inhibin B. Accurate quantification of these hormones is crucial in the investigation of individuals with a suspected or newly diagnosed DSD and for their management over the life span.

The authors give clear descriptions of the technological pitfalls associated with laboratory assays for these peptide hormones and relevant factors for quality assurance. Age and sex related reference values are of paramount importance. There are dynamic changes over the first few months of life, during mini-puberty, as well as in puberty and adult life. For example in infancy, compared to girls, boys have higher concentrations of LH, lower concentrations of FSH, and hence high LH/FSH ratio. Hormonal measurements during this early window of life can be used to assess gonadal and reproductive function.

Measuring the combination of gonadotropins, AMH, and/or Inhibin B provides a rapid and powerful tool to differentiate between primary and secondary failure of the HPG axis in the initial evaluation of the patient with DSD. The authors describe in detail the typical hormone patterns seen in different DSD. Clinical guidance is given on the interpretation of the results of peptide hormone analysis for different forms of DSD. This is a very informative and useful paper, a must for every clinician working in the field of DSD and sexual development.

Basic Research in Gender Dysphoria

6.13. Behavioral and neurobiological effects of GnRH agonist treatment in mice - potential implications for puberty suppression in transgender individuals

Anacker C, Sydnor E, Chen BK, LaGamma CC, McGowan JC, Mastrodonato A, Hunsberger HC, Shores R, Dixon RS, McEwen BS, Byne W, Meyer-Bahlburg HFL, Bockting W, Ehrhardt AA, Denny CA

Neuropsychopharmacology. 2021 Apr;46(5):882–890.
doi:10.1038/s41386-020-00826-1. PMID: 32919399.

This mouse study addresses the question of the psychological effects of treatment with the GnRH analogue (GnRHa) leuprolide. Six-week-old, i.e. early-pubertal, male and female mice were injected daily with leuprolide (20 μg) or saline for 6 weeks. The mice were subjected to a number of behavioral tests, and hormonal stress response was assessed. The effects on reproductive function, social and affective behaviour, cognition, and brain activity were studied.

A main focus has previously been the effects of GnRHa on bone mineral density in adolescents receiving GnRHa in the context of gender dysphoria. Neuropsychiatric and neurobiological effects, other than halting puberty and possibly alleviating the stress of gender dysphoria and allowing for thorough psychological assessment, have been increasingly discussed, as GnRH receptors are particularly abundant in the hippocampus and the limbic system.

These data show for the first time that GnRH agonist treatment after puberty onset has sex-specific effects on social and affective behavior, stress regulation, and neural activity in mice. More specifically, increased hyperlocomotion, changes in social preference, and increased neuroendocrine stress responses were seen in male mice, while increased hyponeophagia and despair-like behaviour (reactions responsive to antidepressant treatment) were noted in females. In addition, corticosterone response was increased in male but not female mice on exposure to a new situation.

The authors discuss the possibility that increased signs of depression in adolescents with gender dysphoria who are treated with GnRHa may not be readily detectable due to a decrease in depressive symptoms related to the physical changes of the treatment. Although mice and humans differ particularly with regards to hormonal effects in the brain, this study raises further questions about the eventual neuropsychological off-target effects of GnRH agonists as used in adolescents with gender dysphoria.

6.14. Thrombosis Risk in Transgender Adolescents Receiving Gender-Affirming Hormone Therapy

Mullins ES, Geer R, Metcalf M, Piccola J, Lane A, Conard LAE, Mullins TLK
Pediatrics. 2021 Mar 22:e2020023549.
doi:10.1542/peds.2020-023549. PMID: 33753543.

This retrospective, single center chart review study assessed the incidence of arterial or venous thrombosis during gender affirming hormone therapy (GAHT) in 611 transgender adolescents. The prevalence of thrombosis risk factors was also investigated. No increased risk of thrombosis was found over a short time period in trans-females and trans-males, even when thrombosis risk factors were identified in some individuals.

In recent years, the numbers of youth identifying as transgender increased enormously and the requests for hormonal treatments shifted to ever younger ages. Effects and adverse effects of drugs may not be the same in youth as in mature adults. As is well-known for birth control pills, some hormonal treatments significantly increase thrombosis risk. In contrast to the reassuring findings reported here by Mullins et al., data in adults on GAHT suggested significantly increased thrombosis risk for trans-females but not trans-males (1). Duration of hormonal treatment, route of administration and age may have variable influence. Many studies of adverse hormonal treatment effects suffer from limitations such as: small numbers, retrospective design, single centre focus, non-uniform study protocols, or short observation period. Many unsolved questions in transgender medicine, especially with a perspective on long-term health consequences, will only be solved by joining forces and creating an international registry to collect sufficiently large datasets in a standardised manner.

Reference

1. Luuk J J Scheres, Nienke L D Selier, Nienke M Nota, Jeske J K van Diemen, Suzanne C Cannegieter, Martin den Heijer. Effect of gender-affirming hormone use on coagulation profiles in transmen and transwomen. *J Thromb Haemost*. 2021 Apr;19(4):1029–1037.
doi:10.1111/jth.15256.

6.15. Development of Hip Bone Geometry During Gender-Affirming Hormone Therapy in Transgender Adolescents Resembles That of the Experienced Gender When Pubertal Suspension Is Started in Early Puberty

van der Loos MA, Hellinga I, Vlot MC, Klink DT, den Heijer M, Wiepjes CM
J Bone Miner Res. 2021 May;36(5):931–941.
doi:10.1002/jbmr.4262. PMID: 33507568.

This restrospective study of the Cohort of Gender Dysphoria investigated bone geometry parameters of 106 adolescent trans-women and 216 adolescent trans-men during early and late start of gonadotropin-releasing hormone agonist (GnRHa) and gender affirming hormone therapy (GAHT). Transgender youth who started treatment early in puberty developed bone geometry according to their experienced gender, while a later start resulted in bone geometry resembling their gender assigned at birth.

So far, most studies on bone health of transgender youth receiving hormonal treatments focused on bone density and biochemical bone turnover markers, and found differential effects depending on treatments (GnRHa only, GnRHa plus GAHT) and start of intervention (early versus late in puberty; e.g. (1)). Here, van der Loos et al. show nicely that, with earlier diagnosis and treatment of transgender individuals, treatments will modulate the human body differently, given the fact that they will interfere with pubertal development. This is certainly not only true for bone development, but also for all other sex steroid regulated developmental changes seen with puberty, including growth, body composition and neurodevelopment.

Overall, it can be assumed that the typical programming of pubertal development can be best trans-modulated in the direction of experienced gender when endogenous hormones are halted early, and cross-hormone treatment started early (by Tanner stage 2). However, as we learn step by step from interdisciplinary gender medicine

studies in recent years, gender medicine is more complex and is not only modulated by sex steroids. It is known that underlying sex-related genes regulate a vast range of body functions by other means that we have not yet on our focus when it comes to trangender medicine. Long-term follow up of hormonally treated transgender individuals is therefore mandatory to learn more about possible late effects and adapt current treatments.

Reference

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6.16. Use of high dose cyproterone acetate and risk of intracranial meningioma in women: cohort study

Weill A, Nguyen P, Labidi M, Cadier B, Passeri T, Duranteau L, Bernat AL, Yoldjian I, Fontanel S, Froelich S, Coste J
BMJ. 2021 Feb 3;372:n37.
doi:10.1136/bmj.n37. PMID: 33536184

This observational cohort study used data from the French administrative healthcare database during 2007–2015 to assess the risk of meningioma development related to treatment with cyproterone acetate. The cohort comprised 253,777 participants, of whom 54% had high exposure to cyproterone acetate, and 45% had low exposure.

Cyproterone acetate is an effective antiandrogen and is used by women to treat hyperandrogenism of various causes. It is also used for symptom alleviation in trans-women (assigned male at birth). The safety of the drug for the different indications and the respective dose levels used in clinical practice is important to characterise. It has been known since some time that the development of meningioma is related to treatment with cyproterone acetate. The label includes a warning of this risk since 2007, and since 2011 it is formally contraindicated to resume cyproterone acetate after treatment of meningioma. The presence of progesterone receptors on meningiomas provides a possible biological mechanism.

High dose exposure to cyproterone acetate (defined as cumulative dose $>$ 3g in 6 months), was compared to low dose exposure ($<$ 3 g in 6 months). The primary outcome was surgery or radiotherapy for meningioma. Trans-women (male to female) on high dose cyproterone acetate were reported separately.

The relative risk of meningioma was 5.2 (95% confidence interval: 3.2–8.6) in the high dose group, and there was a cumulative dose-effect. Those with long term high dose exposure had a hazard ratio $>$ 20 (12.8–35). The location of the meningioma differed between groups. The high dose group had an almost 47-fold increased risk of anterior skull base meningioma. In trans-women, the incidence of meningioma was 20.7 per 100 000 person years, as compared to none in non-exposed trans-men.

This study confirms the risk for high dose long-term use of cyproterone acetate in men, women, and trans individuals. This risk should be remembered in any clinical situation when treatment with cyproterone acetate is considered. However, there was no observed risk for low dose cyproterone acetate use in women.

Gender Dysphoria - Reviews

6.17. Fertility Counseling for Transgender Adolescents: A Review

Lai TC, McDougall R, Feldman D, Elder CV, Pang KC
J Adolesc Health. 2020 Jun;66(6):658–665.
doi:10.1016/j.jadohealth.2020.01.007.
https://www.ncbi.nlm.nih.gov/pubmed/32115323

This systematic review is inspired by the large discordance between the high number of transgender individuals who express a desire for genetic parenthood compared to the very low number who ultimately seek fertility preservation. Possible hurdles for effective fertility counseling are explored.

The literature search learned that up to 70% of trans individuals aspire to parenthood, and many desire biologically related offspring. However, according to most studies, only 0-10% of transgender girls and boys pursue fertility preservation, but with wide differences across centres. The reasons are manifold. Many clinicians lack specific knowledge and training for effective fertility preservation counseling. Hormonal treatments need to be postponed or interrupted. The technologies required for gamete cryopreservation are extremely stressful for adolescents and may increase their gender dysphoria, whereas cryopreservation of immature gonadal tissue remains highly experimental. In addition, many of those who sought fertility preservation or pregnancy report negative experiences. Cost may also be an important factor, as most fertility procedures in trans individuals will not be reimbursed. But perhaps the most important and challenging factor is the developmental inability of teens to consider future parenthood. Impaired mental health status may further limit an adolescent's ability to make such long-term decisions.

Despite their wish for biological offspring, many adolescents ultimately seek alternatives, such as adoption, fostering or donor gametes. These authors conclude that the development of clinical practice guidelines for fertility preservation counseling in young trans individuals, together with education and professional development, are needed to make progress in this important area of transgender care.

7. Puberty

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Preface

Some of this year's studies help us further understand the role of MKRN3 in puberty and better define the phenotype associated with MKRN3 mutations. Large cohort studies bring more information regarding secular trends in central precocious puberty, premature thelarche and adrenarche, but also gynecomastia. This chapter also highlights new discoveries regarding the effect of prenatal stress on pubertal timing and the results of the first phase 3 trial of 6-monthly subcutaneous leuprolide acetate.

Clinical Guidance

7.1. Cranial MRI abnormalities and long-term follow-up of the lesions in 770 girls with central precocious puberty

Helvacıoğlu D, Demircioğlu Turan S, Güran T, Atay Z, Dağçınar A, Bezen D, Karakılıç Özturan E, Darendeliler F, Yüksel A, Dursun F, Kılınç S, Semiz S, Abalı S, Yıldız M, Önder A, Bereket A
J Clin Endocrinol Metab. 2021 Mar 25:dgab190.
doi:10.1210/clinem/dgab190. PMID: 33765130.
https://academic.oup.com/jcem/article-abstract/106/7/e2557/6188450?redirectedFrom=fulltext

In brief: This multicentre cohort study explores the frequency, long-term outcomes and potential predictors of central nervous system (CNS) lesions in 770 Turkish girls with central precocious puberty (CPP). The authors conclude that cranial MRI remains justified in all girls with CPP.

Comment: The proportion of girls with CPP with a CNS abnormality varies between 0-27% (1-4). Such abnormalities can be hamartomas, hydrocephaly, cysts, neoplasia, trauma, inflammatory diseases or developmental abnormalities. The 2007 ESPE-LWPES consensus discussed that the MRI requirement for girls who present CPP between ages 6-8 years is controversial (5). Girls with CPP $<$ 6 years, with rapid pubertal progression or neurological symptoms are more likely to have a CNS abnormality. However, a small proportion of girls without neurological symptoms or very early presentation might still have CNS abnormalities on MRI.

The current study retrospectively analysed a cohort of 770 girls with CPP in 9 Turkish reference centres. 13.5% had an abnormal brain MRI, of whom 2.8% had a previously known lesion, and 3.8% had a causally related lesion. Two neoplastic lesions were identified, one low grade glioma and one meningioma. Five patients (0.6%) presented with pathologies that required neurosurgery. The most common brain abnormality was hamartoma, consistent with previous studies (4, 6). Age at breast development $<$ 6 years and LH/FSH ratio $<$ 0.6 were positively associated with a cerebral lesion, although those indicators were not robust enough to be used in clinical decision making. The prevalence of brain abnormalities in this cohort was comparable to previous studies (1,4, 6). Long term follow-up identified a low proportion of neoplasia (0.25%).

In summary, this retrospective study identified a new and causal MRI abnormality in 1:14 girls with CPP $<$ 6 years, and 1:31 girls with CPP $>$ 6 years. The authors conclude that, in the absence of strong clinical or biological predictors, cranial MRI remains justified in all girls with CPP. This raises the question of MRI accessibility in low income countries.

Reference

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7.2. Genotype-phenotype correlations in central precocious puberty caused by *MKRN3* mutations

Seraphim CE, Canton APM, Montenegro L, Piovesan MR, Macedo DB, Cunha M, Guimaraes A, Ramos CO, Benedetti AFF, de Castro Leal A, Gagliardi PC, Antonini SR, Gryngarten M, Arcari AJ, Abreu AP, Kaiser UB, Soriano-Guillén L, Escribano- Muñoz A, Corripio R, Labarta JI, Travieso-Suárez L, Ortiz-Cabrera NV, Argente J, Mendonca BB, Brito VN, Latronico AC

J Clin Endocrinol Metab. 2021 Mar 25;106(4):1041–1050.
doi:10.1210/clinem/dgaa955. PMID: 33383582.
https://academic.oup.com/jcem/article-abstract/106/4/1041/6056669?redirectedFrom=fulltext

In brief: This paper describes the clinical and hormonal features of a large cohort of patients with central precocious puberty (CPP) caused by mutations in *MKRN3*. The authors found that phenotypic features of patients with *MKRN3* mutations is similar to those with idiopathic CPP.

Comment: Inactivating mutations in *MKRN3* were first identified in 2013 in families presenting with CPP (1). Later studies identified *MKRN3*, a maternally imprinted gene encoding the makorin RING-finger protein-3, as an essential inhibitory component of the gene network governing puberty (2-4). *MKRN3* mutations that cause CPP include frameshift, stop gain and missense mutations affecting the coding or gene promoter region (5). *MKRN3* mutation is currently the most common monogenic cause of familial CPP (5).

In this study, a multi-ethnic cohort of 716 patients with familial or idiopathic CPP was screened for *MKRN3* mutations using Sanger sequencing and compared to 156 Brazilian girls with idiopathic CPP. Forty-five girls and 26 boys from 36 unrelated families presented loss-of-function mutations. Among the patients with *MKRN3* mutations, first pubertal signs occurred at 6.2 ± 1.2 years in girls and 7.1 ± 1.5 years in boys, and bone age advancement was 2.0 ± 1.6 and 1.8 ± 1.3 years, respectively. Basal LH levels were 1.9 ± 1.8 IU/l in girls and 1.6 ± 1.2 IU/l in boys. The patients with severe mutations (frameshift mutations, stop gain variants or promoter region deletions) had greater bone age advancement and higher basal LH levels than patients with missense mutations. Girls with *MKRN3* mutations had a shorter delay between puberty onset and first evaluation than girls with idiopathic CPP, independently of family history.

Overall, this study suggests that the phenotypic presentation of patients with *MKRN3* mutations is similar to those with idiopathic CPP. They present a shorter time between pubertal onset and first evaluation, as well as higher FSH levels. Severe mutations (as predicted by *in silico* analysis) lead to greater bone age advancement and higher basal LH levels.

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Latronico AC, Kaiser UB. (2013) Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med* 368:2467–2475.
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7.3. Phase 3 trial of a small-volume subcutaneous 6-month duration leuprolide acetate treatment for central precocious puberty

Klein KO, Freire A, Gryngarten MG, Kletter GB, Benson M, Miller BS, Dajani TS, Eugster EA, Mauras N
J Clin Endocrinol Metab. 2020 Oct 1;105(10):e3660–71.
doi:10.1210/clinem/dgaa479.
https://academic.oup.com/jcem/article/105/10/e3660/5879679

In brief: This phase 3 multi-centre, open-label, single-arm study explores the efficacy, pharmacokinetics and safety of 6-monthly 45-mg subcutaneous leuprolide acetate in 59 patients with central precocious puberty (CPP). Six-monthly leuprolide acetate appears to be a promising treatment to suppress pubertal hormones and progression of secondary sexual features.

Comment: CPP is classically treated with GnRH agonists. Available treatments include intramuscular leuprolide acetate injections, intramuscular triptorelin injections and subcutaneous histrelin acetate implants (1).

This phase 3 study evaluated the pharmacokinetics, safety and efficacy of 6-monthly 45-mg subcutaneous leuprolide acetate. Fifty-nine patients (57 girls and 2 boys) received 2 injections of leuprolide acetate and were evaluated at weeks 0, 24 and 48. Post-stimulation LH was suppressed (<4 IU/L) in 87% and 88% of children at weeks 24 and 48, respectively. Growth velocity regressed in >50% of patients at weeks 24 and 48, and average bone age advancement slightly but significantly regressed. Breast development regressed in almost all girls (55/57) and genital development regressed from G3 to G2 after 2 injections in both treated boys. An initial burst release of leuprolide was reported between 1 and 6 hours post-injection, followed by stable levels from weeks 12 to 44. Injections were well tolerated and no withdrawal was reported. Two serious adverse effects were documented (wheezing and rash) but considered unrelated.

Although short in duration, this first phase 3 study indicates that a 6-monthly leuprolide acetate treatment represents a promising treatment to suppress pubertal hormones and progression of secondary signs of sexual maturation. Such a convenient administration might improve feasibility and adherence.

Reference

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7.4. Marked increase in incident gynecomastia: A 20-year national registry study, 1998 to 2017

Koch T, Bräuner EV, Busch AS, Hickey M, Juul A
J Clin Endocrinol Metab. 2020;105(10):dgaa440.
doi:10.1210/clinem/dgaa440
https://academic.oup.com/jcem/article/105/10/3134/5868100

In brief: Based on a 20-year Danish registry, this study reports a marked increase in incidence of gynecomastia between 1998 to 2017, 5-fold among post-pubertal males age 16–20 years, and 11-fold among males age 61–80 years.
Comment: Gynecomastia is a frequent condition affecting 32–65% of men depending on age and diagnostic criteria (1). It is considered to result from an imbalance between estrogens and androgens (2).

All Danish citizen are registered in a unique Danish National Patient Registry established in 1977. Reporting medical events is compulsory and provides exceptional data regarding national incidence of diseases. The authors documented an average 20-year incidence of gynecomastia of 3.4 per 10 000 men per year. All age groups showed a strong secular increase. The highest overall incidence was reported in postpubertal men (16-20 years) with a 5-fold increase in 20 years. A greater than 10-fold increase was documented in the age groups 10 to 15, 21 to 40, 41 to 60 and 61 to 80 years.

This is the first national report of increasing incidence in gynecomastia. Although gynecomastia is not a serious condition, it is a likely marker of a change in exogenous or endogenous sex steroid environment which is associated with other health risks such as prostate cancer or metabolic syndrome. Although data were not collected on risk factors, the authors suggest increasing obesity incidence (3) as well as abuse of anabolic steroids (4) as the likely main contributing factors and also raise the question of a role for endocrine-disrupting chemicals.

Reference

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7.5. Trends in the Incidence of central precocious puberty and normal variant puberty among children in denmark, 1998 to 2017

Bräuner EV, Busch AS, Eckert-Lind C, Koch T, Hickey M, Juul A
JAMA Netw Open. 2020;3(10):e2015665.
doi:10.1001/jamanetworkopen.2020.15665.
https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2771377

In brief: This Danish population-based 20-year cohort study provides strong epidemiological data supporting substantial increases in central precocious puberty (CPP) and normal early variants of puberty.

Comment: The secular trend towards younger onset of puberty has been reported in the general population for the last 30 years (1–3). However, because of scarce epidemiological data, the question remains whether there are also parallel secular trends in the incidence of CPP, premature thelarche (PT) and adrenarche (PA), which are normal puberty variants (4).

The authors searched the Danish National Patient Registry and included 8596 children registered between 1998 and 2017 with a diagnosis of CPP, PT or PA. Incidences were stratified according to immigration group: Danish origin, second-generation immigrants or first-generation immigrants. Substantial upwards trends in the annual incidence of CPP, PT and PA were documented in girls of Danish origin. CPP showed a 6-fold increase during the 20-year period, PT 33-fold and PA 18-fold. A similar but slightly weaker trend was reported in boys of Danish origin. The annual incidence of CPP and PA was substantially higher in girls of non-Danish origin.

This study is the first to report specific data regarding national incidence of CPP, PA and PT in Denmark. It demonstrates substantial increases, not only in normal variants of puberty (PA and PT) but also in CPP during

the last 20 years. These findings raise the question of a need to change the international reference for normal ages at puberty.

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7.6. GnRH stimulation testing and serum inhibin B in males: insufficient specificity for discriminating between congenital hypogonadotropic hypogonadism from constitutional delay of growth and puberty

Mosbah H, Bouvattier C, Maione L, Trabado S, De Filippo G, Cartes A, Donzeau A, Chanson P, Brailly-Tabard S, Dwyer AA, Coutant R, Young J

Hum Reprod. 2020 Oct 1;35(10):2312–2322.
doi:10.1093/humrep/deaa185. PMID: 32862222.
https://academic.oup.com/humrep/article/35/10/2312/5899242

In brief: This study shows that both the GnRH stimulation test and serum inhibin B have insufficient specificity to discriminate congenital hypogonadotropic hypogonadism (CHH) from constitutional delay of growth and puberty (CDGP).

Comment: CDGP is common and difficult to differentiate from CHH, in particular when CHH is not accompanied by cryptorchidism/micropenis or additional syndromic features (1). This study included 74 males with CDGP aged 14-18 years and 127 males with CHH. CDGP was confirmed by complete spontaneous puberty after the age of 18 years. LH response to GnRH was very variable among CHH patients and was positively correlated with testicular volume. LH response in CDGP patients was also variable and overlapped with CHH patients. Notably, no CDGP patient had an LH peak <4 IU/L. Inhibin B was significantly lower in CHH patients than in CDGP, but values often overlapped. All CDGP patients had inhibin B levels >35 pg/ml.

This study presents a large cohort of male patients for which the diagnosis of CHH or CDGP had been carefully confirmed. Testicular volume and basal testosterone did not significantly differ between both groups. Overlapping values of stimulated LH and inhibin B suggest that neither test is able to discriminate between CDGP and CHH patients with milder GnRH deficiency. The authors propose to offer genetic testing to adolescents with low gonadotropins as mutations in CHH genes are very rare in patients with CDGP (2).

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7.7. Prenatal exposure to maternal stressful life events and earlier age at menarche: the Raine study

Bräuner EV, Koch T, Juul A, Doherty DA, Hart R, Hickey M

Hum Reprod. 2021 Jun 18;36(7):1959–1969.
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https://academic.oup.com/humrep/article-abstract/36/7/1959/6179308?redirectedFrom=fulltext

In brief: This large prospective population-based pregnancy cohort study identifies an association between maternal stressful life events during gestation and earlier age at menarche in the daughters.

Comment: Earlier age at menarche is a known risk factor for adverse physical and psychological outcomes (1–2). Therefore, understanding its potential early drivers is important for public health. Literature suggests that childhood stress impacts reproductive function in females and leads to earlier puberty timing (3–5). However, data regarding prenatal stress and pubertal onset is much more scarce.

The authors studied an Australian cohort of 753 mother-daughter pairs. Women were recruited between their 16th and 20th gestational week from 1989 to 1991. 77% of women prospectively reported at least one stressful life event during the pregnancy (63% during early gestation; 54% during late gestation). Median age at menarche in girls exposed to prenatal stressful life events was significantly earlier than non-exposed girls. This association between stressful life events and earlier age at menarche followed a non-linear U-shaped response, meaning that one stressful event was associated with a 3.4 month earlier age at menarche, whilst exposure to 2 or more stressful events were associated with a 1.7 month earlier menarche. The association was not explained by childhood BMI. This data suggests a direct effect of prenatal stress on age at menarche, potentially due to an earlier activation of the hypothalamic-pituitary-adrenal axis. The hypothesis is supported by previous studies that identified an association between maternal stress and altered HPA axis in the offspring (6-7). The non-linear U-shaped response suggests that the first exposure to prenatal stress exerts a greater effect on fetal reproductive development than following exposures, possibly via dramatic initial changes in the HPA axis.

Further studies are needed to understand the underlying mechanisms of this association between prenatal stress and pubertal precocity in girls. However, these results support growing evidence for prenatal determinants of reproductive life with direct implications for women's physical and psychological health across the life-span.

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Basic Science

7.8. Oral contraceptive use, especially during puberty, alters resting state functional connectivity

Sharma R, Fang Z, Smith A, Ismail N

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doi:10.1016/j.yhbeh.2020.104849.
https://www.sciencedirect.com/science/article/abs/pii/S0018506X20301756?via%3Dihub

In brief: This study used functional MRI to compare resting state functional connectivity in women who started oral contraception during puberty or adulthood. It shows that pubertal onset of oral contraception is associated with higher connectivity in specific cortical regions.

Comment: Puberty represents a crucial window of sensitivity regarding the organizing effects of sex steroids on brain development, in particular in females (1). Recent evidence suggests that oral contraception induces structural and functional changes in the adult brain. Women taking oral contraception show region-specific increases and decreases in grey or white matter and functional differences in memory and emotion (2, 3).
This study included 75 women aged 18 to 26 years. Of these, 12 were pubertal/adolescent users of oral contraception who had started within 6 months following menarche, a further 12 were adult users, and the others had never used oral contraception. Women taking oral contraception were tested in the 'active phase' of combined ethinyl estradiol and progestin. Functional MRI showed that oral contraception increased connectivity in the frontal regions. Pubertal onset of oral contraception was associated with higher connectivity in the salience network (bilateral insula, anterior cingulate cortex, mid-cingulate cortex, superior temporal gyrus, and parts of the dorsolateral prefrontal cortex). These results suggest that the use of exogenous sex steroids may alter the physiological neuronal reorganization and pruning that takes place during puberty. This raises question regarding the potential effects of early oral contraception on mental health in adolescent and later in life.

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7.9. ERα signaling in GHRH/Kiss1 dual-phenotype neurons plays sex-specific roles in growth and puberty

Garcia-Galiano D, Cara AL, Tata Z, Allen SJ, Myers MG Jr, Schipani E, Elias CF
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doi:10.1523/JNEUROSCI.2069-20.2020. PMID: 33158965.
https://www.jneurosci.org/content/40/49/9455.long

In brief: These authors used two transgenic mouse models to assess the direct action of estrogens and androgens on GHRH neurons to control growth, pubertal development and reproductive physiology.

Growth hormone (GH) release is critical for normal progression of sexual maturation. Women with GH deficiency show impaired sexual maturation and/or fertility (1). However, the existence of a steroid-sensitive neuronal population involved in this crosstalk between growth and puberty remains mysterious. GH secretion is regulated by two neuronal populations located in the hypothalamus. One population releases somatostatin and has an inhibitory action on GH secretion and the other synthesizes GH-releasing hormone (GHRH) which is stimulatory. Hypothalamic GHRH neurons express estrogen receptor α (ERα) (2) but no detectable levels of androgen receptor (AR) (3). GH stimulates sex steroid synthesis and follicle development by increasing ovarian sensitivity to gonadotropins. However, the existence of a feedback by gonadal steroids onto GHRH neurons to control GH secretion during puberty was still unknown.

To address this issue, the authors generated two transgenic mouse models which harboured a deletion of ERα or AR in GHRH cells. They showed the crucial role of ERα in GHRH neurons, as its deletion disrupts growth in both sexes and pubertal progression in females. By contrast, deletion of AR in GHRH cells causes only a delay in female pubertal completion without impacting growth. As hypophysiotropic GHRH neurons are mostly located in the arcuate nucleus, the authors investigated the potential crosstalk between GHRH and Kiss1 neurons. Using dual-reporter genes and developmental analysis of GHRH and Kiss1 expression, they showed that a subset of GHRH/ERα neurons appear to shift phenotype to become Kiss1/ERα neurons in adult females. In contrast, GHRH and Kiss1 did not colocalize in male mice. This observation could explain the sex difference in GH release pattern and in gonadal steroid control of growth and puberty. In summary, this study provides new insights on the direct action of estrogen on growth and puberty. GHRH/Kiss1 neurons appear to play a role in the crosstalk between the somatotropic and gonadotropic axes during female puberty.

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7.10. Central ceramide signaling mediates obesity-induced precocious Puberty

Heras V, Castellano JM, Fernandois D, Velasco I, Rodríguez-Vazquez E, Roa J, Vazquez MJ, Ruiz-Pino F, Rubio M, Pineda R, Torres E, Avendaño MS, Paredes A, Pinilla L, Belsham D, Diéguez C, Gaytán F, Casals N, López M, Tena-Sempere M
Cell Metab. 2020 Dec 1;32(6):951–966.e8.
doi:10.1016/j.cmet.2020.10.001. PMID: 33080217.
https://www.sciencedirect.com/science/article/pii/S1550413120305349?via%3Dihub

In brief: This study reveals the critical role of hypothalamic ceramide synthesis in the induction of precocious puberty in obese female rats.

Comment: Recent years have brought evidence on the link between the rising prevalence of child obesity and the advancement of puberty timing, especially in girls (1). Even if recent data indicate a contribution of key regulators such as leptin, ghrelin, kisspeptin or SIRT1, the underlying mechanisms are not yet fully understood. Ceramides are a large family of lipid-signalling molecules with major metabolic roles (2). They act as transmitters for the central actions of leptin and ghrelin (3, 4).

This study aimed to determine whether hypothalamic ceramides play a role in the central control of puberty and contribute to its disruption in obesity. Using a model of early overnutrition, the authors showed that early-onset obesity increased hypothalamic ceramide content and advanced puberty in female rats. Using pharmacological tools, stimulation of ceramide synthesis induced early pubertal onset, while its blockade delayed puberty and prevented kisspeptin activating signals. These effects were sexually dimorphic, as no change was observed in pubertal male rats. This observation is consistent with the stronger association between obesity and early puberty in girls than boys. Additionally, the authors reported deregulated ovarian sympathetic tone in early-onset obesity. This phenomenon appeared to involve the paraventricular nucleus (PVN), as early-onset obesity enhanced PVN expression of SPTLC1, a key enzyme for ceramide synthesis, and advanced the maturation of the ovarian noradrenergic system. The blockade of ceramide synthesis in the PVN reversed obesity-induced puberty. In conclusion, this study describes a new pathway explaining precocious puberty in conditions of obesity in female. It includes de novo ceramide synthesis in the PVN and sympathetic ovarian innervation.

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7.11. MKRN3 inhibits the reproductive axis through actions in kisspeptin-expressing neurons

Abreu AP, Toro CA, Song YB, Navarro VM, Bosch MA, Eren A, Liang JN, Carroll RS, Latronico AC, Rønnekleiv OK, Aylwin CF, Lomniczi A, Ojeda S, Kaiser UB

J Clin Invest. 2020 Aug 3;130(8):4486–4500.
doi:10.1172/JCI136564. PMID: 32407292.
https://www.jci.org/articles/view/136564

In brief: This study in rats and non-human primate models investigated the mechanisms by which MKRN3 regulates pubertal onset in rats and non-human primates.

Comment: Pubertal timing is influenced by genetic, nutritional, environmental and socioeconomic factors. The importance of genetic determinants is clearly illustrated by MKRN3 mutations leading to central precocious puberty (CPP) (1). MKRN3 appears to act as a brake on GnRH secretion during childhood, as loss of function mutations lead to CPP (1) and MKRN3 expression markedly decreases in mouse medial basal hypothalamus before puberty (2). However, the mechanism by which MKRN3 deficiency induces early activation of GnRH secretion remains unknown.

Using rats and non-human primate models, this study characterizes MKRN3 expression and action. The authors confirmed the decrease in MKRN3 expression in the hypothalamus of prepubertal rats and nonhuman primates, as previously reported in mice. This decrease occurs independently of gonadal activation and suggests a primary role for MKRN3 early in life. Although not explored here, such developmental decrease in expression could involve miR30, recently identified as a repressor of MKRN3 (3). The authors found MKRN3 expression in KISS1 neurons and showed its ability to inhibit KISS1 and TAC3 promoter activity. This action requires MKRN3 ubiquitinase activity, as mutations in the RING finger domain prevented MKRN3 inhibitory action on KISS1 and TAC3 promoter activity. This finding is clinically relevant as most of the MKRN3 missense mutations associated with CPP are located in the RING finger domain (1). In summary, this study supports a role for MKRN3 in the central control of GnRH release through the inhibition of *KISS1* and *TAC3* transcription in Kiss1 neurons.

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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, 2020 and May 31, 2021. 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 2020, unless progress in the field has been incremental. Emerging themes for this year's chapter include: i) The role of neuropeptide substance P in the regulation of aldosterone secretion; ii) Kisspeptin deficiency leads to abnormal adrenal glands and excess steroid hormone secretion; iii) Urinary GC-MS steroid metabotyping in treated children with congenital adrenal hyperplasia; iv) Efficacy and safety of setmelanotide, an MC4R agonist, in individuals with severe obesity due to LEPR or POMC deficiency; v) Efficacy and safety of osilodrostat in patients with Cushing's disease (LINC 3): a multicentre phase III study with a double-blind, randomized withdrawal phase; vi) First-trimester prenatal Dexamethasone treatment is associated with alterations in brain structure at adult age; and vii) Perturbed beta-cell function and lipid profile after early prenatal Dexamethasone exposure in individuals without CAH.

Mechanism of the Year: Neuropeptide Substance P Regulates Aldosterone Secretion

8.1. The neuropeptide substance P regulates aldosterone secretion in human adrenals

Wils J, Duparc C, Cailleux AF, Lopez AG, Guiheneuf C, Boutelet I, Boyer HG, Dubessy C, Cherifi S, Cauliez B, Gobet F, Defortescu G, Ménard JF, Louiset E, Lefebvre H
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https://pubmed.ncbi.nlm.nih.gov/32471973/

The authors conducted an experimental study in male human volunteers to examine the effects of a neurokinin type 1 receptor (NK1R) antagonist on aldosterone secretion. The findings show the presence of Substance P (SP)-positive nerve fibres in the human adrenal cortex in the vicinity of aldosterone-producing cells and with a role in mineralocorticoid synthesis.

Aldosterone plays a major role in the maintenance of water and mineral homeostasis and in the regulation of blood pressure. It is stimulated by the renin angiotensin system (RAS) and plasma potassium concentrations. However, other factors also appear to regulate its secretion. SP belongs to the family of tachykinins, which also includes neurokinins A and B (NKA and NKB), hemokinin-1 and endokinins. Although evidence suggests that

SP may stimulate steroidogenesis and exert trophic actions on the rat adrenal cortex (**1, 2**), its role in the regulation of the aldosterone production remains unclear.

SP exerts a stimulatory tone on aldosterone secretion through a neurocrine mechanism involving the neurokinin type 1 receptor (NK1R), which is expressed by zona glomerulosa cells (**3**). The action of SP seems to be complementary to that of Angiotensin II (Ang II), which is involved in the aldosterone response to upright posture, while SP may mainly control basal aldosterone production. At the cellular level, binding of SP to the NK1R induces an activation of the ERK pathway, however, it has a minor effect on the calcium signaling pathway in contradistinction to Ang II. SP activation of aldosterone secretion may also be involved in pathophysiologic processes characterized by idiopathic aldosteronism, such as obesity and sleep apnea, which are associated with an increase in the autonomous nervous activity (**4, 5**). Therefore, SP blockade may offer alternative therapeutic pathways to both mineralocorticoid receptor antagonists. whose utilization is hampered by their antiandrogenic properties, and aldosterone synthase inhibitors, whose administration leads to accumulation of steroid precursors with mineralocorticoid activity.

Further to the above, the authors tested the hypothesis that aprepitant, a NK1R antagonist, reduces aldosterone production independently of the renin angiotensin system (RAS). They performed a prospective proof-of-concept, double blind, cross-over and placebo-controlled study in healthy adult male volunteers. Participants received during two 7-day treatment periods aprepitant or placebo in a random order at a 2-week interval. Primary endpoint was the plasma aldosterone concentrations during posture test. Secondary endpoints included basal aldosterone alterations, plasma aldosterone variation during metoclopramide and hypoglycemia tests, and basal and stimulated alterations of renin, cortisol and ACTH concentrations during the three different stimulatory tests. Serum transaminase concentrations were determined as a safety measure. Aprepitant decreased aldosterone production by ~30% but did not influence the aldosterone response to upright position. These findings indicate that the autonomic nervous system exerts a direct stimulatory tone on mineralocorticoid synthesis through SP, and therefore plays a role in the maintenance of hydromineral homeostasis.

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New Mechanisms

8.2. Kisspeptin deficiency leads to abnormal adrenal glands and excess steroid hormone secretion

Berthon A, Settas N, Delaney A, Giannakou A, Demidowich A, Faucz FR, Seminara SB, Chen ME, Stratakis CA

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https://pubmed.ncbi.nlm.nih.gov/33089319/

The authors performed an experimental study in Kiss1 knock-out mice, followed by an observational study of patients with adrenal tumors. The findings indicate that KISS1/KISS1R signaling may be involved in obesity, metabolic disorders and even gonadal steroid hormone perturbations.

The kisspeptin receptor (KISS1R) and its physiological ligand, kisspeptin (KISS1), play an essential role in gonadotropin-releasing hormone (GnRH) secretion. Inactivating variants in the KISS1R gene are detected in

patients with idiopathic hypogonadotropic hypogonadism (IHH) (**1**), while Kiss1r knockout (KO) mice (Kiss1r−/−) develop IHH, and similar to the human patients, respond to exogenous GnRH treatment (**1**). Furthermore, KO mice for the KISS1R ligand, kisspeptin (Kiss1−/−) develop a similar phenotype, confirming that KISS1 is the physiological ligand of KISS1R and that both molecules are required for GnRH release (**2, 3**). Recent studies have demonstrated that KISS1 is expressed widely in fetal adrenal cortex and that kisspeptin treatment induces dehydroepiandrosterone sulphate (DHEAS) secretion in a human adrenocortical carcinoma cell line and by human fetal adrenal cells (**4**).

First, the authors evaluated the adrenal pathology and secretion in Kiss1 KO mice. They found that Kiss1 deletion leads to persistence of the fetal X-zone in both male and female mice (as indicated by the continuing expression of the Akr1c18, Pik3c8, Inha and Cyp17a1 markers) and this was associated with hypersecretion of corticosterone and aldosterone. Although corticosterone concentrations normalized in older animals, hyperaldosteronism persisted. They then screened human patients with hypercortisolism or hyperaldosteronism caused by adrenal tumors. Interestingly, among the patients with steroid hormone hypersecretion, they identified one missense KISS1 and three KISS1R variants (two missense and one synonymous), which had all been previously described in patients with IHH or Kallmann syndrome.

These data suggest that KISS1 and/or KISS1R are involved in the adrenocortical development and hormonal secretion. In older adrenal cortex, in both humans and mice, hyperaldosteronism may be the consequence of KISS1/KISS1R deficiency, although stronger data are needed to conclude a causative effect. Finally, these data also indicate that KISS1/KISS1R signaling may be involved in obesity, metabolic disorders and even gonadal steroid hormone perturbations (i.e. in polycystic ovarian syndrome) in a sexually dimorphic manner, as seen in Kiss1−/− mice studied here.

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Important for Clinical Practice

8.3. Modified-release hydrocortisone in congenital adrenal hyperplasia

Merke DP, Mallappa A, Arlt W, Brac de la Perriere A, Lindén Hirschberg A, Juul A, Newell-Price J, Perry CG, Prete A, Rees DA, Reisch N, Stikkelbroeck N, Touraine P, Maltby K, Treasure FP, Porter J, Ross RJ

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The authors report the findings of a 6-month, randomized, phase 3 trial, with a single arm extension, to investigate the efficacy, safety and tolerability of modified release hydrocortisone (MC-HC) replacement therapy versus standard glucocorticoid replacement therapy in 122 adult patients with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD). The primary end-point was a change in 24-h 17-OHP concentrations. MC-HC improved morning and early afternoon biochemical control of CAH compared with standard therapy.

Standard glucocorticoid therapy in CAH regularly fails to control hyperandrogenism, leading to virilization,

glucocorticoid overexposure and poor health outcomes. This modified-release hydrocortisone formulation (MR-HC) has a delayed-release action. When given twice daily, it mimics the physiological overnight rise in cortisol (**1, 2**), and improved CAH control in a phase 2 study (**2**).

This was the first randomized, controlled trial of glucocorticoid treatment in patients with CAH due to 21-OHD. Patients who received MR-HC had superior hormonal control during the morning and early afternoon than those on standard therapy, and this was sustained over 18 months of follow-up, with a higher proportion of patients (91%) having a morning (09:00h) serum 17OHP concentration $<$ 1200 ng/dL (36 nmol/L) than on standard treatment (71%) ($P = 0.002$). MR-HC improved the clinically relevant end point of morning biochemical control, with reduced AUC and 17OHP amplitude. The improvement in biochemical control was maintained at 18 months, with 80% displaying good control for 17OHP and 96% for androstenedione vs 52% and 45% at baseline, despite reduction of the hydrocortisone dose by 33%, to doses typical for adrenal replacement therapy. After 18 months on MC-HC, the median HC dose was 20 mg. MR-HC also led to menses restoration in 8 patients (1 on standard therapy), and 3 patient and 4 partner pregnancies (none on standard therapy). The number of adrenal crisis were not reduced.

These findings indicate that MR-HC improved morning and early afternoon biochemical control of CAH due to 21-OHD compared with standard glucocorticoid therapy. This improvement was sustained for 18 months on hydrocortisone doses typical for adrenal replacement therapy and lower than doses normally used in CAH. MR-HC was safe and well-tolerated.

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8.4. Urinary GC-MS steroid metabotyping in treated children with congenital adrenal hyperplasia

Kamrath C, Hartmann MF, Pons-Kühnemann J, Wudy SA

Metabolism. 2020; 112: 154354.
https://pubmed.ncbi.nlm.nih.gov/32916150/

In order to better define treatment groups and improve treatment monitoring, the authors performed a retrospective metabotyping analysis using 24-h GC–MS urinary steroid metabolome measurements in young prepubertal children ($n = 109$; age 7.0–1.6 years) with classical congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD), on treatment with hydrocortisone and fludrocortisone. They identified four unique metabolomic profiles, which correspond to degree of CAH treatment control.

The aim of treatment of classical CAH due to 21-OHD is two-fold: to provide sufficient glucocorticoid replacement to prevent adrenal crises, and to suppress the excess adrenal androgen production. However, in clinical practice this is often difficult and patients may develop in tandem hypercortisolism and/or hyperandrogenism. Urinary steroid metabolome analysis by GC–MS is a non-invasive diagnostic approach that provides qualitative and quantitative data on the global excretion of steroid hormone metabolites (**1**). In case of 24-h urine collections, this method provides timely integral information on the daily steroid excretion rates of the whole spectrum of adrenal steroids in patients with CAH. In children with CAH, GC–MS based 24-h urinary steroid metabolome analysis is appropriate to monitor metabolic control of treatment because it determines the metabolites of the classic androgens and their precursors, such as androstenedione and testosterone, and allows assessment of the highly potent adrenal-derived 11-oxygenated androgens (**2**). In addition, urinary steroid metabolome analysis reflects cortisol exposure in treated patients (**3**). Therefore, 24-h urinary steroid metabolome analysis could help to identify not only patients with adequate or inadequate

metabolic control, but also those patients with treatment failure. The classification of individuals into subgroups with similar metabolic patterns is called metabotyping.

In this study, 24-h urinary steroid metabolite excretions were transformed into CAH-specific *z*-scores. Subjects were divided into groups (metabotypes) by k-means clustering algorithm. Four unique metabotypes were generated. Metabotype 1 [$N=21$ (19%)] revealed adequate metabolic control with low cortisol metabolites and suppressed androgen and 17α-hydroxyprogesterone (17-OHP) metabolites. Metabotype 2 [$N=23$ (21%)] showed overtreatment consisting of a constellation of elevated urinary cortisol metabolites and low metabolites of androgens and 17-OHP. Metabotype 3 [$N=32$ (29%)] showed undertreatment with low cortisol metabolites and elevated metabolites of androgens and 17OHP. Metabotype 4 [$N=33$ (30%)] indicated treatment failure as evidenced by unsuppressed androgen- and 17OHP metabolites despite elevated urinary cortisol metabolites.

Thus, the authors could successfully assess glucocorticoid replacement therapy in patients with CAH to identify clinically important treatment groups. Identifying patients with poor compliance and/or patients with indications of over- or undertreatment is extremely important in the management of CAH. Optimizing glucocorticoid replacement therapy is crucial, given that suboptimal treatment and poor compliance may lead to long-term adverse effects and poor health outcomes. Therefore, metabotyping may be an additional tool to monitor glucocorticoid replacement therapy in patients with CAH.

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8.5. Improved urinary cortisol metabolome in addison disease: A prospective trial of dual-release hydrocortisone

Espiard S, McQueen J, Sherlock M, Ragnarsson O, Bergthorsdottir R, Burman P, Dahlqvist P, Ekman B, Engström BE, Skrtic S, Wahlberg J, Stewart PM, Johannsson G

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https://pubmed.ncbi.nlm.nih.gov/33236103/

Here, the authors performed a randomized, 12-week, crossover study in order to assess cortisol metabolism during dual-release hydrocortisone (DR-HC) and conventional hydrocortisone (TID-HC) therapy in patients with primary adrenal insufficiency ($n=50$). Healthy controls ($n=124$) were included for comparison. DR-HC led to improvements in both 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and 11β-HSD2 activities, as assessed by urinary metabolite profiles.

Treatment of adrenal insufficiency using conventional hydrocortisone (HC) therapy requires several doses to achieve sufficient coverage during the day, usually: a higher dose in the morning, a lower dose around midday, and a third dose late afternoon (**1**). However, patients with both primary and secondary adrenal insufficiency still demonstrate increased morbidity and mortality owing to cardiovascular diseases and infection (**2, 3**). It has been speculated that the exposure to supraphysiologic concentrations of cortisol and the non-physiologic cortisol profile are responsible for these adverse health outcomes. Two new modified release HC formulations have been designed to produce a more physiologic circadian cortisol exposure: a once-daily dual-release (DR-HC) tablet based on an immediate-release coating with an extended-release core (**4**); and a twice-daily modified-release tablet based on a multilayer design with an external coat providing delayed and sustained release of HC before final release of an inner HC coat, which is under development for the management of congenital adrenal hyperplasia (**5**) (**see paper 8.3**). The DR-HC formulation has been studied in primary and secondary adrenal insufficiency with observed improvement in cardiovascular risk factors and quality of life. Urinary corticosteroid metabolites were measured by gas chromatography/mass spectrometry on 24-hour urinary collections. The results showed that patients receiving cortisol replacement therapy exhibit an alternate

in cortisol metabolism profile. Total cortisol metabolites decreased during DR-HC compared to TID-HC ($P < 0.001$) and reached control values. During DR-HC treatment, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activity was reduced compared to TID-HC ($P < 0.05$), but remained higher than in controls. 11β-HSD2 activity was decreased with TID-HC vs controls ($P < 0.01$) but normalized with DR-HC. These findings demonstrate that the urinary cortisol metabolome shows significant abnormalities in patients receiving conventional TID-HC replacement therapy, with increased 11β-HSD1 activity, which relate to increased long-term exposure to glucocorticoids and may account for the unfavorable metabolic phenotype in patients with primary adrenal insufficiency. Its change toward normalization with DR-HC may mediate beneficial metabolic effects. Finally, the urinary cortisol metabolome may serve as a tool to assess optimal cortisol replacement therapy.

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8.6. Reproductive and perinatal outcomes in women with congenital adrenal hyperplasia: A population-based cohort study

Hirschberg AL, Gidlöf S, Falhammar H, Frisén L, Almqvist C, Nordenskjöld A, Nordenström A

J Clin Endocrinol Metab. 2021; 106(2): e957–e965.
https://pubmed.ncbi.nlm.nih.gov/33135723/

The authors investigated the reproductive and perinatal outcomes in women with congenital adrenal hyperplasia (CAH) compared with control women in the largest such population-based study to date. Women with CAH had lower birth rates, were more likely to develop gestational diabetes, and had other adverse perinatal outcomes.

CAH is one of the most common monogenic autosomal recessive disorders. Its mild form has a prevalence of 1-10%, depending on the population. The disorder is characterized by impaired glucocorticoid and often mineralocorticoid secretion, and increased adrenal androgen production. Reportedly, both men and women with CAH exhibit reduced fertility and birth rate. In women with CAH, there are many possible underlying causes. Under- and overtreatment with glucocorticoids may disturb normal ovulation by causing hormone imbalances, e.g. increased androgen production. But other causes, such as anatomical barriers and psychosocial factors, may also play an important role.

This study included 272 women with CAH due to 21-hydroxylase deficiency (median age 31 years) and 27,200 controls (100 controls per patient) matched by sex, age, and place of birth. Outcome measures, i.e., pregnancy, births and neonatal outcomes were retrieved from the Swedish Medical Birth Registry. On average, women with CAH had fewer children and a lower proportion of birth rate, especially patients with the most severe phenotypes. Patients with CAH were also older at the birth of their first child and were more prone to develop gestational diabetes. Cesarean section was the most common mode of delivery in CAH patients, especially in women with the most severe phenotype. Perinatal outcome was comparable with the controls in general, but the simple virilizing CAH subgroup showed a slightly increased risk of small for gestational age infants. This study provides valuable information regarding the reproductive and perinatal outcomes of women with CAH due to 21-hydroxylase deficiency.

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8.7. Targeted metabolomics as a tool in discriminating endocrine from primary hypertension

Erlic Z, Reel P, Reel S, Amar L, Pecori A, Larsen CK, Tetti M, Pamporaki C, Prehn C, Adamski J, Prejbisz A, Ceccato F, Scaroni C, Kroiss M, Dennedy MC, Deinum J, Langton K, Mulatero P, Reincke M, Lenzini L, Gimenez-Roqueplo AP, Assié G, Blanchard A, Zennaro MC, Jefferson E, Beuschlein F

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https://pubmed.ncbi.nlm.nih.gov/33382876/

In this multicentre patient cohort study, the authors investigated the use of targeted metabolomics to discriminate primary hypertension (PHT) from endocrine forms of hypertension (EHT). They identified 16 metabolites that help to discriminate between PHT and EHT.

Arterial hypertension represents a global epidemic with an estimated prevalence ranging from 25% to 50%, according to region, population age and definition. Among secondary forms of hypertension, those caused by endocrine disorders are the most challenging to diagnose. The prevalence of EHT, such as primary hyperaldosteronism (PA), hormonally active pheochromocytoma/paraganglioma (PPGL) and Cushing syndrome (CS), is difficult to estimate. EHT remains largely unrecognized, even though the early diagnosis and treatment is effective and cost-effective (**1**). Metabolomic profiling is a relatively new strategy for the parallel and high-throughput identification and quantification of dozens to hundreds of low molecular weight molecules (metabolites). Targeted metabolomics refers to the targeted identification of previously identified specific metabolites (low molecular weight molecules). (**2**). Targeted metabolomics have been successfully used to investigate endocrine conditions associated with secondary hypertension, such as CS and PPGL (**3, 4**).

These authors performed retrospective analyses of 282 adult patients (52% female; mean age 49 years) with proven PHT ($n=59$) or EHT ($n=223$) from a European multicenter study (ENSAT-HT). They used liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) and flow injection analysis–electrospray ionization–tandem mass spectrometry (FIAESI-MS/MS) on stored blood samples to identify discriminating metabolites between the two forms of hypertension in a "Targeted Metabolomics" approach. Identified factors were assessed via a "classical approach" (univariate and multivariate analyses) and a "machine learning approach" (MLA) (random forest). From 155 eligible metabolites, 16 metabolites (C9, C16, C16:1, C18:1, C18:2, arginine, aspartate, glutamate, ornithine, spermidine, lysoPCaC16:0, lysoPCaC20:4, lysoPCaC24:0, PCaeC42:0, SM C18:1, SM C20:2) were found by both statistical approaches as discriminating between PHT and EHT (31 by the classical approach; 27 by MLA).

There is a strong clinical need for this approach. Under current recommendations, 50% of patients with arterial hypertension are eligible for screening for EHT (**5, 6**). New strategies are needed to preselect patients for referral to endocrine clinics. The lack of published data on the diagnostic performance of current recommendations EHT screening limits the possibility of comparing them with the proposed new method. However, based on the established prevalence of arterial hypertension (30%) and estimation that 10% of arterial hypertension patients have an underlying endocrine cause, the authors extrapolate a positive and negative predictive value of 4.3% and 98.6%, respectively – which suggests good performance as a screening tool. Furthermore, the capability of this methodology to identify specific metabolites in specific clinical entities can provide mechanistic links. In this regard, the authors highlight that in both primary and endocrine hypertension, high concentrations of long-chain acylcarnitines that have been associated with cardiovascular morbidity (**7**).

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Clinical Trials – New Treatments

8.8. Efficacy and safety of setmelanotide, an MC4R agonist, in individuals with severe obesity due to LEPR or POMC deficiency: single-arm, open-label, multicentre, phase 3 trials

Clément K, van den Akker E, Argente J, Bahm A, Chung WK, Connors H, De Waele K, Farooqi IS, Gonneau-Lejeune J, Gordon G, Kohlsdorf K, Poitou C, Puder L, Swain J, Stewart M, Yuan G, Wabitsch M, Kühnen P; Setmelanotide POMC and LEPR Phase 3 Trial Investigators

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This article reports the results of two single-arm, open-label, multicentre, phase 3 trials of the MC4R agonist, setmelanotide, in patients with severe obesity due to pro-opiomelanocortin (POMC) deficiency or leptin receptor (LEPR) deficiency. Mean % change in bodyweight after ∼1 year was −25·6% in patients with POMC deficiency and −12·5% in those with LEPR deficiency.

Melanocortin 4 receptor (MC4R) is a key component of the leptin-melanocortin pathway and plays an important role in the regulation of appetite and body weight. Severe early-onset obesity can be caused by biallelic variants in genes that affect MC4R signalling. These trials were conducted in 10 hospitals in North America (Canada and the USA) and Europe (Belgium, France, Germany, the Netherlands, and UK). The POMC trial (NCT02896192) included individuals aged 6+ years with obesity caused by POMC deficiency, defined as homozygous or compound heterozygous variants in POMC or PCSK1. The LEPR trial (NCT03287960) included individuals aged 6+ years with obesity caused by LEPR deficiency, defined as homozygous or compound heterozygous variants in LEPR. In both trials, setmelanotide produced significant weight loss and reduction in hunger scores in after ∼1 year of treatment. Setmelanotide was well tolerated in all individuals, and no new safety concerns were observed. Patients in both groups reported injection site reactions.

Effectiveness was greater in POMC patients than in LEPR patients. The primary outcome, weight loss of 10+ %, was observed in 80% of POMC and 45% of LEPR patients, and this was also reflected in mean % changes in bodyweight (−25·6% vs −12·5%, respectively). Furthermore, all POMC patients reported skin hyperpigmentation compared to 36% of LEPR patients.The authors comment on the potentially different aspects of POMC and LEPR deficiency. They posit that setmelanotide as an MC4R agonist is potentially able to completely restore signalling in POMC obesity (**1**). By contrast, in LEPR, setmelanotide might only partially

restore signalling, given that apart from POMC neurons LEPR is also expressed on agouti-related peptide-positive neurons (**2**).

In the POMC trial, nausea was reported in 5 participants and vomiting in 3 participants. Five serious adverse events (depression, major depression, acute adrenocortical insufficiency, pneumonia, and pleurisy) were reported in 4 participants but none was considered to be related to setmelanotide. No treatment emergent adverse events led to study drug withdrawal or death. In addition, 1 participant reported suicidal ideation at baseline but not at ∼1 year. Another participant developed suicidal ideation at ∼1 year.

In the LEPR trial, nausea and vomiting were reported in 4 participants that both resolved without sequelae. Four serious adverse events (cholecystitis, suicidal ideation, gastric banding reversal, and road traffic accident leading to death) were reported in 3 participants; none was considered to be related to setmelanotide. One participant discontinued the trial because of grade 1 hypereosinophilia, which was considered to be possibly related to setmelanotide and resolved following discontinuation. In both studied groups, no treatment-related cardiovascular adverse events were reported, and there was no evidence that setmelanotide was associated with changes in blood pressure or heart rate. No cases of suicidal ideation and behaviour were reported at either baseline or at ∼1 year.

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8.9. Efficacy and safety of osilodrostat in patients with Cushing's disease (LINC 3): a multicentre phase III study with a double-blind, randomised withdrawal phase

Pivonello R, Fleseriu M, Newell-Price J, Bertagna X, Findling J, Shimatsu A, Gu F, Auchus R, Leelawattana R, Lee EJ, Kim JH, Lacroix A, Laplanche A, O'Connell P, Tauchmanova L, Pedroncelli AM, Biller BMK; LINC 3 investigators

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The authors report the outcomes from the pivotal phase 3 trial in patients with Cushing's disease of osilodrostat (a potent oral inhibitor of cytochrome P450 11B1, [mitochondrial 11β-hydroxylase]). Twice-daily osilodrostat rapidly reduced mean 24-h urine free cortisol (UFC) and sustained this reduction alongside improvements in clinical signs of hypercortisolism.

Cushing's disease is a rare endocrine disorder characterised by increased cortisol secretion and severe complications. Therapies for cortisol reduction are often necessary.

This prospective, multicentre, open-label study, included a double-blind randomised withdrawal phase after a 24-week, open-label, single-arm treatment period. Patients with active Cushing's disease of pituitary origin were recruited from 66 hospitals in 19 countries. The study protocol included 3 separate periods. In period 1, open-label osilodrostat was initiated in all participants and adjusted every 2 weeks on the basis of mean 24-h UFC concentration and safety until week 12. In period 2, all participants continued on osilodrostat at the therapeutic dose determined by period 1. In period 3, participants with mean 24-h UFC concentration $<=$ ULN at week 24, were randomly assigned (1:1, stratified by osilodrostat dose and history of pituitary irradiation) to continue osilodrostat or switch to placebo for 8 weeks. In a final period, all participants were given open-label osilodrostat until core-study end (week 48). The primary objective was to compare the efficacy of osilodrostat versus placebo at the end of period 3.

The study enrolled 137 patients (median age: 40 years; 77% female), of whom 72 (53%) were eligible for randomisation to continuation or withdrawal (phase 3). Patients who continued osilodrostat were more likely to maintain a complete response at week 34 ($P<0.0001$). Most common adverse events (in $>25\%$ of patients) included nausea, headache, fatigue and adrenal insufficiency. Hypocortisolism occurred in 70 (51%) patients

and adverse events related to adrenal hormone precursors occurred in 58 (42%) patients. One patient died, unrelated to study drug, after the core study phase. Regarding clinical benefits, patients on osilodrostat showed significant improvement in weight, BMI, fasting plasma glucose, systolic and diastolic blood pressure, and total cholesterol concentrations, apparent soon after initiation of treatment, and sustained until the end of the study. Therefore, osilodrostat is an effective new treatment option for the management of Cushing's disease.

New Genes

8.10. GWAS for autoimmune addison's disease identifies multiple risk loci and highlights AIRE in disease susceptibility

Eriksson D, Røyrvik EC, Aranda-Guillén M, Berger AH, Landegren N, Artaza H, Hallgren Å, Grytaas MA, Ström S, Bratland E, Botusan IR, Oftedal BE, Breivik L, Vaudel M, Helgeland Ø, Falorni A, Jørgensen AP, Hulting AL, Svartberg J, Ekwall O, Fougner KJ, Wahlberg J, Nedrebø BG, Dahlqvist P; Norwegian Addison Registry Study Group; Swedish Addison Registry Study Group, Knappskog PM, Wolff ASB, Bensing S, Johansson S, Kämpe O, Husebye ES
Nat Commun. 2021 Feb 11;12(1):959.
https://pubmed.ncbi.nlm.nih.gov/33574239/

The authors report a genome-wide association study (GWAS) of autoimmune Addison's disease (AAD) in 1223 cases (defined as autoimmune adrenal failure plus positive serum autoantibodies against 21-hydroxylase) and 4097 healthy controls. Patients with APS-1 were identified and excluded. They identified 9 genome-wide significant genomic loci and explained 35–41% of the additive genetic heritability of AAD.

Autoimmune Addison's disease (AAD) is the most common cause of primary adrenal failure in the Western world. It requires lifelong steroid hormone replacement therapy and is fatal if untreated. Autoimmunity is often apparent from the presence of other associated autoimmune diseases, and is confirmed by the presence of autoantibodies against the adrenal enzyme, 21-hydroxylase.

Besides the major risk locus at the HLA region, they identified risk variants in or near: PTPN22, CTLA4, LPP, BACH2, SH2B3, SIGLEC5, UBASH3A, and AIRE. Of these, in 5 loci an association has previously been described (PTPN22, CTLA4, HLA, AIRE, and BACH2), while 4 loci were novel (LPP, SH2B3, SIGLEC5, and UBASH3A). Of note, they found AAD associations with two LD-independent protein-coding variants in AIRE: one novel (rs74203920) and one previously reported (rs2075876) (**1**). These associations underline the importance of AIRE expression to maintain immune tolerance. The authors offer the interesting example of Down syndrome, where AIRE gene duplication potentially leads to altered expression in the thymus (affecting homeostasis and function of thymic epithelial cells that affect thymic selection processes), impaired central tolerance and increased risks of autoimmune diseases (**2**). This study also identified risk loci in genes involved in antigen presentation and recognition, and hence in thymocyte maturation.

The authors hypothesize that AAD with positive 21-hydroxylase antibodies has a rather homogenous disease etiology with relatively low polygenicity compared to other diseases, explaining at least in part the high heritability estimates (**3**). The study highlights the importance of the complex network of antigen presentation and immunomodulation that underlie autoimmune disease development. These findings underscore the importance of future studies in identifying and developing preventive treatment strategies.

References

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New Hope

8.11. New Horizons: Novel adrenal regenerative therapies

Bornstein SR, Malyukov M, Heller C, Ziegler CG, Ruiz-Babot G, Schedl A, Ludwig B, Steenblock C
J Clin Endocrinol Metab. 2020; 105(9): 3103–3107.
https://pubmed.ncbi.nlm.nih.gov/32629476/

In this narrative review, the authors discuss the potential role of novel regenerative therapies for the treatment of adrenal insufficiency, including gene therapy and cell replacement strategies. More specifically, the authors discuss the heterogeneity in adrenal function in patients with Addison disease, including numerous case reports of spontaneous remission. The authors cite recent studies showing that, shortly after the onset of Addison disease, residual adrenal function might be restored using B-lymphocyte-depleting immunotherapy and ACTH treatment. The rationale is that ACTH stimulates the differentiation of the persisting progenitor or stem cells, while B-cell depletion prevents destruction of the newly differentiated cells (**1**). Furthermore, the cell turnover of the adrenal cortex limits the time-span of the effectiveness of gene therapy (**2**). A more promising strategy is adrenal cell transplantation, which is potentially curative. Research foci in this strategy include alternative cell sources, novel biomaterials that have been developed to improve cell viability of engineered cells upon implantation, and cellular therapies based on pluripotent stem cells (**3**).

Finally, the authors discuss the potential use of adrenal cells as a source for regenerative therapies of nonadrenal neurodegenerative diseases. More specifically, chromaffin cells from the adrenal medulla have been considered as a potential source of dopamine producing cells to treat neurodegenerative conditions, such as Parkinson disease (**4**).

Reference

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New Concerns

8.12. First-Trimester prenatal dexamethasone treatment is associated with alterations in brain structure at adult age

Van't Westeinde A, Karlsson L, Nordenström A, Padilla N, Lajic S
J Clin Endocrinol Metab. 2020; 105(8):dgaa340.
https://pubmed.ncbi.nlm.nih.gov/32497228/

Here, the authors investigated whether prenatal treatment with dexamethasone (DEX) in the first trimester is associated with alterations of brain morphology on MRI scans. Observed MRI alterations were not linked to any alterations in cognitive function or mood, but were associated with DNA methylation in genes that were previously found to be differentially methylated in non-CAH DEX-treated individuals.

Prenatal treatment with glucocorticoids is used in several clinical settings. In the context of congenital adrenal hyperplasia (CAH), prenatal dexamethasone (DEX) has been used successfully in order to ameliorate the virilization of the affected female fetus. However, long term outcome, metabolic, cognitive or other, is an area for concern. Excessive exposure to glucocorticoids during prenatal development may have detrimental effects

on the development of the human brain through various mechanisms, especially in glucocorticoid receptor rich regions of the brain.

In this observational study, the authors investigated brain morphology by MRI scans in adult individuals without CAH, but who had a family history of CAH and were therefore treated prenatally with DEX ($n = 19$). They were compared to apparently healthy population controls ($n = 43$). Prenatal DEX-exposed individuals showed bilateral enlargement of the amygdala and increased surface area and volume of the left superior frontal gyrus. Moreover, DEX-exposed individuals exhibited widespread alterations in white-matter microstructure, mainly in the superior longitudinal fasciculi and corticospinal tracts. Increased DNA methylation for one CpG site in the gene FKBP5 was significantly associated with mean radial diffusivity. DEX-exposed subjects did not exhibit any differences in measures of cognition or behavior and these outcomes were not associated with alterations in brain structure.

This study provides the first evidence that prenatal treatment with DEX is associated with alterations in brain structure that persists into adult life. Epigenetic mechanisms, here measured as differences in DNA methylation, may be an important factor in explaining how DEX exerts its effects on brain development. These findings provide new important information about prenatal with DEX.

8.13. Perturbed beta-cell function and lipid profile after early prenatal dexamethasone exposure in individuals without CAH

Wallensteen L, Karlsson L, Messina V, Nordenström A, Lajic S
J Clin Endocrinol Metab. 2020;105(7): e2439-48.
https://pubmed.ncbi.nlm.nih.gov/32433752/

Here, the authors investigated the effects of dexamethasone (DEX) on metabolism in individuals without CAH but treated with DEX during the first trimester of fetal life. Prenatal DEX exposure was associated with decreased beta-cell function and higher cholesterol concentrations.

Prenatal treatment with DEX successfully ameliorates the virilization of the female fetus with CAH. However, the long-term outcomes on metabolic, somatic and cognitive health are of concern, given that many fetuses (all treated males, and females without CAH) are unnecessarily exposed. This observational study included 40 prenatal DEX-exposed participants and 75 population controls. Fasting blood samples were sampled from all participants and the following parameters were analysed: blood count, renal function, glucose homeostasis, and serum lipid profiles.

Prenatal DEX-exposed adults showed lower HOMA-β index (insulin secretion) and higher blood glucose concentrations, especially in younger participants and girls. Only one other study (**1**) has investigated glucose metabolism in prenatal DEX-exposed individuals. That study also found an association with decreased insulin secretion. Therefore, the current findings strengthen the evidence for a possible adverse effect on beta cell function. Furthermore, in the current study, older participants had significantly higher concentrations of total cholesterol and LDL cholesterol. This new evidence adds to other existing concerns regarding the long-term safety of prenatal DEX treatment in pregnancies at risk of CAH.

Reference

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8.14. DNA Methylation of steroidogenic enzymes in benign adrenocortical tumors: New insights in aldosterone-producing Adenomas

Di Dalmazi G, Morandi L, Rubin B, Pilon C, Asioli S, Vicennati V, De Leo A, Ambrosi F, Santini D, Pagotto U, Maffeis V, Fassina A, Fallo F

J Clin Endocrinol Metab. 2020 Dec 1;105(12): dgaa585.

https://pubmed.ncbi.nlm.nih.gov/32844182/

The aim of this histological study was to investigate DNA methylation and expression of genes encoding steroidogenic enzymes in benign adrenocortical tumors. The findings suggest that DNA methylation plays a regulatory role in CYP11B2 expression and may contribute to aldosterone hypersecretion in aldosterone-producing adrenocortical adenomas (APA).

DNA methylation of CpG islands in promoter and non-promoter regions is one of the most studied heritable regulatory mechanisms and has been recognized in regulatory process of expression of genes involved in tumorigenesis. This cross sectional study examined DNA methylation in fresh-frozen tissues from patients with benign adrenocortical adenomas ($n=48$) and compared its sub-types (non-functioning, $n=9$; autonomous cortisol secretion, $n=9$; Cushing syndrome, $n=17$; aldosterone-producing [APA], $n=13$; adrenal cortex adjacent to APA, $n=12$). The authors performed targeted analysis of methylation of steroidogenic enzymes in benign adrenocortical tumors according to their functional status.

CYP11B2 methylation levels were lower in APA than in other adrenal tissues. Methylation levels of remaining genes were comparable among groups. Overall, CYP11B2 expression and DNA methylation were negatively correlated. In FFPE paired APA/APCC samples, CYP11B2 methylation level was lower in APA than in concurrent APCCs. Lower CYP11B2 methylation levels in APA than in APCCs may suggest an APCC-to-APA switch via progressive CYP11B2 demethylation. Conversely, DNA methylation seems not to be relevant in regulating the expression of genes encoding steroidogenic enzymes other than CYP11B2.

8.15. Metformin inhibits the activation of melanocortin receptors 2 and 3 in vitro: A possible mechanism for its anti-androgenic and weight balancing effects in vivo?

Parween S, Rihs S, Flück CE

J Steroid Biochem Mol Biol. 2020; 200:105684.

https://pubmed.ncbi.nlm.nih.gov/32360359/

In this mouse cell model, the authors show that metformin appears to directly inhibit signaling of MC2R (the ACTH receptor) and also MC3R.

Metformin is used to treat type 2 diabetes and obesity, and may reduce the androgen excess in women with polycystic ovary syndrome (PCOS) or congenital adrenal hyperplasia (CAH). The mechanisms of its action are manifold. Metformin inhibits the cellular stress response at the level of the mitochondrial OXPHOS system and through AMPK-dependent and independent mechanisms. It also decreases pituitary ACTH secretion and reduces the ACTH-stimulated adrenal secretion. The authors previously reported that metformin inhibits complex I and the activities of the steroidogenic enzymes 3β-hydroxysteroid dehydrogenase (HSD3B2) and CYP17/17,20-lyse, enzymes essential for androgen production (**1**). MC2R is expressed primarily in the adrenal cortex and controls steroidogenesis and adrenal growth. MC3R and MC4R play essential roles in energy homeostasis and are expressed in brain and spinal cord. MC3R is important in regulating energy homeostasis and feeding, especially under stressful conditions.

The studies were performed in an adrenal OS3 cell model. Mouse adrenal OS3 cells, which do not express MC2R, were co-transfected to expressing human MC2R and luciferase (a fluorescent marker) and then stimulated with ACTH. In these cells, metformin inhibited ACTH-induced MC2R activation. A 3-fold increase in MC2R mRNA expression with ACTH stimulation, which was abolished to basal levels by metformin. By

contrast, there was no effect of metformin on MRAP expression. This shows that metformin directly blocks ACTH stimulated MC2R expression. No significant effect of metformin was found on MC1R and MC4R activity. Metformin did not shift the α-MSH stimulated MC3R CRC, but produced 40% inhibition of the maximal response (Rmax), suggesting non-competitive inhibition. MC3R is involved in energy balance and seems to act as a rheostat when the metabolism is challenged.

The findings suggest that inhibition of MC2R and MC3R are mechanisms whereby metformin acts to produce weight loss and attenuate the androgen excess in PCOS and CAH.

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Reviews

8.16. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency

Merke DP, Auchus RJ

N Engl J Med. 2020; 383(13): 1248–1261.

https://pubmed.ncbi.nlm.nih.gov/32966723/

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is one of the most common autosomal recessive disorders. Patients with CAH have cortisol deficiency and in its most severe form, a potentially lethal aldosterone deficiency.

In this review, the authors describe the current knowledge regarding the genetic background of CAH, including CAH-X, a syndrome caused by disruption of the gene *CYP21A2,* leading to the 21-hydroxylase deficiency, and a gene located nearby, *TNXB*. The authors further elaborate on the diagnosis and clinical presentation of CAH during different stages of life. While the vast majority of patients with CAH survive, the disorder presents with various challenges in the long-term care and adverse outcomes may occur throughout the patient's lifetime. These adverse outcomes may affect several aspects in health from metabolic to behavioral and cognitive effects. The main factor responsible for these outcomes is the difficulty in mimicking the normal circadian cortisol secretion. The authors further describe the clinical challenges of managing patients on a long-term basis and the current opinions regarding treatment. In summary, this article provides a comprehensive overview regarding the pathophysiological and genetic features of CAH. The review further summarizes the current views regarding its diagnosis, treatment, and management.

8.17. Pathogenesis and treatment of primary aldosteronism

Zennaro MC, Boulkroun S, Fernandes-Rosa FL

Nat Rev Endocrinol. 2020; 16(10): 578–589.

https://pubmed.ncbi.nlm.nih.gov/32724183/

In this review, the authors discuss the pathogenesis and treatment of primary aldosteronism (PA), the most frequent form of secondary hypertension (affecting 5% of patients with hypertension in primary care and 10–20% of those referred to specialist care). The pathophysiological basis of PA is autonomous aldosterone production (autonomous from its physiological regulators and inappropriate to the salt and blood volume status of the individual) from the adrenal gland, which leads to hypertension, hypokalemia and metabolic alkalosis. Patients with PA are at higher risk for cardiovascular disease compared to patients with essential hypertension matched for age and blood pressure. In most cases, PA is attributed to a unilateral aldosterone-producing adenoma (APA) or to bilateral adrenal hyperplasia. Many cases of PA are sporadic, while ∼6% have a familial form of the disease. Familial forms of hyper-aldosteronism (FH-I, FH-II and FH-III) result from germline

mutations. In unilateral APA, somatic mutations are found in the same genes that are associated with familial forms of primary aldosteronism, and the use of next generation sequencing increases the detection of mutations. Most genetic abnormalities increase intracellular calcium signaling in the adrenal zona glomerulosa, increasing aldosterone production, and making it autonomous. The underlying mechanisms leading to activation of calcium signaling are different for each type of channel and pump. PA is curable. However, it is largely underdiagnosed, and this prevents patients from receiving appropriate targeted treatments and results in cardiovascular complications. New approaches are currently being developed to achieve more rapid and precise diagnosis, for more efficient targeted treatment in mutation carriers, and improved management of affected family members.

9. Oncology and Chronic Disease

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Preface

As in previous years, most of the selected papers concern the medium and long-term complications of cancer therapy. Issues related to long-term surveillance strategies represent an emerging topic of discussion. In comparison with previous years, this year we have found few truly original and innovative studies. Rather, the attention of the researchers seems to be focused on confirming and consolidating acquired data, in particular:

- The development of subsequent neoplasms in childhood cancer survivors does not correlate with the use of substitutive therapy with growth hormone, but seems to be primarily associated with the use of radiotherapy.
- The hormone deficiencies resulting from cancer treatment are predominantly secondary to radiotherapy. Chemotherapeutic drugs cause a minor long-term endocrine damage, although some of them (particularly alkylating agents) have a significant gonadotoxic effect.
- Novel genetic markers influencing detrimental effects of chemotherapy on long-term ovarian function have been reported. The identification of these markers can improve the development of risk prediction models. Fertility preservation programs, including ovarian tissue cryopreservation, would benefit from personalized counselling directed to adolescent and young adults at higher risk of gonadotoxicity.

Cancer Treatment and the Risk of Second Neoplasia

9.1. Risk factors of subsequent central nervous system tumors after childhood and adolescent cancers: findings from the french childhood cancer survivor study

Journy NMY, Zrafi WS, Bolle S, Fresneau B, Alapetite C, Allodji RS, Berchery D, Haddy N, Kobayashi I, Labbé M, Pacquement H, Pluchart C, Schwartz B, Souchard V, Thomas-Teinturier C, Veres C, Vu-Bezin G, Diallo I, de Vathaire F.
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Cancer Epidemiol Biomarkers Prev. 2021; 30: 133–141.
https://pubmed.ncbi.nlm.nih.gov/33033142/

This retrospective study of 152 patients and 604 matched-controls within the FCCSS (French Childhood Cancer Survivor Study) cohort aimed to identify clinical and therapeutic factors associated with long-term risk of subsequent primary neoplasm (SPN) in the central nervous system (CNS), occurring at least 5 years after the primary cancer diagnosis.

Treatment of CNS tumors predisposes to SPN. In patients treated with cranial irradiation, the risk of SPN increases linearly with radiation doses. Other factors that contribute to this risk are still not completely understood (1–3). A better knowledge of the risk factors for CNS SPN is essential to define guidelines for long-term surveillance of childhood cancer survivors, improve early detection and treatment.

The study included individuals diagnosed in 1946–2000 with a solid cancer or lymphoma at age < 21 years. The type of first cancer and treatment modalities (radiotherapy, chemotherapy, and growth hormone) were analyzed. Genetic syndromes associated with increased risk of carcinogenesis were considered

(neurofibromatosis types 1 and 2, Turcot, Gorlin, Li-Fraumeni, Klinefelter, Rubinstein–Taybi, Turner, Bloom syndrome, tuberous sclerosis, colon polyposis, and bilateral or familial retinoblastoma).

The risk of subsequent meningioma was 16x higher among CNS tumor survivors compared with other cancer survivors. Meningioma risk, after adjustment for potentially predisposing syndromes and primary CNS tumor, increased with higher radiation doses and cumulative doses of alkylating agents, but no association was found with growth hormone therapy. Meningioma risk was higher in patients who were younger at the primary cancer diagnosis, but did not vary over time. The risk of subsequent glioma was 10x higher among CNS tumor survivors and those with a predisposing genetic syndrome. There was no or a moderate reduction in these risks after adjustment for cumulative radiation dose. The excess Odds ratio per Gy (EOR/Gy) increased among older individuals at the time of the primary cancer diagnosis and with a shorter follow-up time. The risk of glioma was higher in patients treated with epipodophyllotoxins, without any dose–response relationship. The sample size was too small to investigate the relationship between growth hormone therapy and subsequent glioma.

Follow-up of individuals with genetic growth disorders or acromegaly showed an association between growth factors and carcinogenesis, and thus, indirectly raised concerns about growth hormone treatment in CNS tumor survivors. Cranial irradiation increases CNS SPN risk and can also induce growth hormone deficiency, thus, it represents an important confounding factor. Very few studies have evaluated the effect of growth hormone therapy while accounting for radiation doses (4). The impact of growth hormone therapy in the long-term risk of CNS SPN need to be investigated in large populations with a prolonged follow up. Up to now, no study has included an adequate number of cases to produce statistically robust results.

Reference

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9.2. Influence of growth hormone therapy on the occurrence of a second neoplasm in survivors of childhood cancer

Thomas-Teinturier C, Oliver-Petit I, Pacquement H, Fresneau B, Allodji RS, Veres C, Bolle S, Berchery D, Demoor-Goldschmidt CHaddy N, Diallo I, de Vathaire F.
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Eur J Endocrinol. 2020; 183: 471–480.
https://pubmed.ncbi.nlm.nih.gov/32738133/

This cohort and nested case–control study analyzed the impact of growth hormone (GH) treatment on the risk of second neoplasm (SN) in a French cohort of 2852 childhood cancer survivors (CCS) treated before 1986. In total, 126 (64.3%) survivors who received GH had been treated for a brain tumor, 22 (11.2%) for a retinoblastoma, 20 (10.2%) for a lymphoma and 28 (14.3%) for another type of solid tumor; 374 survivors developed a SN, including 40 who had received GH therapy. In multivariate analysis, GH treatment did not increase the risk of secondary non-meningioma brain tumors, secondary non-brain cancer, or meningioma. Slight non-significant increases in the risk of meningioma (1.6-fold) were observed after an exposure to GH of less than 4 years vs 2.3-fold after a longer exposure.

CCS receiving radiation therapy have a high risk of developing a subsequent radiation-induced SN. Radiation dose confounds the relationship between GH and the occurrence of a second CNS neoplasm, as higher doses are associated with both a higher likelihood of both GH deficiency and SN. Previous studies on CCS suggested that meningioma is diagnosed earlier in GH-treated survivors (1), but it is unclear if this is simply due to more careful follow up in GH-treated patients. The risk of meningioma is linearly related to radiation dose (568-fold higher risk at >30 Gy) (2).

In this study, the small number of meningioma cases compared to the numerous cofactors influencing the risk of meningioma (such as higher cranial radiation dose, younger age at cancer diagnosis and associated cancer predisposition syndromes), could have limited statistical power. The impact of GH dose, IGF-1 levels during GH treatment and the use of cadaveric vs recombinant GH were not analyzed. Finally, these data may not be confirmed in patients treated with new techniques of radiation therapy. A recent meta-analysis showed that GH therapy does not increase the risk of SN and improves final height, lipid profiles and quality of life in survivors of childhood cancer (3).

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Childhood Cancer and Thyroid Disease

9.3. Presentation and outcome of subsequent thyroid cancer among childhood cancer survivors compared to sporadic thyroid cancer: a matched national study

Clement SC, Lebbink CA, Klein Hesselink MS, Teepen JC, Links TP, Ronckers CM, van Santen HM.
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Eur J Endocrinol. 2020; 183: 169–180.
https://pubmed.ncbi.nlm.nih.gov/32449692/

This retrospective study analyzed subsequent differentiated thyroid cancer (DTC) in in a group of CCS ($n = 31$) matched with patients affected by sporadic DTC (n = 93) to compare clinical presentation and outcomes. CCS with subsequent DTC were identified by two source populations: the DCOG-LATER cohort of more than 6000 5-year CCS diagnosed with a primary tumor between 1963 and 2001, and The Netherlands Cancer Registry, for cases diagnosed after 2001. Unsurprisingly, CCS with subsequent DTC presented with smaller and more frequently bilateral tumors. DTC can probably be detected when they are smaller in CCS, because these patients receive careful long-term follow-up. On the other hand, DTC are more often bilateral in CCS because thyroid irradiation results in a diffuse toxicity.

Childhood cancer survivors (CCS), are at risk of subsequent malignancies, approximately 10% of these involve the thyroid gland. The occurrence of a differentiated thyroid cancer in CCS is predominantly related to radiotherapy; the risk increases linearly with estimated radiation dose to the thyroid gland, with a plateau at ~10–30 Gy and a decline at higher doses (1).

Two aspects of this study are noteworthy. First, one-third of CCS with subsequent DTC had been treated with chemotherapy only, which prompts reflection on the intrinsic risk due to chemotherapy. Second, subsequent DTC were detected by routine neck palpation (46.2%), self-identified mass (34.6%), or by chance (19.2%). Notably, none was diagnosed by ultrasound screening, the use of which is still debated (2-4). Admittedly, this retrospective study presents a dated cohort (1963-2015). Evidence in recent years consider ultrasound superior to palpation for thyroid cancer detection in high-risk CCS (5).

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Thyroid Disorders in Inflammatory Bowel Diseases

9.4. Infliximab therapy could decrease the risk of the development of thyroid disorders in pediatric patients with Crohn's Disease

Furtak A, Wedrychowicz AM, Sladek M, Wedrychowicz A, Fyderek K, Starzyk J.
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Front Endocrinol (Lausanne). 2020; 11: 558897.
https://pubmed.ncbi.nlm.nih.gov/33042019/

This patient cohort study evaluated the prevalence of autoimmune thyroid diseases (AITDs), defined as decreased thyroid echogenicity in 61 children with Crohn's disease, 25 infliximab (IFX)-naïve (control group) and 36 IFX-treated patients (mean duration of IFX therapy 13.9 ± 16.6 months). The same researcher performed all the thyroid ultrasound scans. In doubtful cases, a second specialist performed a verification scan. AITD prevalence was found higher in IFX-naive patients (7/25) compared to IFX-treated patients (1/36). Thyroid function tests were normal and thyroid autoantibodies were negative in all patients of both groups.

Tumor necrosis factor alpha (TNF-alpha) plays a role in the pathogenesis of autoimmune diseases. Therefore, anti-TNF-alpha agents, such as infliximab (IFX) used in Crohn's disease, could theoretically modify the outcome of concurrent autoimmune diseases or even protect against them. A higher prevalence of AITD in ulcerative colitis patients has been suggested, but no association with Crohn's disease. According to published data, the prevalence of thyroid dysfunction is similar or lower in Crohn's disease patients, compared to the general population (1).

Limitations of this study are the small sample size and the definition of AITD. Actually, while it is true that the lack of autoantibodies does not exclude an AITD, most patients with AITD show high autoantibody titres. Differently from what the authors state, presented data do not seem sufficient to extrapolate a protective role of IFX on the development of autoimmune thyroiditis. The reason why a higher prevalence of decreased thyroid echogenicity is found in IFX-naïve patients than IFX-treated patients, despite the shorter disease duration in the former (mean CD duration: 25 versus 53 months), remains unclear.

Reference

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Surveillance Strategies of Endocrine Complications

9.5. Beyond premature ovarian Insufficiency: staging reproductive aging in adolescent and young adult cancer survivors.

Medica ACO, Whitcomb BW, Shliakhsitsava K, Dietz AC, Pinson K, Lam C, Romero SAD, Sluss P, Sammel MD, Su HI.
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https://pubmed.ncbi.nlm.nih.gov/33141175/

This cross-sectional study applied a commonly used system to classify reproductive aging (Stages of Reproductive Aging Workshop +10 or 'STRAW +10') to 338 adolescents and young adult (AYA) cancer

survivors (1). The study aimed to evaluate if STRAW +10 correctly identifies premature ovarian failure in this population and to assess the relationship between cancer treatment and reproductive aging stage.

The STRAW classification system was first described in 2001 to stage ovarian aging based on menstrual cycle characteristics and FSH levels. Several studies then highlighted the critical changes in hypothalamic-pituitary and ovarian function that occur before and after the final menstrual period. These advances led to an update in 2011 that expanded the STRAW system from 7 to 10 criteria (STRAW +10). In particular, anti-müllerian hormone (AMH) levels and antral follicle count (AFC) were added as qualitative markers of fertility potential during the late reproductive life (1). Short reproductive lifespan is common in AYA cancer survivors, in particular those who received gonadal irradiation and/or alkylating chemotherapy and were older at the time of cancer treatment. Current guidelines for these patients recommend to monitor ovarian function by menstrual patterns, and to measure endocrine biomarkers only if menstrual changes appear. However, it is well known that ovarian reserve declines before the appearance of menstrual changes.

In this study, patients were initially classified by menstrual pattern, then according to FSH and AMH levels. The proportion of individuals in each reproductive aging stage was compared to evaluate the concordance of these two methods. Within the reproductive phase, the addition of endocrine biomarkers distinguished between peak and late reproductive aging. The agreement between classification by menstrual pattern alone vs menstrual pattern with ovarian reserve testing was high for the reproductive and postmenopausal stages. However, the menopausal transition was highly discrepant between the two approaches. After adjustment for age and cancer recurrence, cyclophosphamide equivalent dosing (CED) remained significantly associated with the risk of menopausal transition and postmenopause.

This study highlights that the STRAW +10 system does not correctly identify premature ovarian failure in AYA survivors. Current guidelines for the evaluation of reproductive health should be revised, in particular for patients in low-risk groups. Large observational studies are needed to understand the timing and progression of reproductive aging in young cancer survivors. Predicting premature ovarian failure is essential to correctly inform patients about the fertility, improve quality of life and prevent disease-related complications (i.e cardiometabolic risk and bone impairment).

Reference

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9.6. Female reproductive function after treatment of childhood acute lymphoblastic leukemia

Roshandel R, van Dijk M, Overbeek A, Kaspers G, Lambalk C, Beerendonk C, Bresters D, van der Heiden-van der Loo M, van den Heuvel-Eibrink M, Kremer L, Loonen J, van der Pal H, Ronckers C, Tissing W, Versluys B, van Leeuwen F, van den Berg M, van Dulmen-den Broeder E; LATER-VEVO Study Group.
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Pediatr Blood Cancer. 2021; 68: e28894.
https://pubmed.ncbi.nlm.nih.gov/33459500/

This cross-sectional observational study examined reproductive function in a nationwide cohort of female childhood acute lymphoblastic leukemia (ALL) survivors.

Self-reported reproductive characteristics (age at menarche, virginity status, desire for children, pregnancy rates, and adverse pregnancy outcomes) were assessed by a questionnaire in 357 adult 5-year ALL survivors, treated between 1964 and 2002, and 836 controls. Ovarian function was assessed by anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), inhibin B and antral follicle count (AFC). ALL survivors were significantly more likely to have low AMH and low AFC than controls. However, ALL survivors treated with chemotherapy (CT) only had overall self-reported reproductive outcomes, pregnancy rates and ovarian function markers similar to controls. Whereas, ALL survivors treated with CT + radiotherapy (RT) had lower pregnancy rates.

Patients treated with cranial RT have an increased risk of neuropsychological sequelae, which potentially reduce the chances of becoming parents. However, the reduced pregnancy risks among ALL survivors treated with CT

+ RT could also result from hypothalamic-pituitary damage (after cranial RT) or ovarian damage (after TBI). A previous study of ALL survivors showed that risk of miscarriage is increased in patients treated with craniospinal RT, but not in patients treated with cranial RT only (1). Recent advances in ALL treatment have made the use of spinal RT obsolete (and RT is not included in standard risk protocols), therefore the risk of miscarriage is probably not increased in more recent ALL survivors. Female childhood ALL patients need to be informed of the late effects of treatment on fertility. In particular, survivors who were treated with RT should be advised not to delay childbearing due to their reduced reproductive lifespan. Survivors not treated with RT seem not at risk of a diminished reproductive function.

Strengths of this study include the large number of participants, but there are also some limitations. Participants who underwent the study of ovarian function markers were significantly younger than questionnaire-only participants. This could possibly have over- or underestimated the prevalence of impaired reproductive function. Additionally, the self-reported outcomes may have been influenced by recall bias. Finally, the study population included relatively young women, and data on menopausal status needs to be confirmed, because not all women with premature menopause may have been identified.

Reference

1. Green DM, Whitton JA, StovallM, *et al*. Pregnancy outcome of female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. *Am J Obstet Gynecol*. 2002; 187: 1070–80.

9.7. Hormonal replacement therapy in adolescents and young women with chemo- or radio-induced premature ovarian insufficiency: Practical recommendations.

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Blood Rev. 2021; 45: 100730.
https://pubmed.ncbi.nlm.nih.gov/32654893/

This practical treatment protocol was developed by an experienced multidisciplinary team following a critical and updated systematic review of the literature.

Hypogonadism is a common finding after antineoplastic treatment. It can either result from a primary gonadal disorder (due to pelvic irradiation and alkylating agents) or from hypothalamic-pituitary ovarian axis damage (due to cranial or craniospinal radiotherapy), or both. Hematopoietic stem cell transplantation (HSCT) is an independent risk factor for premature ovarian insufficiency (POI); the incidence of POI ranges from 44 to 100% among HSCT childhood recipients. Few studies have investigated iatrogenic POI in children and young adolescents (1).

The clinical approach described here was extrapolated from published evidence on adult women with POI due to multiple etiologies. In post-pubertal patients with incipient iatrogenic POI, if no contraception is requested, hormone replacement therapy via 17βE-based transdermal patches is strongly recommended as the first line approach. Combined oral contraceptives should be prescribed only in patients who wish for contraception as a priority. In girls presenting with pubertal delay and a hormonal picture consistent with hypergonadotropic (primary) hypogonadism following childhood cancer +/− HSCT, transdermal 17βE is the recommended first line to induce puberty. Puberty induction should be started between ages 11–13 years, when FSH levels are > 10 U/l, in analogy with management of Turner Syndrome. Estrogen doses should be increased 6-monthly with the aim of completing pubertal development in ~2 years, as in normal girls. Progestin therapy should be added when the first menstrual bleed occurs.

Reference

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Genetic Susceptibility to Treatment-Related Gonadotoxicity

9.8. Possible modification of BRSK1 on the risk of alkylating chemotherapy-related reduced ovarian function

van der Kooi ALF, van Dijk M, Broer L, van den Berg MH, Laven JSE, van Leeuwen FE, Lambalk CB, Overbeek A, Loonen JJ, van der Pal HJ, Tissing WJ, Versluys B, Bresters D, Beerendonk CCM, Ronckers CR, van der Heiden-van der Loo M, Kaspers GL, de Vries ACH, Robison LL, Hudson MM, Chemaitilly W, Byrne J, Berger C, Clemens E, Dirksen U, Falck Winther J, Fosså SD, Grabow D, Haupt R, Kaiser M, Kepak T, Kruseova J, Modan-Moses D, Pluijm SMF, Spix C, Zolk O, Kaatsch P, Krijthe JH, Kremer LC, Yasui Y, Brooke RJ, Uitterlinden AG, van den Heuvel-Eibrink M, Mvan Dulmen-den Broeder E.
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Hum Reprod. 2021; 36: 1120–1133.
https://pubmed.ncbi.nlm.nih.gov/33582778/

This multi-centre retrospective cohort study identifies that common genetic variants in DNA repair genes modify the damage induced by alkylating agents on ovarian function, as assessed by low AMH levels.

Alkylating agents are known to induce apoptosis of cancer cells by damaging DNA replication and transcription and inhibiting cellular metabolism (1). Previous studies in female childhood cancer survivors (CCS) showed that alkylating agents are strongly associated with reduced ovarian function, as measured by low anti-Mullerian hormone (AMH) levels. However, there is wide inter-individual variability in the detrimental effect of alkylating agents on adult ovarian function. Genetic variants in genes involved in DNA repair may be responsible for this variability.

This study reports a strong modifying effect of the G allele of the common single-nucleotide polymorphism rs11668344 in *BRSK1* gene (rs11668344 A > G) on the ovarian toxicity induced by alkylating agents. The modifying impact results in 2.5-fold increased odds of gonadotoxicity in CCS carrying one G allele, and 3-fold increased odds in CCS carrying two G alleles, compared to CCS without this allele. The BR Serine/Threonine Kinase 1 (*BRSK1*) gene is recognized to be involved in DNA damage checkpoint response. The efficiency of the DNA damage sensing and repair system is crucial when treatments with alkylating agents corresponding to a high cyclophosphamide equivalent dose (CED) are needed. Due to a less efficient response to DNA damage, cancer patients carrying the G allele of rs11668344 in BRSK1 may experience increased risk of gonadotoxicity.

To our knowledge, this is the first study to report a specific genetic factor that influences the damaging effects of chemotherapy on long-term ovarian function. The identification of genetic markers that can predict the extent of gonadal damage induced by alkylating agents can potentially inform the development of risk prediction models. Fertility preservation programs, including ovarian tissue cryopreservation, would benefit from personalized counselling directed to adolescent and young adults identified to be at high risk of gonadotoxicity.

Reference

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Reproductive Outcomes in Childhood Cancer Survivors

9.9. Risk of induced abortions in childhood cancer survivors

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Cancer. 2021; 127: 1439–1447.
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This nationwide, Finnish registry-based study identified a lower likelihood of primary pregnancy (relative risk = 0.72; 95% CI, 0.64–0.80) in 1357 childhood cancer survivors (CCS) diagnosed in 1971-2012 and 6658 age-matched general population controls. However the risk of induced abortion was similar in both groups.

Parenthood is diminished in CCS, as a consequence of both impaired fertility and concern about their offspring's health. Induced abortions in CCS have been evaluated in comparison to siblings and/or general population, with contrasting results, probably due to the age of the study population and cultural differences regarding induced abortion (1-3). Here, data from four Finnish registries for cancer, births, and induced abortions were merged to identify 420 primary pregnancies in CCSs and 2508 primary pregnancies in age-matched population controls. The risk of primary pregnancy was reduced in CCS compared to controls (relative risk $=0.72$; 95% CI, 0.64-0.80), whereas the risk of a primary pregnancy resulting in an induced abortion was similar in CCSs and controls, including when stratifying by decade of diagnosis and cancer treatment. These results suggest that female CCS who become pregnant are as willing as their peers to continue their pregnancy and become parents. The overall reduced probability of pregnancy highlights the persisting need for interventions to preserve fertility at the time of cancer diagnosis.

The registries used in this study lacked detailed information on cancer treatment (radiation dose and field, specific chemotherapeutic agents) and were based only on the first 4 months of therapy. For these reasons, the association between cancer therapies and the probability of pregnancy could not be completely defined. Moreover, social difficulties can influence the decision to terminate a pregnancy and data on socioeconomic status were not available for the majority of both CCSs and controls. Using the same approach, it would be useful to analyze frequency and outcome of pregnancy, in relation to long-term therapy, disease-related complications, education and socioeconomic status.

Reference

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3. Anderson R, Brewster D, Wood R, *et al*. The impact of cancer on subsequent chance of pregnancy: a population-based analysis. *Hum Reprod*. 2018; 33:1281–90.

9.10. Health outcomes in offspring born to survivors of childhood cancers following assisted reproductive technologies.

Sommerhäuser G, Borgmann-Staudt A, Astrahantseff K, Baust K, Calaminus G, Dittrich R, Fernández-González MJ, Hölling H, König CJ, Schilling R, Schuster T, Lotz L, Balcerek M.

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J Cancer Surviv. 2021; 15: 259–272.

https://pubmed.ncbi.nlm.nih.gov/32844376/

Long-term treatment effects are possible reasons for reduced fertility and adverse pregnancy outcomes in childhood cancer survivors (CCS) (1). This observational study reports perinatal and health outcomes of offspring born to CCS using assisted reproductive technologies (ART). CCS were almost 2-fold more likely to use ART compared to the general population (4.6% vs. 2.6%).

Among offspring born to CCS, multiple sibling births and low birth weight were significantly more common following ART than after spontaneous conception. The high prevalence of multiple sibling births after ART in CCS was similar to the 34% reported in the general population. ART did not increase the prevalence of childhood cancer or congenital malformations in offspring born to CCS. A mildly increased prevalence of moderate preterm births (32 to 37 gestational weeks) in the offspring of CCS was detected. The differences in perinatal outcomes were completely explained by differences in multiple sibling birth and other known confounders.

Patients included in this study were treated between 1980 and 1999, before the implementation of the guidelines for fertility preservation. Only few patients cryopreserved oocytes/sperm prior to cancer treatment. Most CCS (58.7%) were treated in the 1980s, with higher doses of radiation and alkylating agents than used now. These findings appear particularly reassuring for current patients treated with less toxic protocols. The results are also reassuring because only a mild increase of moderate preterm birth was demonstrated in survivor offspring and most medical consequences occur in severely preterm infants.

In this study, the prevalence of childhood cancer and congenital malformations, whether conceived by ART or spontaneous conception, was not higher in CCS offspring than in the general population. A meta-analysis published in 2013, had reported a slightly elevated overall cancer risk in children born after ART. Fertility impairment and potential epigenetic defects in the gametes, rather than the ART procedure itself, were supposed to be the main predisposing factors (2).

The study has some limitations. The recruitment based on previous surveys identifying CCS with biological children (required by the German Society for Pediatric Oncology and Hematology to reduce the study burden for survivors) potentially caused a selection bias. The questionnaire-based setting reduces data accuracy by recall bias. Moreover, factors as maternal age, body mass index, infections or other maternal diseases, which clearly influence pregnancy outcome and perinatal events, were not analyzed.

Reference
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2. Hargreave M, *et al*. Fertility treatment and childhood cancer risk: a systematic metaanalysis. *Fertil Steril*. 2013; 100: 150–61.

Cancer Treatment, Growth and Growth Hormone Therapy

9.11. Height after photon craniospinal irradiation in pediatric patients treated for central nervous system embryonal tumors

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Pediatr Blood Cancer. 2020; 67: e28617.
https://pubmed.ncbi.nlm.nih.gov/32715632/

This retrospective single-centre study aimed to develop a final height prediction model based on data from 212 long-term survivors (aged 5-20.4 years) of childhood central nervous system (CNS) tumors who received craniospinal irradiation (CSI) and with a median follow up of 10.2 years. Mean final height *Z*-scores at 18 years of age, compared to United States standards, were −1.3 for female and −1.5 for male survivors. Prediction models showed that height was impaired in: females, African American background, high CSI dose ($\geq$ 36 Gy), and younger age at CSI. In contrast, factors associated with higher growth rates before age 15 years were: replacement therapy for growth hormone (GH) deficiency and central adrenal insufficiency. Growth after age 15 years in male survivors was associated with treatment for gonadotropin deficiency.

CSI is a key element for the treatment of childhood CNS tumors that can spread to neuroaxis. The risk of short stature following CSI is well known and is due to both a direct radiation-induced bone damage of the spine and to GH deficiency. A previous study (1) reported that change in height after GH treatment was positively associated with male sex, GH dose, and lower bone age at therapy start; while concurrent endocrine disorders and radiation dose to the spine negatively affected change in height. The same study suggested that early onset of female puberty reduced the available time for effective GH-replacement therapy.

This study is interesting but has some limitations. First, it lacked data on puberty, which is important for both growth spurt and timing of GH treatment. Second, stature was not correlated to parental height, which might account for the differences among ethnicities. Third, the impact of GH dose and duration were not discussed. Moreover, this study did not consider the differences between proton and photon therapy. A recent study (2) showed a significantly reduced incidence of hypothyroidism, sex hormone deficiency and the need of replacement therapy in patients treated with proton therapy, but median follow-up was relatively short. Patients in the proton group were young and puberty had not been reached in many of them. This issue needs to be analyzed in future studies of growth following CSI.

Reference
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2. Eaton BR, Esiashvili N, Kim S, et al. Endocrine outcomes with proton and photon radiotherapy for standard riskmedulloblastoma. *Neuro Oncol*. 2016; 18: 881–7.

9.12 Response to GH treatment after radiation therapy depends on location of irradiation

Rose SR, Carlsson M, Grimberg A, Aydin F, Albanese A, Hokken-Koelega ACS, Camacho-Hubner C. Response to GH Treatment After Radiation Therapy Depends on Location of Irradiation.
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J Clin Endocrinol Metab. 2020; 105: e3730–41.
https://pubmed.ncbi.nlm.nih.gov/32706856/

This retrospective analysis of the *Pfizer International Growth Database (KIGS)* included 1149 cancer survivors with growth hormone deficiency (GHD) who had received growth hormone therapy (GHT) for at least 5 years. Patients with craniopharyngioma had the best 5-year growth response to GHT with a delta height SD score of 1.6, compared to 0.9 in patients with medulloblastoma. By contrast, delta height SD score was only 0.3 in patients with leukemia who received TBI, and 0.5 in patients with leukemia without RT.

Many patients with craniopharyngioma already present at diagnosis with poor linear growth, suggesting that GHD was a direct result of tumor location in the hypothalamic-pituitary region. On the contrary, GHD following medulloblastoma or leukemia is an effect of treatment (i.e. RT). GHT is often started in craniopharyngioma survivors by 1 year after surgery, but is often delayed in survivors of medulloblastoma or leukemia. Several small studies have observed that vertebral growth is significantly impaired after spinal RT or TBI and contributes to disproportionate short stature.

In this study, leukemia survivors who had received conditioning regimen with TBI for bone marrow transplantation experienced the most severely impaired growth response to GHT at 1 and 5 years. In general, patients who were pubertal at GHT start showed a better height growth response than those who were prepubertal at GHT start, probably because the pubertal spurt enhanced the response to GHT. However, pubertal patients with leukemia receiving TBI, were an exception in that they showed a worse growth response than prepubertal patients. All these data support the hypothesis that TBI impairs the pubertal spurt by direct damage to the epiphyses and bony matrix.

The large sample size in each tumor group and the long GHT duration are clear strengths of this study. Nevertheless, the retrospective analysis of registry data lacks details of imaging, treatment modalities, body proportions, onset and tempo of puberty, and other pituitary hormone disfunctions. Details about body proportions would be particularly useful in evaluating the response to GHT in medulloblastoma patients who are treated with craniospinal RT. These patients may exhibit suboptimal height gains as well as disproportionately poor growth of the trunk relative to the legs, due to a direct radiation damage to the spine.

Cardiometabolic Risk in Chronic Disease

9.13. Biomarkers of cardiometabolic complications in survivors of childhood acute lymphoblastic leukemia

Morel S, Léveillé P, Samoilenko M, Franco A, England J, Malaquin N, Tu V, Cardin GB, Drouin S, Rodier F, Lippé S, Krajinovic M, Laverdière C, Sinnett D, Lefebvre G, Levy E, Marcil V.
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Sci Rep. 2020 Dec 9; 10: 21507.
https://pubmed.ncbi.nlm.nih.gov/33299020/

This cross-sectional study of 246 childhood acute lymphoblastic leukemia (cALL) survivors aimed to analyze the relationships between various blood biomarkers and cardiovascular risk, and to test the link between endotoxemia and cardiometabolic complications. A high leptin-adiponectin ratio was associated with obesity, insulin resistance and the metabolic syndrome. Higher levels of plasminogen activator inhibitor-1 and tumor necrosis factor-α were related to obesity. Elevated C-reactive protein levels correlated with insulin resistance and metabolic syndrome. Oxidized-LDL (Ox-LDL) concentrations, as biomarkers of oxidative stress, were associated with dyslipidemia.

Oxidative stress, chronic inflammation, adipose tissue dysfunction, endocrine disorders and accelerated cellular aging have been implicated in the development of cardiometabolic complications in cALL survivors. High circulating levels of adipokines and pro-inflammatory cytokines have been demonstrated during and after chemotherapy. Endothelial dysfunction has been reported in long-term survivors of cALL, contributing to their risk for early cardiovascular disease. The infiltration and retention of low-density lipoprotein (LDL) in the arterial intima triggers the inflammatory process evolving to plaque formation. Modification of LDL through oxidation causes endothelial cells to express leucocyte adhesion molecules leading to the progression of atherosclerosis.

This study includes a large number of biomarkers of endotoxemia, inflammation, oxidative stress and endothelial function in a well-characterized cohort of cALL survivors. The monocentric design, with a relatively small sample size and the absence of a control group of healthy subjects are limitations. Additionally, all the studied subjects were Caucasian and the results may not be generalizable to other ethnicities, because epidemiological studies have clearly shown that biomarkers of cardiometabolic risk can significantly vary in different ethnic groups.

9.14. Incidence and risk factors of obesity in childhood solid-organ transplant recipients

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This retrospective study analyzed the incidence and risk factors for obesity in a large ($n = 410$), single center cohort of pediatric solid-organ transplant recipients, who received their first transplant between January 1, 2002 and December 31, 2011 (median transplant age 8.9 years; median follow-up time 3.6 years). Within 5-years post-transplant, 1:4 developed obesity with higher risk in those: overweight at transplant, age < 5 at transplant, kidney transplant recipients, and higher cumulative prednisone exposure.

With the progressive improvement of survival of patients with childhood cancer, the burden of long-term comorbidities is increasingly underlined. Obesity is an increasing healthcare problem with clear cardiovascular consequences (1). In cross-sectional analyses of pediatric transplant recipients, obesity prevalence ranges from 13 to 34% and it is associated with a worse graft survival and function (2).

The study stratified the risk of obesity in a heterogeneous population, identifying specific organ groups in which further examination of organ-specific risk factors may be warranted. Across all organ groups, those who developed obesity did so within the first year post-transplant, emphasizing the need to carefully screen for obesity early after transplantation. Corticosteroid reduction and avoidance protocols for patients receiving solid organ transplant have been developed in recent years to minimize the risk of obesity and metabolic syndrome (3). The increased prevalence in kidney recipients is ascribed to the absence in these patients of the severe wasting and cachexia, which is generally associated with chronic lung, liver and heart disease.

Limitations of this study are its retrospective design and the absence of data on physical activity and nutrition. Abdominal distribution of adiposity, an essential feature of metabolic syndrome, and other metabolic syndrome components were not evaluated. Abdominal adiposity, usually assessed by weight-to-height ratio, is common in cancer survivors, while BMI underestimates obesity in these patients. A major strength of this study is the large

sample size, with data collected in a universal healthcare system that captures data on all patients with healthcare and medication coverage.

Reference

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Latrogenic Adrenal Insufficiency and Chronic Steroid Rherapy

9.15. Risk of adrenal insufficiency following intra-articular or periarticular corticosteroid injections among children with chronic arthritis

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This retrospective study evaluated the prevalence of adrenal insufficiency after a single intra-articular corticosteroid injection (IACI) in 60 children with chronic arthritis. Adrenal insufficiency was diagnosed in 30% (18/60) and was associated with higher doses of injected corticosteroid. Median duration of adrenal insufficiency was 181 days, among the nine patients who were followed up to the resolution of adrenal insufficiency. Four patients developed symptoms that were suggestive of adrenal insufficiency (fatigue, nausea and/or abdominal pain).

It is well known that non-systemic steroid formulations are able to inhibit the hypothalamic-pituitary adrenal (HPA) axis and cause iatrogenic central adrenal insufficiency. Adrenal insufficiency after IACI has long been demonstrated in adults (2) but there are very few data in children. As a meta-analysis (1) on the subject stated: *1) Adrenal insufficiency after discontinuation of glucocorticoid occurs frequently; 2) there is no administration form, dosing, treatment duration, or underlying disease for which adrenal insufficiency can be excluded with certainty, although higher dose and longer use give the highest risk; 3) the threshold to test corticosteroid users for adrenal insufficiency should be low in clinical practice, especially for those patients with nonspecific symptoms after cessation*.

In this study, children were screened by measuring a morning cortisol about 2 weeks after IACI. Those with cortisol $<$ 500 nmol/l (Siemens assay) or $<$ 375 nmol/l (Roche assay), underwent a low dose ACTH stimulation test (ACTH 1 µg intravenously, with cortisol measurements at time 0 and 30 min). Adrenal insufficiency was diagnosed as: morning cortisol $<$50 nmol/l; peak cortisol at ACTH stimulation test $<$ 375 nmol/l (Roche) or $<$500 nmol/l (Siemens); or AI diagnosis by a pediatric endocrinologist based on clinical judgment plus a low morning cortisol value (but $>$50 nmol/l). distribution of adiposity,

The topic of this study deserves the attention of all clinicians who prescribe steroids, not only endocrinologists, because adrenal insufficiency is a potentially life-threatening complication. Possible study limitations are its retrospective design, which limited the evaluation of signs and symptoms suggestive of adrenal insufficiency and its estimated duration; the questionable definition of adrenal insufficiency, although diagnostic criteria are still controversial (3); finally, the relatively small sample size, including patients treated with different types of corticosteroid drugs.

Reference

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10. Type 1 Diabetes Mellitus

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Preface

As has been the case in many areas of child and adolescent health and in medicine in general, the recent Covid19 pandemic has affected children and adolescents with type 1 diabetes (T1D) and their families very much. In addition, health system resources have been allocated to adult care infectious disease and pulmonary medicine departments. Pediatric care and pediatric health care structures have suffered (1-3). Still, a number of important publications have been made available within the last year which shed new light on many aspects of T1D in early life and will allow to improve the care for children and adolescents with T1D.

Reference

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10.1. Did the COVID-19 lockdown affect the incidence of pediatric type 1 diabetes in Germany?

Tittel SR, Rosenbauer J, Kamrath C, Ziegler J, Reschke F, Hammersen J, Mönkemöller K, Pappa A, Kapellen T, Holl RW, DPV Initiative

Diabetes Care. 2020;43(11):e172–e173.
doi:10.2337/dc20-1633

During the first phase of the COVID-19 pandemic, the DPV (the German diabetes registry database) study group asked whether or not the COVID-19 lockdown had affected the incidence of pediatric T1D in Germany (1). More than 210 German pediatric diabetes centers contributed their data on pediatric patients with T1D with an onset at ages between 6 months and <18 years diagnosed between 13 March and 13 May in each year between 2011 and 2020. The latter period corresponds to the first COVID-19 lockdown period in Germany in 2020. T1D incidence increased from 16.4 [95% CI 14.7–18.2] in 2011 to 22.2 [20.3–24.2] in 2019 ($P=0.04$). The incidence in 2020, based on 532 cases among 13.6 million subjects <18 years, was 23.4 [21.5–25.5] and did not differ significantly from the prediction (22.1 [20.4–23.9]). It can be concluded that T1D incidence in 2020 followed the increasing trend observed between 2011 and 2019 without any observable up- or downward deviation.

There is no sign that the COVID-19 pandemic influenced the incidence of diabetes in youth in the short term. Therefore, direct diabetogenic and even islet destroying effects of the COVID-19 virus itself seem unlikely. However, subtle and or indirect effects cannot be excluded given the relatively low COVID-19 infection rate in children in Germany during the study period. In addition and importantly it remains unclear whether or not elevated stress levels and/or reduced infection rates may actually outweigh each other regarding any putative effects on the onset of T1D in children and adolescents or as an alternative there may indeed be no measurable impact on incidence. In addition, there may be a time delay between immunologic factors or infections and the

onset of T1D. Epidemiologic differences, confounding factors and delays in reporting are known to have occurred during lockdown periods and may have influenced these results (1).

Reference

1. Vogel M, Beger C, Gausche R, Jurkutat A, Pfaeffle R, Körner A, Meigen C, Poulain T, Kiess W. COVID-19 pandemic and families' utilization of well-child clinics and pediatric practices attendance in Germany. *BMC Res Notes*. 2021;14(1):140. doi: 10.1186/s13104-021-05562-3.

10.2. Impact of lockdown in COVID-19 on glycemic control in patients with type 1 diabetes mellitus

Verma A, Rajput R, Verma S, Balania VKB, Jangra B
Diabetes Metab Syndr. 2020;14(5):1213–1216.
doi:10.1016/j.dsx.2020.07.016

In this small cross-sectional study on 52 pediactric patients with T1D, a structured questionnaire was administered on follow up within 15 days after lockdown during the COVID-19 pandemic. Of these 52 patients, 36.5% had hyperglycemic and 15.3% had hypoglycemic episodes. Insulin dose was missed in 26.9%, glucose monitoring was not performed routinely anymore in 36.5%, and 17.4% were not diet compliant during the lockdown period. Average blood glucose was higher during lockdown 276.9 ± 64.7 mg/dl as compared to the pre-lockdown phase 212.3 ± 57.9 mg/dl in the same patients. Mean HbA1c values were also much higher during lockdown ($10 \pm 1.5\%$) than pre-lockdown ($8.8 \pm 1.3\%$) and this difference was indeed statistically significant ($P < 0.05$).

There are numerous reports of suggested and potentially direct or indirect effects of the COVIC-19 infection or lockdown measures on diabetes control in children. According to questionnaires administered to these T1D patients and their caretakers, glycemic control of worsened during the lockdown period mainly due to non-availability of insulin and/or glucostrips. These data very strongly suggest that societies should learn their lessons from the pandemic and be aware of the fact that there is indeed a need for preparedness for epidemics/pandemics in the future so that complications in those already affected by disease can be kept at a low level (1).

Reference

1. Kiess W, Poulain T, Jurkutat A, Kirstein A, Vogel M. Covid19 pandemic and pediatric endocrinology and metabolism - are we through with it? *J Pediatr Endocrinol Metab*. 2021;34(5):535–537. doi: 10.1515/jpem-2021-2133.

10.3. Caring for children and adolescents with type 1 diabetes mellitus: Italian Society for Pediatric Endocrinology and Diabetology (ISPED) statements during COVID-19 pandemia

d'Annunzio G, Maffeis C, Cherubini V, Rabbone I, Scaramuzza A, Schiaffini R, Minuto N, Piccolo G, Maghnie M
Diabetes Res Clin Pract. 2020;168:108372.
doi:10.1016/j.diabres.2020.108372

Here, the Italian Society for Pediatric Endocrinology and Diabetology carried out a literature search in Medline and Embase and also Diabetes Societies websites until May 21st, 2020 for guidelines and recommendations on T1D management during the COVID-19 pandemic. They state that there is currently no evidence to suggest a higher risk of COVID-19 infection in children with diabetes than in unaffected peers. In addition, contrary to adult patients with diabetes, there are no reports suggesting that diabetes is associated with poor outcomes of COVID-19 infection in children and adolescents.

Many learned scientific societies have reacted to the COVID-19 pandemic swiftly and have issued standard operational procedures on how to manage diseases and best utilize medical services during the pandemic and/or

lockdown periods. Despite the potential risk that the COVID-19 infection may develop into a more severe form in people with diabetes including children, this risk is considered to be very low when diabetes is well-controlled. However, several practical recommendations are made: children and adolescents affected by T1D, and/or by other chronic conditions, need uninterrupted access to insulin, drugs, supplies, such as insulin pumps, pens and syringes and technology and continuous specialist medical care, especially in stressful situations. Since the lockdown had actually reduced the access to outpatient clinics, many Italian pediatric diabetologists had rescheduled all routine checks as telephone and video-link consultations during the peak pandemic and lockdown periods. It seems that these measures helped to keep children and adolescents with diabetes in good metabolic control and to prevent an increase in acute or chronic adverse events.

10.4. Extended family history of type 1 diabetes in HLA-predisposed children with and without islet autoantibodies

Kuusela S, Keskinen P, Pokka T, Knip M, Ilonen J, Vähäsalo P, Veijola R
Pediatr Diabetes. 2020;21(8):1447–1456.
doi:10.1111/pedi.13122

Family histories suggest strong degrees of inheritance of T1D in some children, especially in populations with an overall high risk of autoimmunity.

This paper from the Finnish T1D Prediction and Prevention (DIPP) study looked at subjects carrying high HLA-conferred risk for T1D. A family history of T1D in relatives outside the core family was a significant risk factor for islet autoimmunity and progression to clinical T1D in HLA susceptible children.

Case children ($N=343$) were identified when they were found to be positive for at least one islet autoantibody. They were matched by age, gender and class II HLA genotype to control children ($N=343$) who tested negative for islet autoantibodies at the time of data collection. An extended family history of T1D was obtained using structured questionnaires in all subjects.

Among children who were autoantibody positive and progressed to T1D, 62.2% (28/45) had at least one relative with T1D. Interestingly, more of these children (26/45, 57.8%) had such a relative outside the core family, compared to 30.7% of children with no autoantibodies ($P=.001$), 35.2% of those with only classical islet cell antibodies ($P=0.006$), and 35.2% of non-progressors with biochemical autoantibodies ($P=0.011$). A positive history of T1D in the paternal extended family was more common in children with multiple biochemical autoantibodies compared to those with only one biochemical autoantibody ($P=0.010$). However, no association was found between the specificity of the first appearing autoantibody and family history of T1D.

These data suggest that in addition to HLA genotypes, other genetic factors play a major role in influencing islet autoimmunity and/or progression to overt T1D.

10.5. Type 1 diabetes mellitus and its oral tolerance therapy

Mao RF, Chen YY, Zhang J, Chang X, Wang YF
World J Diabetes. 2020;11(10):400–415.
doi:10.4239/wjd.v11.i10.400

Based on a review of the proposed mechanisms of the development of T1DM, the authors provide an overview of oral tolerance therapies for T1DM conducted in both animal models and clinical trials. This key paper also outlines its future perspectives.

As a mainly T cell-mediated autoimmune disease, T1D is characterized by insulin deficiency resulting from the destruction of pancreatic β-cells. The understanding of many aspects of T1DM, such as its epidemiology, pathobiology, pathogenesis, clinical manifestations, and complications, has been greatly promoted by extensive research performed during only the past decades. Unfortunately, these findings have not yet been translated into

an effective treatment. The ideal treatment should efficiently and safely restore the destroyed immune balance in a long-lasting manner, preventing or even topping the destruction of β-cells. As a type of immune hyporesponsiveness to the orally administrated antigen, oral tolerance may be induced by enhancement of regulatory T cells (Tregs) or by anergy/deletion of other T cells, depending on the dosage of orally administrated antigen. Acting as an antigen-specific immunotherapy, oral tolerance therapy for T1DM has been mainly performed in animal models, although some clinical trials have been completed or are ongoing. Some of these actually use orally administered insulin powder as the key antigen.

10.6. Yield of a public health screening of children for islet autoantibodies in Bavaria, Germany

Ziegler A, Kick K, Bonifacio E, Haupt F, Hippich M, Dunstheimer D, Lang M, Laub O, Warncke K, Lange K, Assfalg R, Jolink M, Winkler C, Achenbach P, Fr1da Study Group
JAMA. 2020;323(4):339–351.
doi:10.1001/jama.2019.21565

This study aimed to determine the prevalence of pre-symptomatic T1D in young children participating in a public health screening program for islet autoantibodies and the risk for progression to clinical T1D in children carrying multiple autoantibodies. On screening, 0.03% were found to have clinical T1D. Of the 0.31% who were found to have pre-symptomatic T1D, 24.9% developed clinical T1D during the 3-year follow-up.

Public health screening for T1D in its pre-symptomatic stages may reduce disease severity and burden on a population level. In this study, screening for islet autoantibodies was offered to children aged 1.75 to 5.99 years in Bavaria, Germany, between 2015 and 2019 by primary care pediatricians during routine well-baby visits. Children with multiple islet autoantibodies were invited to participate in a program of diabetes education, metabolic staging, assessment of psychological stress associated with the diagnosis, and prospective follow-up for progression to clinical T1D. The primary outcome was pre-symptomatic T1D, defined as 2 or more islet autoantibodies, with categorization into Stage 1 (normoglycemia), Stage 2 (dysglycemia), or Stage 3 (clinical T1D).

In total, 90,632 children were screened (median [interquartile range {IQR}] age, 3.1 [2.1–4.2] years). Of these, 280 (0.31%) had pre-symptomatic T1D, including 196 (0.22%) with Stage 1, 17 (0.02%) with Stage 2, and 26 (0.03%) with Stage 3 (and 41 were not staged). After a median 2.4 years follow-up, another 36 children developed Stage 3 T1D. The 3-year cumulative risk for Stage 3 T1D among the 280 children with pre-symptomatic T1D was 24.9% ([95% CI, 18.5%–30.7%]; 54 cases; annualized rate, 9.0%). Only two children developed diabetic ketoacidosis. Median (IQR) psychological stress scores were modestly higher at the time of staging in mothers of children with pre-symptomatic T1D (3 [1-7]) compared with mothers of children without islet autoantibodies (2 [1-4]) ($P=0.002$), but declined after 12 months of follow-up (2 [0-4]) ($P<0.001$).

This study shows both the potential and benefits of early detection of presymptomatic islet cell autoimmunity as well as the potential risks and drawbacks as shown by severe distress in the families once the diagnosis of asymptomatic albeit proven had been made early.

10.7. Circulating metabolites in progression to islet autoimmunity and type 1 diabetes

Lamichhane S, Kemppainen E, Trošt K, Siljander H, Hyöty H, Ilonen J, Toppari J, Veijola R, Hyötyläinen T, Knip M, Orešič M
Diabetologia. 2019;62(12):2287–2297.
doi:10.1007/s00125-019-04980-0

This study identified different circulatory metabolite profiles in children who subsequently progress to T1D compared to children who progress to islet autoimmunity but not T1D, and antibody-negative control children.

In addition to altered T cell immunity and autoantibody appearance, metabolic dysregulation may precede the onset of T1D. However, these metabolic disturbances and their specific role in disease initiation remain poorly

understood. In this study, polar metabolites were measured using two-dimensional gas chromatography (GC) high-speed time of flight mass spectrometry (MS) in 415 longitudinal plasma samples in a cohort of children. Three study groups were compared: children who progressed to T1D; children who seroconverted to one islet autoantibody but not to T1D; and an antibody-negative control group.

In early infancy, progression to T1D was associated with lower amino acids, sugar derivatives and fatty acid metabolites, including catabolites of microbial origin, compared to control children. Methionine remained persistently upregulated in those who progressed to T1D. The appearance of islet autoantibodies was associated with decreased glutamic and aspartic acids.

These findings suggest that children who later progress to T1D already have a unique metabolic profile, which is, altered again and even more so with the appearance of islet autoantibodies. These findings may add to the tools that are available for early prediction of the T1D, such as the presence of autoantibodies and HLA typing.

10.8. Absence of islet autoantibodies and modestly raised glucose values at diabetes diagnosis should Lead to testing for MODY: lessons from a 5-year pediatric Swedish National Cohort study

Carlsson A, Shepherd M, Ellard S, Weedon M, Lernmark A, Forsander G, Colclough K, Brahimi Q, Valtonen-Andre C, Ivarsson SA, Elding Larsson H, Samuelsson U, Örtqvist E, Groop L, Ludvigsson J, Marcus C, Hattersley AT

Diabetes Care. 2020; 43:82–89.

doi:10.2337/dc19-0747.

It is often difficult to identify maturity-onset diabetes of the young (MODY) in pediatric patients close to diabetes onset. Hence, misdiagnosis and unnecessary insulin treatment are still common.

This study reports the discriminatory clinical features at diabetes onset in Swedish pediatric patients (age 1–18 years) with glucokinase (GCK), hepatocyte nuclear factor-1A (HNF1A), and HNF4A forms of MODY. Children ($n = 3{,}933$) diagnosed with diabetes between May 2005 to December 2010, were identified from the national consecutive prospective cohort, Better Diabetes Diagnosis. Clinical data, islet autoantibodies (GAD insulinoma antigen-2, zinc transporter 8, and insulin autoantibodies), HLA type, and C-peptide were collected at diagnosis. GCK, HNF1A, and HNF4A genes were sequenced through either routine clinical or research testing.

The minimal prevalence of MODY was 1.2%. Discriminatory factors for MODY at diagnosis included negativity for four islet autoantibodies (100% in MODY vs. 11% in non-MODY), lower HbA1c (7.0% vs. 10.7% [53 vs. 93 mmol/mol]), lower plasma glucose (11.7 vs. 26.7 mmol/l), more likely parental diabetes (63% vs. 12%), and less likely diabetic ketoacidosis (0% vs. 15%). Sequencing of the 303 autoantibody-negative patients identified 46 patients with MODY (detection rate 15%). Limiting testing to the 73 patients with islet autoantibody-negativity and HbA1c $< 7.5\%$ (58 mmol/mol) at diagnosis identified 36/46 (78%) patients with MODY (detection rate 49%). On follow-up, the 46 patients with MODY had excellent glycemic control (mean HbA1c 6.4%; 47 mmol/mol) with 42/46 (91%) not on insulin treatment.

At diagnosis of diabetes, absence of all islet autoantibodies and modest hyperglycemia should indicate DNA sequencing for MODY. Testing all 12% patients who are negative for four islet autoantibodies may be a more effective strategy for not missing MODY. Patients with MODY have excellent long-term glycemic control even without insulin and therefore it mandatory to identify these patients in all countries.

10.9. Serum 25-hydroxyvitamin D concentration in childhood and risk of islet autoimmunity and type 1 diabetes: the TRIGR nested case-control ancillary study

Miettinen ME, Niinistö S, Erlund I, Cuthbertson D, Nucci AM, Honkanen J, Vaarala O, Hyöty H, Krischer JP, Knip M, Virtanen SM; TRIGR Investigators

Diabetologia. 2020;63(4):780–787.

doi:10.1007/s00125-019-05077-4.

This multicenter multinational study, in children with high genetic risk of T1D, identified an association between lower serum 25-hydroxyvitamin D (25OHD) concentrations and subsequent appearance of islet autoimmunity or T1D.
The Trial to Reduce IDDM in the Genetically at Risk (TRIGR) ancillary study (Divia) included from children in 15 countries. Case children ($n=244$) were defined as those positive for 2+ of 4 autoantibodies. For each case, two control children were matched for country and date of birth ($n=488$). Serum 25OHD levels were measured repeatedly in infancy and childhood and were related to age at the first seroconversion and calendar age. Analyses were adjusted for month of sample collection, human leucocyte antigen genotype, maternal T1D and sex.

Serum 25OHD concentrations were lower 18 months before first seroconversion in children with islet autoimmunity, than at the equivalent ages in controls (mean 57.7 vs 64.8 nmol/l, $P=0.007$). Of the case children, 144 developed T1D – their serum 25OHD concentrations were also lower 18 months before first seroconversion (58.0 vs 65.0 nmol/l, $P=0.018$) and at calendar age 12 months (70.1 vs 75.9 nmol/l, $P=0.031$) than in controls. These results suggest that indeed early postnatal vitamin D levels may be related to and even confer protection against the development of T1D.

Whether or not this may be of clinical relevance or could even be used as a preventive measure against the disease is still very uncertain indeed. In such observational studies, there are dozens of diseases associated with lower Vitamin D levels, and very few are confirmed by randomized controlled trials. There may be many potential confounding factors, including geography (latitude), lifestyle (sun exposure), BMI, supplement use etc.

10.10. Risk of psychiatric disorders and suicide attempts in emerging adults with diabetes

Robinson ME, Simard M, Larocque I, Shah J, Nakhla M, Rahme E
Diabetes Care. 2020;43(2):484–486.
doi:10.2337/dc19-1487.

This retrospective study in Quebec, Canada included 3,544 adolescents and young adults with diabetes and 1 388 397 without diabetes. It concludes that between the ages of 15 and 25 years, the risks of psychiatric disorders and suicide attempts were substantially higher in adolescents and young adults with diabetes than those without diabetes.

The authors linked routine databases of adolescents (age 15 years) and without prior psychiatric disorders between 1997 and 2015, and followed to age 25 years. Individuals with diabetes were more likely to suffer from a mood disorder (diagnosed in the emergency department or hospital) (adjusted hazard ratio 1.33 [95% CI 1.19–1.50]), attempted suicide (3.25 [1.79–5.88]), visit a psychiatrist (1.82 [1.67–1.98]), and experience any type of psychiatric disorder (1.29 [1.21–1.37]) compared with those without diabetes.

It remains important to understand how this risk is conferred to subjects with diabetes and whether or not the burden of disease contributes substantially to the development of psychiatric illness. Meanwhile, clinicians caring for you adults and adolescents with diabetes should be very aware of the risk of psychiatric disease in the patients they care for. A multidisciplinary approach to care for individuals with diabetes and psychiatric issues should be developed.

10.11. Use of factory-calibrated real-time continuous glucose monitoring improves time in target and HbA1c in a multiethnic cohort of adolescents and young adults with type 1 diabetes: the MILLENNIALS study

Thabit H, Prabhu JN, Mubita W, Fullwood C, Azmi S, Urwin A, Doughty I, Leelarathna L
Diabetes Care. 2020;43(10):2537–2543.
doi:10.2337/dc20-0736.

This paper describes a randomized crossover trial in young people with T1D (16–24 years old) comparing two 8-week study periods: the factory-calibrated Dexcom G6 CGM system versus routine self-monitoring of blood glucose (SMBG). CGM improved time within glucose target and reduced HbA1c by 0.76%.

The primary outcome, time within glucose range 70-180 mg/dL, was higher during CGM than self-monitoring (mean 35.7% vs. 24.6%; mean difference 11.1%). CGM use reduced mean sensor glucose (219.7 mg/dl vs. 251.9 mg/dl; mean difference −32.2 mg/dl) and time above range (61.7% vs. 73.6%; mean difference 11.9%). HbA1c levels were reduced by 0.76%; $P < 0.001$). Times spent below range (<70 mg/dl and <54 mg/dl) were low and similar during both study periods. Sensor wear was 84% during the CGM period. In is concluded that CGM use in young people with T1D does indeed improve time in glucose target and also HbA1c levels as compared to those with SMBG.

International T1D registries have shown that HbA1c levels in patients with T1D are highest in young adult. Improving their glycemic control remains a challenge. These findings propose the Dexcom G6 CGM system to potentially improve glycemic control in young adults with diabetes.

10.12. Cognitive function following diabetic ketoacidosis in children with new-onset or previously diagnosed type 1 diabetes

Ghetti S, Kuppermann N, Rewers A, Myers SR, Schunk JE, Stoner MJ, Garro A, Quayle KS, Brown KM, Trainor JL, Tzimenatos L, De Piero AD, McManemy JK, Nigrovic LE, Kwok MY, Perry CS 3rd, Olsen CS, Casper TC, Glaser NS; Pediatric emergency care applied research network (PECARN) DKA FLUID study group

Diabetes Care. 2020;43(11):2768–2775.
doi:10.2337/dc20-0187.

In this observational analysis of a patient cohort, a single diabetic ketoacidosis (DKA) episode was associated with lower cognition and memory both in children with newly diagnosed T1D and in children with known T1D.

Children ($N=758$) were 6-18 years old, taking part in a multisite randomized trial evaluating different DKA intravenous fluid protocols. Of these, 392 children had DKA at T1D onset. DKA was moderate/severe in 430 children and mild in 328 children. Neurocognitive assessment was carried out 2–6 months after the DKA episode. The controls were 376 children with T1D, who had no previous DKA exposure. Models were adjusted for hypoglycemia, diabetes duration, and socioeconomic status.

Across all patients, moderate/severe DKA was associated with lower intelligence quotient (IQ) ($\beta=-0.12$, $P < 0.001$), item-color recall ($\beta=-0.08$, $P=0.010$), and forward digit span ($\beta=-0.06$, $P=0.04$). Among newly diagnosed patients, moderate/severe DKA was associated with lower item-color recall ($\beta=-0.08$, $P=0.04$). Among previously diagnosed patients, repeated DKA exposure and higher HbA1c were independently associated with lower IQ ($\beta=-0.10$ and $\beta=-0.09$, respectively, $P < 0.01$) and higher HbA1c was associated with lower item-color recall ($\beta=-0.10$, $P=0.007$).

The surprising findings indicate that even one single DKA episode is associated with lower IQ and memory soon after T1D onset. Similar reductions were detectable in children with known diabetes, suggesting that DKA and putatively other diabetes related disturbances may influence cognition and that these may be exacerbated in children with chronic hyperglycemia. A limitation of the study design is that cognition and memory were not assessed prior to DKA – it is possible that reductions were pre-existing and potentially contributed to their higher risk of DKA and high HbA1c.

10.13. Type 1 diabetes can present before the age of 6 months and is characterized by autoimmunity and rapid loss of beta cells

Johnson MB, Patel KA, De Franco E, Hagopian W, Killian M, McDonald TJ, Tree TIM, Domingo-Vila C, Hudson M, Hammersley S, Dobbs R; EXE-T1D Consortium, Ellard S, Flanagan SE, Hattersley AT, Oram RA

Diabetologia. 2020;63(12):2605–2615.
doi:10.1007/s00125-020-05276-4.

Diabetes diagnosed at <6 months of age is often of monogenic origin. However, 10-15% of affected infants do not have a pathogenic variant in one of the 26 known neonatal diabetes genes. In this study, 166 infants diagnosed at <6 months of age without such pathogenic variants showed all the the classic features of T1D. They were compared to infants with monogenic neonatal diabetes ($n=164$) and children with T1D diagnosed at age 6–24 months ($n=152$). T1D genetic risk score (T1D-GRS), islet autoantibodies, C-peptide and clinical features were assessed and recorded.

An excess of infants with high T1D-GRS was found: 38% (63/166) had a T1D-GRS >95th centile of healthy individuals (5% would be expected by chance). Infants with a high T1D-GRS had a similar rate of autoantibody positivity to infants with T1D diagnosed at age 6–24 months (41% vs 58%, $P=0.2$), and had markedly reduced C-peptide levels (median <3 pmol/l within 1 year of diagnosis), indicating rapid loss of insulin secretion. These infants also had reduced birthweights (median z score −0.89), especially among infants diagnosed with T1D at <3 months (median z score −1.98).

Comprehensive genetic testing for all neonatal diabetes genes remains essential for all infants diagnosed with diabetes at <6 months of age. However, these findings provide strong evidence that T1D can present even before age 6 months. These infants show the classic features of T1D: high polygenic genetic risk, autoimmunity and rapid beta cell loss. Furthermore, the association with reduced birthweight raises the possibility of reduced insulin secretion already in utero. This population may represent a subgroup of patients with a high potential for early comorbidities and related disease.

10.14. Targeted pharmacological therapy restores β-cell function for diabetes remission

Sachs S, Bastidas-Ponce A, Tritschler S, Bakhti M, Böttcher A, Sánchez-Garrido MA, Tarquis-Medina M, Kleinert M, Fischer K, Jall S, Harger A, Bader E, Roscioni S, Ussar S, Feuchtinger A, Yesildag B, Neelakandhan A, Jensen CB, Cornu M, Yang B, Finan B, Di Marchi RD, Tschöp MH, Theis FJ, Hofmann SM, Müller TD, Lickert H
Nature Metabolism. 2020;2(2):192–209.
doi:10.1038/s42255-020-0171-3.

This experimental mouse study examined whether or not dedifferentiated β cells could be reversed or targeted by pharmacological intervention for diabetes remission. They identified evidence for β-cell dedifferentiation and dysfunction which could be reversed by single and combined pharmacological approaches.

Dedifferentiation is a process whereby mature differentiated cells transition back to a less mature state to allow potential for regeneration, but with the loss of some cell functions. Dedifferentiation of insulin-secreting β cells in pancreatic islets has been proposed to be a major mechanism of β-cell dysfunction. In this study, mice with streptozotocin-induced diabetes were used as a model to study β-cell dedifferentiation. Single-cell RNA sequencing (scRNA-seq) of islet cells identified markers and pathways associated with β-cell dedifferentiation and dysfunction.

Administration of insulin triggered insulin receptor pathway activation in β cells and restored cell maturation and function, leading to islet differentiation and diabetes remission. Additionally, β-cell selective delivery of oestrogen combined with Glucagon-like peptide-1 (GLP-1-oestrogen) decreased insulin requirements by 60%, triggered oestrogen-specific activation of the endoplasmic-reticulum-associated protein degradation system, and further increased β-cell survival and even regeneration. The GLP-1-oestrogen combination also protected human β cells against cytokine-induced dysfunction.

This study not only describes mechanisms of β-cell dedifferentiation and regeneration, but also reveals potential pharmacological entry points to target dedifferentiated β cells for diabetes remission and cure.

11. Obesity and Weight Regulation

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Preface

In analogy to previous years, in this year's chapter we can present only ~1% of the acquired publications according to our search criteria in Pubmed. The last year has again been extremely exciting for the field of obesity and weight regulation and it was a significant step into the future in terms of scientific output. Of course, one of the well-known phenomena of the past year has been a marked increase in child and adolescent body weights and the prevalence of obesity, as well as changes in lifestyle behaviors, during the Coronavirus pandemic lockdown (1, 2, 3). On the other side, it has been observed that the BMI or body fat mass of patients contributes significantly to the severity of the course of COVID-19 disease. In this context, new findings on the pathophysiological basis of this observation have been published (see paper XX).

The Yearbook 2021 chapter on obesity and weight regulation comprises further exciting articles covering a broad research area. Particular mention should be made of the new insights into the structure and function of the satiety receptor, MC4R, the high prevalence of functionally relevant variants, and the possible effective pharmacological therapy for affected patients.

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The COVID-19 Pandemic and Obesity

11.1. Characteristics of hospitalized children with SARS-CoV-2 in the New York city metropolitan area

Verma S, Lumba R, Dapul HM, Gold-von Simson G, Phoon CK, Lighter JL, Farkas JS, Vinci A, Noor A, Raabe VN, Rhee D, Rigaud M, Mally PV, Randis TM, Dreyer B, Ratner AJ, Manno CS, Chopra A

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Hosp Pediatr. 2021 Jan;11(1):71–78.

doi: 10.1542/hpeds.2020-001917.

https://pubmed.ncbi.nlm.nih.gov/33033078/

In a cohort of 82 children (0–21 years) who were hospitalised for severe SARS-CoV-2 associated respiratory illness, obesity was the strongest risk factor for the outcome of critical care. Already during the first COVID-19 wave, it was shown that obesity is a major risk factor for severe COVID-19 infection in adults and caused the age at disease manifestation to shift towards younger ages (1). Recent findings give insights into the underlying mechanism that links obesity to severe COVID-19 infection:

1. Obesity leads to respiratory dysfunction, characterised by increased airway resistance, reduced gas exchange and lung volume and muscle strength, and decreased diaphragmatic excursions (2,3). Transdifferentiation of pulmonary lipofibroblasts into myofibroblasts may contribute to the development of pulmonary fibrosis and thus aggravate the severity of COVID-19 associated lung disease (4).
2. Insulin resistance and chronic subclinical inflammation associated with obesity lead to an increased vulnerability to infection-related lung failure (2).
3. The expression of angiotensin converting enzyme 2 (the functional receptor of SARS-CoV-2) is upregulated in adipocytes from patients with obesity, making adipose tissue a potential target organ and viral reservoir (4).
4. Leptin links metabolism to the immune response by signaling via the Jak/STATand Akt pathways. Increased circulating leptin levels seen in obesity lead to compromised systemic immune response. Leptin is also an important mediator of pulmonary immunity and elevated leptin levels worsen the pulmonary defense against infection (5). Leptin may also increase systemic inflammation via paracrine effects on T cells (6). In a mouse model, administration of antileptin antibody decreased pro-inflammatory events and improved lung pathology and survival (7).

Based on clinical observations and pathophysiological findings, specific recommendations for prevention and care of patients with obesity and COVID-19 disease have recently been published (8,9,10,11).

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New Hope: Increased Diagnostic Yield for Disease Causing MC4R Variants and Pharmacological Treatment Options

11.2. Structure reveals the activation mechanism of the MC4 receptor to initiate satiation signalling

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Isreali et al. describe the molecular structure of the melanocortin 4 receptor (MC4R) complexed with its effector G protein (Gs alpha) and setmelanotide, a pharmacological agonist of MC4R.

MC4R is a known key element in body weight regulation, connecting response to leptin with inhibition of hunger and food intake. Targeted deletion of *Mc4r* in mice induces weight gain (1), and heterozygous loss-of-function mutations in *MC4R* are frequently found in obese children as well as adults (2, 3). Thus, targeting MC4R pharmacologically is a major focus in the development of weight loss therapies. Setmelanotide is the first US FDA-approved MC4R agonist for treatment of obesity due to genetically based deficiencies of pro-opiomelanocortin (POMC), proprotein subtilisin/kexin type 1 (PCSK1), and leptin receptor LEPR.

The current study contributes important data to our understanding of MC4R signalling. Although a recent publication describes the crystal structure of MC4R bound to an antagonist, and thus provides data about the structure of the inactive receptor (4), the architecture of active MC4R had not been described so far. Using single-particle cryogenic electron microscopy, the authors were able to analyse conformational changes and to display crucial amino acids mediating activation of the receptor. This information may be helpful in modelling of pathogenic MC4R mutations and to identify those patients with MC4R defects that would potentially benefit from setmelanotide treatment.

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11.3. Human MC4R variants affect endocytosis, trafficking and dimerization revealing multiple cellular mechanisms involved in weight regulation

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This study demonstrates that MC4R variants found in humans affect receptor endocytosis, trafficking and dimerization and thus reveal multiple cellular mechanisms involved in weight regulation. The findings contributes to our understanding of the complex mechanisms that lie behind the melanocortin 4 receptor's (MC4R) pivotal role in weight regulation. Stimulation of MC4R, a G-protein coupled receptor, is considered to be one of the key factors that reduces appetite and regulates energy homeostasis and body weight. It was previously shown that the binding of pro-opiomelanocortin (POMC) derived melanocyte-stimulating hormone (MSH) to the MC4R leads to increased production of cyclic AMP (cAMP) (1). We are aware of several G-protein related pathways that can be 'drugged' (2) and meanwhile MC4R is a prime target for anti-obesity drugs (3). Informed by the recently published 3D structure of the MC4R (4) novel therapeutic methods to target MC4R can be more efficiently investigated and developed.

These authors aimed to investigate the cellular functioning and trafficking of MC4R by looking at effects of several naturally occurring MC4R variants when experimentally expressed in HEK293 cells, which do not endogenously express MC4R. 48 rare MC4R variants from previously published large cohorts (minor allele frequency [MAF], $<1\%$) (5-7) and 2 more common variants (MAF 1-2%) that have previously been associated with obesity protection (8-11) were studied.

To quantitatively assess and monitor the intracellular and extracellular interaction/signalization of different MC4R pathways, such as plasma membrane (PM) expression, β- arrestin recruitment, G-α interaction, MAPK (mitogen activated protein kinases)/ERK (extracellular signal-regulated kinases) phosphorylation, homodimerization and early (recycling) or late (degradation) endocytosis, enhanced bystander bioluminescence resonance energy transfer (ebBRET) sensors (12) were applied. The effects of these 50 MC4R variants were functionally categorized as either gain of function (GOF) and/or loss of function (LOF) for each of their respective cellular interaction pathways. Interestingly, some pathways might be able to regulate MC4R activity independent of cAMP production. 19 MC4R variants previously shown to be similar to WT or to have none/or limited effects on cAMP production (>85% of WT cAMP production) (13) are shown here to impair one or more pathways. Based on these results, there are obviously novel ways to target MC4R. As an example, targeting the homodimerization process with an allosteric modulator might be an option similar to several experimental therapies that are currently under investigation for central nervous system disorders (14).

By dissecting mechanisms that regulate MC4R with naturally occurring human variants, this study expands our knowledge of MC4R functionality. We believe that further human as well as transgenic animal and cell model studies are needed to further examine the relevance of these new mechanisms. A recent study (3) (see paper 11.2), has shown the interaction between setmelanotide and several naturally occurring human MC4R variants by using cryogenic electron microscopy and thereby helps to better understand the core functioning of MC4R and to discover future pharmacological treatments.

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11.4. Loss-of-function mutations in the melanocortin 4 receptor in a UK birth cohort

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This paper reports the high prevalence of MC4R loss-of-function (LoF) variants in a normal population and their large impact on longitudinally assessed anthropometric traits from birth to young adult life.

This is the first study to examine the prevalence of all non-synonymous MC4R variants in a large sample (5724 persons) of a representative birth cohort, the Avon Longitudinal Study of Parents and Children (ALSPAC). ALSPAC examined over 75% of all children born in the larger Bristol area between 1990-1992, thus these data provide an accurate estimate of true prevalence in a (mainly white) European population.

Obesity is one of the most important non-communicable diseases and has finally now been acknowledged by EU commission as a disease in its own right (1). In contrast to polygenic obesity, monogenic obesity is much rarer. However, the prevalence reported here, 1 in 337, means that LoF MC4R variants can no longer be termed a rare disease.

The longitudinal characterization of participants in ALSPAC (2), provides a unique possibility to compare the development of anthropometric traits between MC4R LoF variant carriers ($n=17$) to non-LoF variant carriers ($n=5707$). They identify a large and stable influence of LoF MC4R variants on BMI, weight and body fat from 5 years onwards. Interestingly, in contrast to previous studies by the same authors (3, 4), no evidence for reduced systolic blood pressure or increased adult height was found after adjusting for age, sex and BMI.

Rare, non-synonymous wild-type like variant carriers ($n=21$) did not differ from wildtype or synonymous, common variant carriers. However, there is no individual analysis for wild-type like carriers offered and LoF was primarily defined as reduced cAMP generation upon MC4R stimulation in a heterologous cell system expressing the MC4R variant, although other MC4R signalling pathways have been described (5, 6). As for some of the here listed wild-type like variants, debates exist on their possible pathogenetic effects. A more detailed analysis would have been welcomed.

In conclusion, LoF MC4R variants conferring a reproducible risk for increased adiposity seem to be more frequent than previously assumed; hence a generous screening approach should be advocated, especially since some variants seem to be rescuable by synthetic MC4R activators, such as Setmelanotide (7).

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11.5. Obesity treatment effect in danish children and adolescents carrying melanocortin-4 receptor mutations

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This study investigated the influence of MC4R variants on treatment effectiveness in a large cohort undergoing an outpatient treatment program. Carriers of MC4R loss-of-function (LoF) variants showed a lack of improvement in BMI, in contrast to non LoF carriers.

As we learn that MC4R LoF variants are more common than expected (1, 2 also in this Yearbook paper 11.4) their impact gains importance, not only on the natural course of BMI, but also on treatment success.

The validated treatment program of the Children's Obesity Clinic (TCOC) in Denmark shows on average a sustained BMI SDS reduction by 0.2-0.3 (3), which is comparable to other European outpatient programs, such as Obeldicks light (4). Remarkably, in this large study ($n=1209$), of the roughly 80% of patients who could be evaluated after a treatment period of on average 1 year (0.5–4.0 years), the 2.5% carriers of MC4R LoF variants ($n=24$) did not reduce their BMI-SDS – in contrast to non LoF carriers ($n=982$). Other treatment approaches, including Obeldicks, had actually found that wildtype MC4R variant carriers show the same BMI SDS reduction as LoF variant carriers, but are unable to uphold treatment success (5). One possible explanation for these divergent observations may be a more extensive treatment approach in former studies.

One challenge of this study was the definition LoF in MC4R variants. While some variants show consistently reduced ability to generate cAMP upon MC4R stimulation in a heterologous cell system expressing the MC4R variant, for others, results vary both on cAMP generation and other measures such as activation of extracellular signal-regulated kinase (ERK) 1/2 or receptor cell surface expression. Hence variants considered here to be functionally relevant have been viewed in other studies as wild-type like (1). Nonetheless, this study shows that knowledge on the genetic background can give both treatment centres and families more realistic expectation about treatment outcomes and may also pave the way for patient-tailored treatment approaches.

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11.6. Child neurobiology impacts success in family-based behavioral treatment for children with obesity

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Schur *et al*. addressed the question, whether responsiveness to visual food cues in functional magnetic resonance imaging (fMRI) is related to the treatment success of a 6-month Family-based Behavioral Treatment (FBT) program in ($n = 37$) obese children.

FBT is one of the few treatment options for children and adolescents with common obesity [1,2]. However, little is known about the factors that contribute to variability in treatment responses [3]. Whereas brain activation in appetitive processing regions has been shown to be altered in obese children and adults [4,5] leading to higher food intake and weight gain [6,7] and to impair the diet and bariatric surgery treatment success in adults [8,9], its influence on treatment success in children has not been addressed so far. The authors performed fMRI scans prior to a 6-month FBT in 9-11-year-old obese children to analyze brain activation in appetite-processing regions in response to (viewing) low- and high-calorie food images before and after a standardized meal.

Pre-meal brain activation was not associated with short- and long-term changes (6 month and 1 year) in BMI *z*-score, whereas the pre- to post-meal reduction in brain activity by high-calorie visual food cues (but not low-calorie-food cues) was related to child BMI *z*-score decline and greater improvements in Healthy Eating-Index scores. Furthermore, they identified clusters of activation associated with BMI *z*-score change: greater pre-meal activation in the ventromedial prefrontal cortex and in the occipital pole correlated with greater BMI *z*-score reduction. In contrast, greater activation by high-calorie food cues in the superior portion of the precuneus, the left lateral occipital cortex and the right lingual gyrus was associated with less BMI *z*-score reduction.

These data focused only on pre-treatment brain activity and causality on weight change cannot be assumed. However, this extensive dataset of functional neuroimaging provides an important contribution to a better understanding of predictors and mechanisms during behavioral treatment and possible reasons for unsuccessful treatment outcomes. These results are important for our understanding of weight regulation. They relieve the patients and support pharmacological therapy approaches that influence the postprandial satiety response.

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11.7. Genetic mapping of etiologic brain cell types for obesity

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Timshel et al. describe a novel strategy for integrating single-cell RNA-sequencing (scRNA-seq) data with GWAS data on BMI associated genetic variants, and thereby identify 26 exclusively neuronal cell types that are significantly enriched for BMI heritability.

The authors developed two scRNA-seq computational toolkits (CELLEX and CELLECT) for genetic identification of likely etiologic cell types and applied them to scRNA-seq data from 727 mouse cell types (380 cell types representing adult mouse organs especially the nervous system and 347 cell types from adult mouse hypothalamus [1,2]). Combining these data with publicly available BMI GWAS summary statistics from > 457 000 individuals in the UK Biobank study [3] they identified 26 BMI GWAS-enriched neuronal cell types mapping to 8 brain regions, which share transcriptional signatures related to obesity. Interestingly, some of the enriched cell types localize to nuclei processing sensory stimuli and directing actions related to feeding behavior and to areas, which are important for learning and memory.

Furthermore, they identified 4 enriched cell types in the hypothalamus, expressing *Sf1* (linked to disrupted leptin-signaling and hyperglycemia [4;5]), *Cckbr* and *Bdnf* (related to *NTRK*-monogenic obesity in humans and to normal energy homeostasis in mice [6]). There was no enrichment in neurons expressing the *Pomc* gene, but nominal enrichment in 4/5 Pomc+ cell populations. In line with previous findings [7,8], no enrichment of genetic variants associated with BMI in non-neuronal cell types was found.

In consideration of the selected scRNA-seq dataset from late postnatal, adult and predominantly wild-type mice, and the limitation to only detect enriched gene expression, these in silico findings provide an important contribution to the understanding of BMI heritability, showing that susceptibility to obesity is enriched in neuronal cell types regulating integration of sensory stimuli, learning and memory, thereby highlighting the need for mechanistic follow-up studies.

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New Treatment Result: Should Bariatric Surgery be done Earlier in Life?

11.8. Outcomes of bariatric surgery in older versus younger adolescents

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This sub-analysis of Teen-LABS study shows very similar improvements over 5 years after bariatric surgery between younger and older adolescents with severe obesity in %BMI change (−22.2% vs. −24.6%, respectively), resolution of hypertension and dyslipidaemia, and quality of life. Importantly, there was a trend towards lower likelihood of nutritional deficiencies in younger adolescents, possibly due to greater control within the home environment. A small difference in remission of T2DM in younger and older adolescents was also observed, but the overall rate of T2DM remission is comparable to published data.

Teen-LABS is a multicentre prospective observational US study, which enrolled 242 adolescents who underwent bariatric surgery (MBS) between 2007 and 2012. Here, they stratified the cohort into 2 age groups: 13–15 years and 16–19 years at the time of surgery. Participants were regularly assessed up to 5 years after MBS. The main outcomes were changes in hypertension, dyslipidaemia, type 2 diabetes, micronutrient status and quality of life.

The prevalence of severe childhood and adolescent obesity and its comorbidities has led to a crisis in treatment response. Although behavioural interventions can be successful for some individuals, the overall results are discouraging when considered as a solution to the large number of obese patients (1-5). In contrast, the effectiveness of bariatric surgery in terms of weight reduction and improved quality of life in adolescents has been clearly demonstrated in clinical trials. Comparative outcome data of bariatric procedures between younger and older adolescent patients could provide important information for choosing the best possible timing of surgery (6).

Strengths of the present work include a large and well-characterised cohort and extensive follow-up. Limitations arise from the non-randomised design, and especially the limited generalisability due to the high proportion of white, female patients. Although long-term data of at least comparable quality over follow-up periods of more than five years are still urgently needed, these Teen-LABS data suggest that in younger adolescents with severe obesity, surgical therapy - as an ultima ratio (last resort) option - should not be rejected solely on the basis of the patient's age.

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Review and Recommendations on ROHHAD(NET) Syndrome

11.9. ROHHAD(NET) syndrome: Systematic review of the clinical timeline and recommendations for diagnosis and prognosis

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Based on a structured summary of a case series of 43 well-defined patients, these authors propose for regular screening and preventive examinations in ROHHAD patients and list the therapeutic options. Although these recommendations are not strictly evidence-based, they are a very valuable support for paediatric endocrinologists and other clinicians who provide care for ROHHAD patients.

ROHHAD(NET) syndrome is a rare obesity syndrome with a high mortality due to cardiovascular arrest. The symptom complex of ROHHAD(NET) syndrome was first described in 2007 (1). Main features include: hyperphagia and obesity, hypoventilation, disorders of temperature and water balance, and other disorders of hypothalamic function, including endocrine deficits, disorders of psychomotor development and tumours of the neural crest. Its cause is unknown, and no aetiology-based therapy is available (2, 3, 4).

Despite the life-threatening complications of ROHHAD(NET), there existed neither clear recommendations for diagnosis nor for the regular screening and preventive examinations. Here, Harvengt et al. meet this challenge and supplement their own case report with a systematic literature review. Two points are noteworthy. First, the authors use the narrow, original definition of ROHHAD(NET) (1) to identify cases and case series of clearly affected patients. Secondly, they use the PRISMA individual patient guideline methodology to analyse chronological clinical data and to identify a characteristic time course of the development of the syndrome. Their analysis of 43 ROHHAD patients shows a median age at onset of excessive weight gain of 3.1 years, followed by a diagnosis of autonomic dysregulation (median age 4.95 years) and central hypoventilation (median age 5.3 years). Overall, 83% of the analysed patients were affected by hypoventilation within the first five years after onset of rapid weight gain. The cumulative data on the occurrence of neural crest tumours are particularly interesting. Overall, 56% received a tumour diagnosis, the majority of which were ganglioneuromas, but also neuroblastomas. In 70% of affected patients, the tumour occurred within 2 years after the onset of weight gain, so there is possibly an age dependency of the tumour risk.

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Mechanism of the Year

11.10. Intercellular mitochondria transfer to macrophages regulates white adipose tissue homeostasis and is impaired in obesity

Brestoff JR, Wilen CB, Moley JR, Li Y, Zou W, Malvin NP, Rowen MN, Saunders BT, Ma H, Mack MR, Hykes BL, Balce DR, Orvedahl A, Williams JW, Rohatgi N, Wang X, McAllaster MR, Handley SA, Kim BS, Doench JG, Zinselmeyer BH, Diamond MS, Virgin HW, Gelman AE, Teitelbaum SL
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Brestoff et al. show, for the first time, that mitochondria are transferred from adipocytes to macrophages and that this event has an impact on systemic metabolic homeostasis.

Within the last years, it has been demonstrated that cells are able to communicate with each other by the exchange of organelles, such as lysosomes and mitochondria, and this gained considerable interest in different fields of medicine. Intercellular exchange of mitochondria can take place by several mechanisms, including tunnelling microtubes, extracellular vesicles, or cell fusion (1). Moreover, mitochondria appear to be transported systemically as well, as they are frequently found in the circulation (2). Whether or not mitochondrial transfer takes place in the adipose tissue had not been investigated so far. The authors used a transplantation mouse model, in which mitochondria in the adipose tissue were specifically labelled. They showed a directed transfer of mitochondria from adipocytes to macrophages, which was dependent on the presence of heparan sulphate on the surface of macrophages. This transport was diminished in high-fat-diet (HDF) obesity, and conversely, inhibition of mitochondrial transfer resulted in metabolic dysfunction.

Overall, these findings clearly demonstrates that adipocyte-macrophage exchange is relevant for obesity development. Still, it would have been desirable to unravel the mechanism of mitochondrial exchange in more detail. As the fluorescent images implicate, it seems that mitochondria are taken up into macrophages by endocytosis, which is the major function of these cells. It would have been interesting to know the mechanisms that prevent macrophages from degrading mitochondria, and instead utilise them. Further research might address whether intercellular organelle transport takes place in humans as well. The presence of mitochondria in the bloodstream (2) suggests that this might be true, and opens up new avenues for the study of intercellular or inter-organ communication in humans.

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Criticism of the Previous BMI Metrics for use in Severely Obese Children

11.11. Evaluation of BMI metrics to assess change in adiposity in children with overweight and moderate and severe obesity

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These authors analysed a cohort of $n = 399$ overweight and obese children (age 2–12 years), who underwent a 1-year lifestyle intervention, to test whether possible alternatives to the BMI z-score (%BMIp95, %BMIp50, BMImz) might correlate better with changes in % total body fat (%BF) as assessed by bioelectrical impendence (BIA).

The findings showed that BMI z-score (calculated according to CDC growth charts) was the least suitable metric to indicate changes in %BF ($R^2 = 0.38$). The association between delta BMI *z*-score and detla %BF was also limited by significant variation across age and weight categories. In contrast, other parameters: detla BMI, delta %BMIp95 and detla %BMIp50 performed better ($R^2 = 0.53$, $R^2 = 0.53$, $R^2 = 0.54$, respectively).

This work adds to previous evidence that shows the BMI *z*-score is a poor indicator to evaluate obesity interventions in children and adolescents (1,2). The calculation of BMI *z*-scores (BMIz) includes a power transformation step to convert the strong positive skew (right sided tail) into a normal distribution – this causes a close bunching of high BMI values and hence under-emphasises even relatively large changes in BMI in overweight and obese individuals. Further work is also needed to quantify changes in body composition using more accurate methods than BIA, and to correlates those changes with alternative weight and height-dependent metrics. Furthermore, the correlations between these parameters and changes in metabolic health parameters needs to be investigated. Regardless of the many remaining open-ended questions, this work shows that the use

of %BMIp95 and of %BMIp50 are more appropriate than BMI z-score in the assessment of an intervention in obese children and adolescents.

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11.12. A longitudinal comparison of alternatives to body mass index *Z*-Scores for children with very high body mass indexes

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The authors analysed BMI data from 10.8 million children and adolescents from the PEDSnet database (USA), collected longitudinally between 1999-2019, to examine the tracking of various BMI metrics (BMIz, BMImz, BMIz extended, %BMIp95, %BMIp50,%BMIp50 adjusted| calculated according to CDC growth charts). The strength of this evaluation is that the tracking of BMI metrics was examined in an enormous cohort. The most important finding was that the tracking of BMIz was lower than for all other BMI metrics in children with obesity as well as for children with extreme obesity (BMI $\geq$ 140% of the 95th percentile at baseline).

This study clearly shows the influence of the applied BMI metrics on the longitudinal BMI course or on the effect of an intervention on BMI in obese children and adolescents. These results are consistent with other literature, in which the use of BMIz in obese children and adolescents in the assessment of the degree of obesity and the effects of an intervention has already been critically discussed (1, 2, 3, 4, 5). Clinicians and researchers should familiarize themselves with the calculation and interpretation of alternatives to BMIz (e.g. %BMIp95, %BMIp50), as they appear to be more suitable for assessing BMI progression (with or without intervention) in obese children and adolescents.

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New Insight into Preconceptional and Prenatal Programming

11.13. Associations between exposure to gestational diabetes mellitus in utero and daily energy intake, brain responses to food cues, and adiposity in children

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Luo *et al*. performed a retrospective cohort study of 159 children (age 7–11 years), including undertaking a food cue task while in the MRI scanner, to investigate whether brain reward systems are altered by in utero exposure to gestational diabetes mellitus (GDM) or maternal obesity, and whether these changes lead to increased food intake and obesity. Exposure to GDM in utero was independently associated with enhanced orbital frontal cortex food cue reactivity, increased energy intake, and increased waist to hip ratio, thereby contributing to the risk for development of obesity.

Notably, GDM exposure before 26 weeks gestation was associated with these offspring outcomes, suggesting that the early timing of GDM exposure may have a crucial effect on fetal programming and the development of the child's brain reward system. Interestingly, maternal prepregnancy BMI was not associated with offspring daily energy intake or food cue reactivity within brain reward areas.

In utero exposure to GDM or maternal obesity contributes to higher risks for childhood obesity, insulin resistance and metabolic syndrome (1, 2). However, little is known regarding the underlying neural mechanisms. Accumulating evidence suggests that altered hypothalamic structure and function as well as altered responses to palatable food or food cues potentiated by the brain's reward system are implicated in the pathogenesis of obesity. Animal and human studies show that in utero exposure to maternal obesity or GDM disrupts the development of the hypothalamus, leading offspring to overeating and obesity (3, 4). To the best of our knowledge, this is the first imaging study that investigated the possible effects of in utero GDM exposure to brain reward regions in the offspring. Future studies including a larger sample with/or a longitudinal follow-up are necessary to assess these findings. In addition, potential sex differences in the effects of GDM exposure on feeding behavior on offspring should be considered. The better understanding of biological pathways linking in utero exposures, such as GDM, to adverse health outcomes later in life will help us design future targets for early identification and prevention.

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11.14. Lifestyle intervention in pregnant women with obesity impacts cord blood DNA methylation, which associates with body composition in the offspring

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These authors performed a randomized trial of obese pregnant women ($n = 425$) to investigate the effects of a pregnancy lifestyle intervention on epigenetic variations in umbilical cord blood, on neonatal body composition (DXA) and on offspring BMI in later life (up to 3 years). Offspring of mothers who received lifestyle intervention during pregnancy had a higher lean mass (+127g, 95%CI: -5-258g) and a higher abdominal lean mass (+59g, 95%CI: 11-108g) compared to offspring of mothers who received standard care.

Furthermore, in umbilical cord blood DNA, 25 sites were differentially methylated according to the pregnancy intervention. 80% of these sites were more methylated in the intervention group and were also positively correlated with neonatal lean mass.

This study stands out from previous studies that have shown differences in cord blood methylation level in dependence on maternal pre-pregnancy overweight/obesity or excessive gestational weight gain (1, 2). As well as the randomized trial design, a notable feature is that newborn body composition was measured by DXA scan within the first 48 hours after birth. This made it possible to study an intervention effect on neonatal body composition, specifically on the lean mass. In order to assess the longer-term effects of the pregnancy lifestyle intervention on offspring body composition, it would have been advantageous to also apply DXA and not only BMI measures at age 3 years. Overall, these findings provide initial evidence that lifestyle interventions during pregnancy may positively influence the body composition of the offspring, although the long-term effects have yet to be demonstrated.

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11.15. Sex-specific programming effects of parental obesity in pre-implantation embryonic development

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These authors investigated the effect of maternal and paternal obesity on gene expression level in the mouse blastocyst. Firstly, they show that female and male offspring were differentially sensitive to an obesogenic maternal environment in the early phase of preimplantation. Male blastocysts from obese mothers showed more severe transcriptome changes than the female blastocysts. It is possible that these changes result in programming effects that persist into postnatal life. Secondly, paternal obesity also resulted in significant sex-specific transcriptome changes of blastocysts. The 49 differentially abundant transcripts (DATs) found in male blastocysts of obese fathers were increased and overlapped completely with the set of upregulated DATs in male blastocysts of obese mothers. In contrast, 47 of 49 DATs were downregulated in female blastocysts when the father was obese. This observation argues for a sex-specific programming effect of paternal obesity.

The observations from this animal experiment support the hypothesis that paternal BMI at the time of conception may also have an important influence on the long-life programming of metabolic diseases in the offspring (1, 2). In epidemiological studies investigating a relationship between parental BMI and lifelong metabolic risk in children, measured data on BMI are often only available for the mothers, and data from the father are missing or were only reported. The observations from this animal study suggest that paternal BMI should also be measured in epidemiological studies and should be included in analyses examining a potential programming effect of parental BMI on lifetime risks of overweight and obesity and associated diseases in the offspring.

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12. Type 2 Diabetes, Metabolic Syndrome and Lipid Metabolism

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Preface

The frequency of type 2 diabetes (T2DM) has markedly increased in the pediatric age group since the end of the 20th century. Risk-based recommendations for screening for prediabetes and T2DM have been assessed. Another approach to identifying adolescents with T2DM uses metabolomic profiling. Decreased bone mineral density was added to the multitude of complications described with a high frequency in adolescents with T2DM. Furthermore, one-fifth of adolescents with T2DM reported elevated depressive symptoms and one-fifth admit thoughts of self-harm. Depression and obesity were found to be associated with lower fluid cognition in adolescents with T2DM. Immigration was found to be a strong risk factor for early-onset T2DM.

Poor outcomes of individuals with the metabolic syndrome (MetS) and T2DM during the COVID-19 pandemic were found to be related to socioeconomic inequalities. Excess consumption of sugar has been implicated in the global epidemic of obesity and MetS. Sugar was demonstrated to fuel the purine degradation pathway, leading to the accumulation of uric acid as kidney stones. The green Mediterranean diet was found to improve MetS parameters in adults; while among adolescents, a high protein/low glycaemic diet was not found to affect insulin resistance.

Type 2 Diabetes

12.1. Screening and diagnosis of prediabetes and diabetes in US children and adolescents

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In brief: This cross-sectional analysis of a nationally representative US sample showed that 25.5% of US children and adolescents (10.6 million in 2016) are eligible for screening for diabetes according to American Diabetes Association (ADA) criteria. The eligibility criteria showed low sensitivity and specificity to detect hyperglycaemia defined by HbA1c $\geq$5.7% (55.5% and 76.3%, respectively) or fasting plasma glucose (FPG) $\geq$100 mg/dl (35.8% and 77.1%, respectively). Confirmed undiagnosed diabetes was rare ($<$0.5% of youth). Hence, the ADA screening approach selects a very large number of children and adolescents, of whom only tiny proportion test positive for diabetes or prediabetes, and a substantial number of children with diabetes but non-eligible for screening are evidently missed.

Comment: Clinical practice guidelines are considered one of the most influential and effective tools for the promotion of evidence-based medicine. The traditional approach to developing clinical guidelines has been labelled GOBSAT (good old boys sitting around a table). While substantial international effort and high-quality evidence are invested in the development of guidelines, considerably less effort has been invested in updating guidelines (1). Hopefully, recent findings such as these will alter the strength of the body of evidence.

In 2000, the ADA and the American Academy of Pediatrics recommended screening for T2DM in high-risk youth aged 10 years and older (or after the onset of puberty). Risk was considered high if overweight plus at least two of the following: non-white race, family history of T2DM (first- or second-degree relatives), maternal

gestational diabetes and signs of insulin resistance (including: acanthosis nigricans, hypertension, dyslipidemia, polycystic ovarian syndrome and small-for-gestational-age). This recommendation was expanded in 2018 to include all youth with overweight plus one or more of these risk factors. This study quantifies the implications of this change: one-quarter of all US children and adolescents are eligible for screening for diabetes by the 2018 criteria, compared only 10% (3.6 million in 2016) according to the pre-2018 criteria.

Unconfirmed undiagnosed diabetes, defined by a single HbA1c of 6.5%, was observed in 0.3% (95% confidence interval [CI]: 0.1%–0.5%) of youth who were eligible for screening, and in 0.1% of those ineligible. A single elevated FPG value did not enable precise prediction of diabetes. Diagnosed diabetes was seen in 0.5% (95% CI: 0.4%–0.7%) of youth (0.2 million), equating to 85% of the total confirmed persons with diabetes. Similarly, the prevalence of prediabetes varied significantly depending on the definition. In conclusion, current screening criteria are not sensitive or specific and may miss many youth with diabetes. This study is important as it shows that clinical recommendations must be critically appraised.

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12.2. Identification of pathognomonic purine synthesis biomarkers by metabolomic profiling of adolescents with obesity and type 2 diabetes

Concepcion J, Chen K, Saito R, Gangoiti J, Mendez E, Nikita ME, Barshop BA, Natarajan L, Sharma K, Kim JJ
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In brief: Metabolite signatures were compared between children with normal weight, obesity, and both obesity and T2DM, by measuring 273 analytes in fasting plasma and a 24-hour urine sample. Twenty-two urine metabolites were uniquely associated with T2DM. Adolescents with T2DM have altered purine nucleotide metabolism, betaine metabolism, and oxidative branched-chain amino acids (BCAAs) and their catabolites.

Comment: Metabolomics, also known as metabolic profiling, is the systematic study of the unique chemical fingerprints of specific cellular processes (1). It involves the measurement of low-molecular-weight metabolites and their intermediates, and reflects the dynamic response to genetic modification and physiological pathways. Metabolite profiling is also useful in detecting incipient adverse events. The prefix ‘meta’ is Greek and means ‘change’. This study describes a targeted, quantitative mass spectrometry-based approach to generate unique urine and plasma metabolite signatures that differentiate youth with obesity and obesity plus T2DM.

Only a few plasma metabolites were uniquely associated with T2DM. However, a clearer unique signature was demonstrated in urine, in which the purine intermediate succinylaminoimidazole carboxamide riboside (SAICA-riboside) was the most increased. Second were betaine metabolites. The largest set of metabolites in the T2DM urine signature were the BCAAs, valine and leucine, and their direct catabolic derivatives, which were all increased in those with T2DM. Aromatic amino acids (phenylalanine, tyrosine and tryptophan) were also increased in the T2DM group. These findings in adolescents corroborate a prospective study in >4000 adults (2). There, the use of nontargeted metabolomics identified 3 purine metabolites associated with risk of developing T2DM (2). Better understanding of the adverse health effects of each of these metabotoxins is needed. Another study on purines in flies presented below (paper 12.9) may shed some insights.

Reference

1. Satheesh G, Ramachandran S, Jaleel A. Metabolomics-Based Prospective Studies and Prediction of Type 2 Diabetes Mellitus Risks. *Metab Syndr Relat Disord*. 2020 Feb;18(1):1–9. doi: 10.1089/met.2019.0047. PMID: 31634052.
2. Ottosson F, Smith E, Gallo W, Fernandez C, Melander O. Purine Metabolites and Carnitine Biosynthesis Intermediates Are Biomarkers for Incident Type 2 Diabetes. *J Clin Endocrinol Metab*. 2019 Oct 1;104(10):4921–4930. doi: 10.1210/jc.2019-00822. PMID: 31502646; PMCID: PMC6804288.

12.3. Bone mass and density in youth with type 2 diabetes, obesity, and healthy weight

Kindler JM, Kelly A, Khoury PR, Levitt Katz LE, Urbina EM, Zemel BS
Diabetes Care. 2020 Oct;43(10):2544–2552.
doi: 10.2337/dc19-2164. PMID: 32778556.

In brief: This cross-sectional study compared bone mineral density (BMD), lean body mass and abdominal visceral fat between 180 individuals with T2DM, 226 with obesity, and 238 with normal weight, aged 10–23 years. The findings suggest that T2DM in youth may have a detrimental effect on bone accrual during the critical window of peak bone mass attainment, irrespective of obesity. Individuals with T2DM and with increased abdominal visceral fat tended to have lower BMD.

Comment: Data regarding bone quantity (BMD) are obtained by the gold standard method of dual X-ray absorptiometry (DXA) and are used to diagnose osteoporosis and assess future risk of fracture. Areal BMD (aBMD), defined as bone mineral content (BMC) per unit area (g/cm^2) is preferred, as it is corrected for body size. Data from adults with T2DM show increased fracture risk despite normal or increased aBMD. This is the first study to assess bone health in youth with T2DM. Whole-body (excluding the head) BMC and aBMD, lean mass, abdominal visceral (cm^2) and subcutaneous (cm^2) fat area, and lean body mass index (LBMI, a measure of skeletal muscle calculated as lean mass (kg)/height (m^2)) were assessed in three groups of youth: normal weight, obesity and T2DM.

Across the age range, BMC and aBMD Z scores were higher in the T2DM and obese groups than in healthy weight controls. However, while the bone Z scores were consistent across the age range in the obese and healthy weight groups, the T2DM group showed an age-related decline in bone Z scores. This finding suggests an adverse impact of diabetes on aBMD during the critical period of bone mass attainment. Longer diabetes duration, nutritional inadequacies, physical inactivity and pharmacologic interventions are factors that may affect this age-related decline. The LBMI Z score as a marker for skeletal muscle mirrored the BMD findings. Accordingly, a higher LBMI Z score was observed in T2DM, but age-related trends resulted in compromised muscle-bone relations in youth with obesity and youth with T2DM.

A multivariate regression model including age, ancestry and sex showed that visceral fat was negatively associated with BMC and aBMD Z scores. This suggests a potential adverse influence of central fat depots on bone development. Skeletal muscle showed no difference between the groups. However, in both the T2DM and obese groups, aBMD *Z* scores were lower for a given LBMI *Z* score than compared to healthy-weight controls. Dietary factors including calcium, vitamin D and physical activity differed between the groups. This suggests that poor diet and physical inactivity might contribute to poor bone health in youth with T2DM. In conclusion, bone assessment in youth with T2DM appears to be an important component of follow-up.

12.4. Multisite examination of depression screening scores and correlates among adolescents and young adults with type 2 diabetes

Monaghan M, Mara CA, Kichler JC, Westen SC, Rawlinson A, Jacobsen LM, Adams RN, Stone JY, Hood KK, Mulvaney SA
Can J Diabetes. 2021 Jan 22:S1499–2671(21)00018-6.
doi: 10.1016/j.jcjd.2021.01.011. PMID: 33722492.

In brief: The presence of depressive symptoms was assessed in 197 adolescents and young adults (ages 12 to 24 years) with T2DM. 19% reported elevated depressive symptoms and 19% admitted thoughts of self-harm. Older age, shorter diabetes duration, higher HbA1c level, being non-Hispanic white, more blood glucose checks per day and being prescribed oral medications were associated with more depressive symptoms.

Comment: The Patient Health Questionnaire (PHQ) is a brief tool for screening, diagnosing and monitoring the severity of depression. It rates the frequency of depressive symptoms over the previous 2 weeks. PHQ-9 is composed of nine items that align with the diagnostic criteria for major depressive disorders and includes a question on the presence and duration of suicide ideation. A further, unscored question assesses the degree to which depressive problems affect functioning. The PHQ is also available in a brief 2-item version to use in screening; higher scores prompt the need to administer the other 7 PHQ-9 items.

Of 197 individuals with T2DM, 63% reported minimal depressive symptoms, 18% had mild, 13% moderate, 5% moderately severe and 1% had severe symptoms. 19% reported thoughts of self-harm. The rates of depressive symptoms in adolescents and young adults with T2DM were twice those of youth with type 1 diabetes assessed in the same clinics. Older age and poor glycaemic control were clinical clues for a higher depressive symptoms.

Most important, only 50% of youth with elevated PHQ scores and only 20% of youth endorsing the harm-to-self item were referred for mental health treatment, and only 10% were in active mental health therapy. These findings corroborate evidence on the lack of identification by primary care clinicians, of up to two-thirds of youth with depression, and the consequent absence of proper care (1). Even when diagnosed, only half the youth were treated appropriately. Moreover, rates were low of completion of specialty mental health referral for youth with a recognized emotional disorder from general medical settings. Major depression in adolescents is recognized as a serious psychiatric illness with extensive acute and chronic morbidity and mortality. It is therefore of utmost importance that paediatricians and paediatric endocrinologists embrace depression screening of all adolescents.

Reference

1. Zuckerbrot RA, *et al*. Guidelines for Adolescent Depression in Primary Care (GLAD-PC): Part I. Practice Preparation, Identification, Assessment, and Initial Management. *Pediatrics*. 2018 Mar;141(3):e20174081. doi: 10.1542/peds.2017-4081. PMID: 29483200.81.

12.5. Cognitive function in adolescents and young adults with youth-onset type 1 versus type 2 diabetes: the search for diabetes in youth study

Shapiro ALB, Dabelea D, Stafford JM, D'Agostino R Jr, Pihoker C, Liese AD, Shah AS, Bellatorre A, Lawrence JM, Henkin L, Saydah S, Wilkening G; SEARCH for Diabetes in Youth Study Group

Diabetes Care. 2021 Apr 26:dc202308.
doi: 10.2337/dc20-2308. PMID: 33905344.

In brief: The multicenter SEARCH for Diabetes in Youth Study assessed cognitive function in adolescents and young adults with type 1 diabetes mellitus (T1DM) ($n=1095$) or T2DM ($n=285$). Mean age was 21.6 ± 5.1 years and mean diabetes duration 11.0 ± 3.4 years. Having T2DM was associated with lower fluid cognitive scores before adjustment for confounders. However, this association was attenuated to non-significance with the model inclusion of a priori confounders and the sub-domain of crystallized cognition scores. In a final combined model, receptive language, waist-to-height ratio and depressive symptoms remained significantly different between groups.

Comment: Intelligence is composed of various interacting abilities. The concepts of fluid and crystallized intelligence were first proposed by Cattell (1), who defined fluid cognition as "the ability to perceive relationships independent of previous specific practice or instruction concerning those relationships." An example of fluid intelligence is puzzle solving. By contrast, crystallized intelligence involves knowledge that comes from prior learning and past experiences, for example reading comprehension and vocabulary tests.

This study assessed subdomains of cognition in adolescents with T2DM or T1DM, compared to the general population. Subdomains of fluid cognition included: cognitive flexibility, working and episodic memory, processing speed and attention/inhibitory control. Subdomains of crystallized cognition included receptive language (Picture Vocabulary Test).

Adolescents with T2DM performed below the national average in fluid cognition. Both the T2DM and T1DM groups performed below the national average with respect to inhibitory control and sustained attention scores. In models unadjusted for covariates, the T2DM group performed significantly worse than the T1DM group in fluid cognition. However, in models that included potential confounders (sex, race/ethnicity, parental education, household income, duration of diabetes, obesity, depression, hypoglycaemic events in the past year and glycaemic control) and also receptive language (crystallized cognition), fluid cognition did not differ between the groups. Receptive language, waist-to-height ratio and depressive symptoms remained significantly different between T2DM and T1DM.

Obesity and depression per se appear to be associated with additional sequelae. Lower fluid intelligence in adolescents has been associated with physical violence (both in the role of victim and victimizer), drug intake

and lower self-esteem (2). Together, these emphasize the importance of programs for early diagnosis of depression and prevention of obesity.

Reference

1. Brown RE. Hebb and Cattell: The Genesis of the Theory of Fluid and Crystallized Intelligence. *Front Hum Neurosci*. 2016; 15;10:606. doi: 10.3389/fnhum.2016.00606. PMID: 28018191.
2. Huepe D, *et al*. Fluid intelligence and psychosocial outcome: from logical problem solving to social adaptation. *PLoS One*. 2011;6(9):e24858. doi: 10.1371/journal.pone.0024858. PMID: 21957464.

12.6. Adolescent BMI and early-onset type 2 diabetes among Ethiopian immigrants and their descendants: a nationwide study

Simchoni M, Hamiel U, Pinhas-Hamiel O, Zucker I, Cukierman-Yaffe T, Lutski M, Derazne E, Beer Z, Behar D, Keinan-Boker L, Mosenzon O, Tzur D, Afek A, Tirosh A, Raz I, Twig G

Cardiovasc Diabetol. 2020 Oct 6;19(1):168.
doi: 10.1186/s12933-020-01143-z. PMID: 33023586.

In brief: The impact of immigration on the risk of early-onset T2DM (before age 40 years) was assessed in a nationwide cohort. Data on 93 806 native Israelis and 27 684 Israelis of Ethiopian origin, assessed at a mean age 17.5 years, were linked to the Israeli National Diabetes Registry. After adjustment for sociodemographic confounders, the hazard ratios for T2DM among Ethiopian men with normal and high BMI were 3.4 (2.3–5.1) and 15.8 (8.3–30.3), respectively, compared to third-generation Israelis with normal BMI. When the analysis was limited to Israeli-born Ethiopian men, the hazard ratios increased to 4.4 (1.7–11.4) and 29.1 (12.9–70.6), respectively.

Comment: The total number of international migrants at mid-year 2020 was 280.6 million, of whom 14.6% were aged <19 years. International migration has increased over time, both numerically and proportionally, at a slightly faster rate than previously anticipated**.** Migration has implications on public health, and addressing migration-associated health threats is crucial.

In the current population-based study, higher incidence of early-onset T2DM was observed in persons of Ethiopian origin than among native Israelis. T2DM was diagnosed at a mean age of 30 years. The crude incidence rate for early-onset T2DM at age 17 years was calculated according to BMI groups. T2DM incidence was higher in Ethiopian vs. native Israeli males in the underweight, normal weight and overweight/obese groups. This suggests increased risk for early-onset T2DM among adolescent males of Ethiopian origin, even those with BMI within the normal range. Those with overweight and obesity were found to be at considerably greater risk.

The incidence of diabetes increased with the time interval since immigration; the risk for early-onset T2DM was higher for those of Ethiopian origin born in Israel than for those born in Ethiopia. This suggests an important contribution of lifestyle changes among Ethiopian immigrants to Israel, who changed their diet to a more Western one, and became less physically active, and also indicate that exposure during postnatal and early childhood are important.

These findings emphasize the need for interventions aimed at obesity prevention, education about healthy lifestyles, and early medical intervention. The findings have implications to other high-income countries that are recipients of immigrants. Ensuring that health services are delivered to migrants in a culturally and linguistically appropriate way is of high priority. https://migrationdataportal.org/data?i=stock_abs_&t=2020.

12.7. The shared risk of diabetes between dog and cat owners and their pets: register based cohort study

Delicano RA, Hammar U, Egenvall A, Westgarth C, Mubanga M, Byberg L, Fall T, Kennedy B

BMJ. 2020 Dec 10;371:m4337.
doi: 10.1136/bmj.m4337.

In brief: Owners of dogs which have with diabetes are more likely to develop T2DM themselves than owners of dogs without diabetes. The underlying mechanisms might include shared diabetogenic health behaviours and environmental exposures. No shared risk of diabetes was found between cat owners and their cats.

Comment: This article was published in BMJ Christmas Special, but do not underestimate it; serious work was invested to reach the conclusions. Prospective data involving 132 783 dogs and 84 143 cats, recorded between 2004 and 2006 by a major Swedish pet insurance company, were linked to Swedish health and drug records to identify incidence of T2DM among pet owners (175 214 dog owners and 89 944 cat owners).

Owners of dogs with diabetes were found to have 38% higher risk of developing T2DM compared to owners of dogs without diabetes. This elevated risk persisted after considering socioeconomic and personal variables, such as age, sex, region of residence, marital status, education level and income; and also the age, sex and breed of the dog. In parallel, the risk of developing diabetes was 28% higher among dogs whose owners had been diagnosed with T2DM compared with dogs whose owners did not have T2DM.

Socioeconomic circumstances were not associated with increased risk. Suggested underlying mechanisms included an impact on dietary habits of the dog owners on their pets' diet through portion control, frequency of feedings and providing table scraps in addition to dog food. Similarly, it is plausible that dog owners and dogs share the same frequency and intensity of exercise, and this could explain the lack of shared T2DM risk between cat owners and their cats.

So, dogs are 'man's best friend'. They can serve as a red light to their owners, to change their lifestyle and prevent T2DM.

Metabolic Syndrome

12.8. COVID-19 and metabolic diseases: a heightened awareness of health inequities and a renewed focus for research priorities

Cefalu WT, Rodgers GP

Cell Metab. 2021 Mar 2;33(3):473–478.
doi: 10.1016/j.cmet.2021.02.006. PMID: 33581046.

In brief: This perspective paper discusses the observed disparities for metabolic diseases in regard to COVID-19. Disparities stem from systemic differences in access to care, environmental exposures and other sociodemographic factors.

Comment: Hopefully, by the time these lines are published, the COVID-19 pandemic will have calmed down and the world will have returned to normality. Nonetheless, lessons need to be learned from this global event. Over the course of the pandemic, it became evident that increasing age, diabetes, obesity, cardiovascular disease, chronic kidney disease and male gender are strongly associated with worse severity of COVID-19 and consequent mortality. The disproportionate effect of COVID-19 on racial minorities also became clear. The mortality ratio was $>$ 3x higher in Black than White populations in the US.

Although Black individuals represent only 31% of US health system users, they represented 70% of patients with COVID-19. Black COVID-19 patients had greater prevalences of metabolic diseases including obesity, diabetes, hypertension and chronic kidney diseases than did White COVID-19 ppatients. However, after adjustment for differences in sociodemographic factors and comorbidities, Black race was not associated with higher in-hospital all-cause mortality.

This study showed that mortality from COVID-19 is not directly influenced by race, but rather by related factors, such as sociodemographic factors, health care access, occupational COVID-19 exposure, and long-standing disparities in nutrition, obesity and the metabolic syndrome. As the world becomes smaller, further viral pandemics are expected. This study highlights the need to improve health equity.

12.9. Sugar-Induced obesity and insulin resistance are uncoupled from shortened survival in drosophila

Van Dam E, van Leeuwen LAG, Dos Santos E, James J, Best L, Lennicke C, Vincent AJ, Marinos G, Foley A, Buricova M, Mokochinski JB, Kramer HB, Lieb W, Laudes M, Franke A, Kaleta C, Cochemé HM
Cell Metab. 2020; 31(4):710–725.e7.
doi: 10.1016/j.cmet.2020.02.016. PMID: 32197072.

In brief: High-sugar diets cause thirst, obesity and metabolic dysregulation, leading to insulin resistance, the MetS, T2DM and shortened lifespan. This study shows that high sugar diets induce dehydration in adult Drosophila. Water supplementation fully rescued the shortened lifespan, but not the metabolic defects, indicating that these are water-independent. High-sugar diets promote the accumulation of uric acid, an end-product of purine catabolism, and the formation of renal stones. These findings were confirmed in a human cohort with metabolomics and dietary data. Thus, the purine pathway is strongly involved in survival.

Comment: High-sugar diets promote the development of obesity, insulin resistance and T2DM, and also shorten survival, which was assumed to be a consequence of these metabolic derangements.

In a series of elegant studies in Drosophila, a chronic high-sugar diet was shown to induce thirst and dehydration, insulin resistance, obesity and decreased lifespan. Water supplementation fully rescued their shortened lifespan; however, the metabolic traits (hyperglycaemia, insulin resistance, increased triglycerides and glycation damage) persisted. Therefore, it was concluded that these metabolic defects are not the direct cause of the shortened lifespan, but that water imbalance is involved. Although gut function is critical to nutritional physiology, the experiments were unable to show a link to the high-sugar shortened lifespan.

The authors further explored the effects of high-sugar diet on renal physiology. Flies fed on high-sugar showed changes in renal morphology, including uric acid deposits in their tubules, and this was fully rescued by water supplementation. High-sugar diets were suggested to provide precursors for purine biosynthesis, thus fuelling the accumulation of uric acid, a waste product of purine catabolism. Administration of allopurinol, an inhibitor that blocks uric acid formation, abolished the increase in uric acid levels and tubule uric acid deposits.

Finally, to assess whether dietary habits are linked to purine metabolism in humans, serum metabolomics data were correlated to dietary records in a cohort of 650 healthy participants. Indeed, the consumption of sugar-rich foods was strongly associated with higher levels of circulating purines and renal dysfunction. Interestingly, metabolomics data showed significant interactions of circulating levels of purines with the consumption of fruits, soft drinks and cereals, but not with the consumption of purine-rich food groups such as fish and meat.

The authors conclude that these findings may explain the ‘fat but fit’ paradox, and provide a novel approach for the discovery of new therapeutic strategies.

12.10. Genome-wide discovery of genetic loci that uncouple excess adiposity from its comorbidities

Huang LO, Rauch A, Mazzaferro E, Preuss M, Carobbio S, Bayrak CS, Chami N, Wang Z, Schick UM, Yang N, Itan Y, Vidal-Puig A, den Hoed M, Mandrup S, Kilpeläinen TO, Loos RJF
Nat Metab. 2021 Feb;3(2):228–243.
doi: 10.1038/s42255-021-00346-2. PMID: 33619380.

In brief: In this genome-wide association study, the authors were able to disentangle the mechanism that uncouples adiposity from its known cardiometabolic complications. They identified 62 genomic loci, at which the same allele is associated with both higher adiposity and lower cardiometabolic risk. Functional analyses implicated several underlying mechanisms. These 62 loci clustered into three functional groups: favourable fat distribution, favourable lipid levels, and lower fasting glucose and lower risk for T2DM.

Comment: There is ongoing controversy regarding the ‘fat but fit’ paradox, as a substantial proportion of individuals with obesity do not have cardiometabolic comorbidities, and some normal-weight individuals have high risk. Previous studies focused on lifestyles and suggested that fitness might mitigate the negative effects of

excess body weight on heart health, and that being "fat but fit" might be associated with similar cardiovascular health to being "thin but unfit." The current study adopted a different approach to explain this paradox.

Data on several traits were analysed in the UK Biobank study, including: BMI, body fat, waist-hip ratio, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, systolic blood pressure and non-fasting glucose. 62 genomic loci were significantly associated with higher body fat, but that also conferred protection from the negative health impacts of obesity. Function analyses showed that the underlying genes had a range of functions, including the regulation and differentiation of adipocytes, and increased peripheral distribution of body fat; also glucose-insulin signalling, inflammation, energy expenditure, fatty acid oxidation and browning of white adipose tissue.

In light of the seeming futility of weight loss interventions for many people, it is of utmost importance to distinguish individuals with excess body weight who are at high risk of developing cardiometabolic diseases, from those who have genetic protection from developing diabetes and cardiovascular diseases.

12.11. The effect of green Mediterranean diet on cardiometabolic risk; a randomised controlled trial

Tsaban G, Yaskolka Meir A, Rinott E, Zelicha H, Kaplan A, Shalev A, Katz A, Rudich A, Tirosh A, Shelef I, Youngster I, Lebovitz S, Israeli N, Shabat M, Brikner D, Pupkin E, Stumvoll M, Thiery J, Ceglarek U, Heiker JT, Körner A, Landgraf K, von Bergen M, Blüher M, Stampfer MJ, Shai I

Heart. 2020 Nov 23:heartjnl-2020-317802.
doi: 10.1136/heartjnl-2020-317802. PMID: 33234670.

In brief: This parallel-arm trial randomly assigned 294 adults with abdominal obesity/dyslipidemia to 1 of 3 dietary guidelines: healthy diet; Mediterranean diet; or 'green Mediterranean' diet. All groups received physical activity guidelines. Weight loss was higher in the two Mediterranean diet groups, but the green Mediterranean diet produced the greatest reduction in waist circumference (−8.6 cm), compared to Mediterranean diet (−6.8 cm; $P=0.033$) and healthy diet (−4.3 cm; $P<0.001$). The green Mediterranean diet also showed greater decreases in LDL-C, diastolic blood pressure, insulin resistance, high-sensitivity C-reactive protein and the 10-year Framingham Risk Score, compared with the healthy diet.

Comment: In 2010, the Mediterranean diet was inscribed into the United Nations Educational, Scientific and Cultural Organization (UNESCO) Representative List of Intangible Cultural Heritage of Humanity. The Mediterranean diet involves knowledge and traditions concerning crops, harvesting, fishing, animal husbandry, conservation, processing, cooking and eating together. The foundation of the Mediterranean diet is vegetables, fruits, herbs, nuts, beans, whole grains, seafood, poultry and eggs; whereas red meat is eaten only occasionally. The Mediterranean diet has been shown to prevent cardiovascular disease. The current study assessed the impact of a 'green Mediterranean' diet.

Participants in the green Mediterranean diet group were guided to consume 3–4 cups/day of green tea and 100 g/day of frozen *Wolffia globosa* as a green shake replacing dinner. Processed and red meat were restricted. *Wolffia globosa* is a flowering plant known commonly as Asian watermeal, or duckweed, or Mankai. It is a very tiny, less than one-millimetre wide, oval-shaped plant with no leaves, stems or roots. Its dry matter has high protein content (> 45%), contains all 9 essential and 6 conditional amino acids, and is rich in polyphenols (flavonoids, phenolic acid), dietary fiber, iron, zinc and vitamin B12.

The green Mediterranean diet showed greater benefits on metabolic syndrome parameters, including waist circumference, lipid profile and overall cardiovascular risk score. In addition, in a later study, the same group reported the impact of the green Mediterranean diet on non-alcoholic fatty liver disease (NAFLD) prevalence, which declined to: 54.8%, 47.9% and 31.5% in the healthy diet, Mediterranean diet and green Mediterranean diet groups, respectively ($P=0.012$) (1). Furthermore, despite similar moderate weight-loss, the green Mediterranean compared to the Mediterranean diet showed almost 2-fold reduction in intrahepatic fat (−38.9% vs. −19.6%, respectively, $P=0.035$, adjusted for weight loss).

Reference

1. Yaskolka Meir A, *et al*. Effect of green-Mediterranean diet on intrahepatic fat: the DIRECT PLUS randomised controlled trial. *Gut*. 2021 Jan 18:gutjnl-2020-323106. PMID: 33461965.

12.12. Effect of a high protein/low glycaemic index diet on insulin resistance in adolescents with overweight/obesity – a preview randomized clinical trial

Dorenbos E, Drummen M, Adam T, Rijks J, Winkens B, Martínez JA, Navas-Carretero S, Stratton G, Swindell N, Stouthart P, Mackintosh K, Mcnarry M, Tremblay A, Fogelholm M, Raben A, Westerterp-Plantenga M, Vreugdenhil A
Pediatr Obes. 2021 Jan;16(1):e12702.
doi: 10.1111/ijpo.12702. PMID: 32681547.

In brief: This randomized parallel trial, conducted in the Netherlands, the United Kingdom and Spain, assessed the impact of a high protein/low glycaemic diet vs. medium protein/medium glycaemic diet in reducing insulin resistance in 126 adolescents with overweight/obesity (mean age 13.6 ± 2.2 years). At 2 years, no effect was shown on insulin resistance. The authors concluded that the null findings were due to infeasibility of the diet, insufficient study retention and poor dietary compliance.

Comment: According to a report published in 2019, 45% of adolescent girls and 30% of adolescent boys aged 16–19 years tried to lose weight in the previous year. For both boys and girls, the proportion increased with weight status category. Parallel to the obesity epidemic, the diet industry is growing. Options include: vegetarian, vegan, plant-based flexitarian, keto, intermittent fasting, Atkins, time-restricted eating, Mediterranean diet, blood type, paleo and high protein/low glycaemic diets. The common dominator of all diets is restricting the number of food choices. The key question is: which diet is most likely to produce permanent weight loss?

Even professionals are affected by trends. Therefore, the current study is important in that it aimed to assess the impact of a high protein/low glycaemic diet on insulin resistance. Its strengths lie in the long follow-up of 2 years, and the assessment of compliance to dietary guidance, and assessment of insulin resistance. The 68 participants randomized to high protein diet received a sample menu with a macronutrient composition of 25%/45%/30% energy from protein/carbohydrate/fat, respectively, with overall glycaemic index $\leq$ 50. The moderate protein group ($n = 58$) received a sample menu with a macronutrient composition of 15%/55%/30% energy from protein/carbohydrate/fat, respectively, and a glycaemic index $\geq$ 56.

During the 2 years intervention period, 43/68 (63%) of participants in the high protein group dropped out, as did 34/58 (59%) of the moderate protein group. Absolute and relative reported protein intakes (as % of total energy) did not differ from baseline after 1 and 2 years; and no difference in dietary intake was observed between the two groups. Similarly, no difference at any time point was observed between the two groups in insulin resistance, glucose parameters, anthropometry or lifestyles.

High protein diets represent a popular alternative to energy restriction for weight maintenance and weight loss. Data from rodents consistently show that the dietary protein:carbohydrate ratio strongly influences weight gain, probably due to the higher satiating effect of protein. However, food intake in humans is a far more complex. The high drop out rate in both groups here and lack of compliance with diet guidance demonstrates the challenges of conducting long-term studies in adolescents.

Reference

1. McDow KB, Nguyen DT, Herrick KA, Akinbami LJ. Attempts to lose weight among adolescents aged 16–19 in the United States, 2013–2016. NCHS Data Brief, no 340. Hyattsville, MD: National Center for Health Statistics. 2019.

Lipids

12.13. Comparison of the mutation spectrum and association with pre and post treatment lipid measures of children with heterozygous familial hypercholesterolaemia (FH) from eight European countries

Futema M, Ramaswami U, Tichy L, Bogsrud MP, Holven KB, Roeters van Lennep J, Wiegman A, Descamps OS, De Leener A, Fastre E, Vrablik M, Freiberger T, Esterbauer H, Dieplinger H, Greber-Platzer S, Medeiros AM, Bourbon M, Mollaki V, Drogari E, Humphries SE
Atherosclerosis. 2021 Feb;319:108–117.
doi: 10.1016/j.atherosclerosis.2021.01.008. PMID: 33508743.

In brief: This multi-centre study included 2866 children with familial hypercholesterolemia (FH) from 8 European countries. The mutation spectrum was assessed, as were associations between gene mutations and clinical characteristics and pre and post-treatment lipid concentrations. The most common causes of FH were LDL receptor gene (*LDLR*) mutations, which were associated with a higher family history of premature coronary heart disease (CHD).

Comment: FH, an autosomal dominant disorder, is the most common inherited cause of premature CHD, and affects ~1:250 people of Northern European descent. Four genes involved in the clearance of LDL-C from the blood are known to cause FH: *LDLR*, which encodes the LDL receptor; the apolipoprotein B gene (*APOB*); gain-of-function mutations in *PCSK9*, encoding proprotein convertase subtilisin/kexin type 9; and a recently reported single mutation in the *APOE* gene.

In this cohort of children with a clinical diagnosis of FH, 88% had a confirmed genetic diagnosis. In all countries, the most common cause of FH was a mutation in *LDLR*. Overall, 297 *LDLR* mutations were reported, with extreme heterogeneity across countries. The prevalence of *APOB* mutations differed across countries, and also within each countries, yet the mutation was the same in 97% of affected individuals. The prevalence of mutations in *PCSK9* was the lowest (0.3% of all mutations).

Higher baseline LDL cholesterol levels were observed in carriers of large insertion/deletion mutations than in carriers of promoter, splicing and missense mutations. LDL cholesterol levels were higher in those with *LDLR* mutations than *APOB* mutations. Nevertheless, responses to lipid-lowering therapy were similar between carriers of the various mutations. Moreover, *LDLR* mutations did not differ significantly in the likelihood of pathogenicity, based on American College of Medical Genetics criteria. However, an analysis based on functional class of the mutation showed differences in the proportions of children who achieved the LDL cholesterol target levels.

As genetic testing is not a part of the diagnostic workup for FH in many low- and middle-income countries, the findings of this work are reassuring. Most cases are due to mutations in *LDLR*, and most patients respond to statin therapy, with a significant decrease in LDL cholesterol. Therefore, the important tasks are clinical and biochemical screening for early diagnosis and early treatment (1). For those patients who respond poorly to statin therapy, genetic tests can be extended.

Reference

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12.14. Effects of apolipoprotein B on the lifespan and risks of major disease including type 2 diabetes: a mendelian randomization analysis using outcomes in first-degree relatives

Tom G Richardson, Qin Wang, Eleanor Sanderson, Anubha Mahajan, Mark I McCarthy, Timothy M Frayling, Mika Ala-Korpela, Allan Sniderman, George Davey Smith, Michael V Holmes

Lancet Healthy Longevity 2021
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In brief: Using a multivariable Mendelian randomization approach, genetic data on lipoprotein lipid levels and disease outcomes indicated that higher Apolipoprotein B (ApoB) levels increase the risks of coronary heart disease, stroke and T2DM, and shorten longevity. ApoB is emerging as a more sensitive measure of disease risks posed by lipoprotein than the conventionally used LDL-C.

Comment: Mendelian randomization is the analytical modelling of selected genetic variants that are randomly distributed at birth, and are known to be reliably associated with a specific risk factor, to obtain causal effect estimates for these risk factors on disease outcomes. Multivariable Mendelian randomization is an extension of the standard Mendelian randomization to consider multiple potential risk factors in a single model.

ApoB is a structural protein that constitutes a major component of the atherogenic lipoproteins: very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and LDL. Each of these lipoprotein

particles carries one ApoB molecule. Therefore, ApoB levels reflect the atherogenic potential of these lipoproteins.

The current study investigated the effects of genetically predicted elevations of ApoB on risks of heart disease, stroke, hypertension, Alzheimer's disease, T2DM and lifespan (the last was reported in first degree relatives). Using multivariable Mendelian randomization, the independent effects of ApoB, LDL-C and triglycerides were simultaneously assessed. More than 400 000 participants reported lifespan information on their first-degree relatives.

Higher ApoB was genetically associated with a shorter lifespan of parents and increased risk for stroke and heart disease. The authors concluded that the number of circulating ApoB particles rather than their lipid content is the critical element for atherogenesis. They suggest that reductions in ApoB should be the primary goal of lipid-lowering, not only because this leads to lower risk of common diseases such as heart disease and stroke, but also because reduced ApoB prolongs lifespan by a period of months to years.

Of note, this article was initially published in *medRxiv* (pronounced "med-archive") an internet site founded in 2019, which displays complete but non-reviewed and unpublished pre-prints of manuscripts in the areas of medicine, clinical research and related health sciences. *medRxiv* provides a platform for researchers to share, comment on and receive feedback on their work before journal submission or publication. As the manuscripts are not yet peer reviewed, the site states that the findings should not be considered for clinical application, nor relied upon as established information. *medRxiv* can save authors time in submitting papers to journals by directly transmitting their manuscript files and metadata. The list of journals that participate is published in https://www.medrxiv.org/content/about-medrxiv.

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2. Vuorio A, Ramaswami U, Holven KB. Editorial: Genetics of Familial Hypercholesterolemia: *New Insight*. 2021; **12**(666).

12.15. Detection of Lipoprotein X (LpX): A challenge in patients with severe hypercholesterolaemia

Ćwiklińska A, Mickiewicz A, Kowalski R, Kortas-Stempak B, Kuchta A, Mucha K, Makowiecki M, Gliwińska A, Lewandowski K, Pęczek L, Fijałkowski M, Gruchała M, Jankowski M
J Med Biochem. 2020 Sep 2;39(3):283–289.
doi: 10.2478/jomb-2019-0038. PMID: 33269016.

In brief: This case report serves as a platform to discuss the differential diagnosis of severe hypercholesterolemia. A young woman with T1DM and autoimmune liver disease presented with an LDL cholesterol value >1000 mg/dl (>26 mmol/l). She had no family history to suggest familial hypercholesterolemia. No mutations were identified in her *LDLR*, *APOB* and *PCSK9* genes. Biochemical analysis showed elevated serum liver enzymes, which led to the detection of abnormal lipoprotein fraction, lipoprotein X (LpX), and prompted appropriate treatment, plasmapheresis.

Comment: Lipoprotein X has a similar density to LDL, making LpX cholesterol indistinguishable from LDL by conventional assay methods. Hence, LpX may be responsible for false elevations in LDL cholesterol. Rather, it is identified using lipoprotein electrophoresis, where LpX runs in a reversely migrating band. LpX is present in the plasma of patients affected by extra and intrahepatic cholestasis, in patients who receive lipid-rich parenteral nutrition, and rarely in lecithin-cholesterol-acyl-transferase deficiency. Cholestasis has been suggested to result in spillage of lipid fractions from bile into plasma, where they combine non-covalently with albumin to form LpX. LpX is rich in phospholipids, albumin and free cholesterol. However, unlike LDL, LpX has no apoB-100 and therefore should be suspected when unusually rapid elevations in LDL-C are encountered with a low apoB concentration.

Clinical studies have shown that cardiovascular disease risk is not increased by Lp-X (1). However, very high Lp-X concentrations may produce hyperviscosity syndrome. Treatment with statins, ezetimibe and PCSK9 inhibitors lack efficacy in reducing Lp-X. As LpX has no apoB, statins do not affect the removal of this lipoprotein by the liver. Furthermore, patients with cholestatic disease might reach toxic statin concentrations. Similarly, due to low levels of apoB, LDL apheresis is not recommended for LpX removal, as the absence of apoB reduces its effectiveness. Instead, plasmapheresis is the treatment for hyperviscosity syndrome due to high LpX.

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12.16. A multidimensional precision medicine approach identifies an autism subtype characterized by dyslipidemia

Luo Y, Eran A, Palmer N, Avillach P, Levy-Moonshine A, Szolovits P, Kohane IS
Nat Med. 2020 Sep;26(9):1375–1379.
doi: 10.1038/s41591-020-1007-0. PMID: 32778826.

In brief: A subtype of autism arising from a cluster of genes that regulate both cholesterol metabolism and brain development was identified by integrating multiple data modalities.

Comment: Autism spectrum disorder (ASD) is now estimated to affect 1:54 children in the USA, 80% of whom are boys. Genomic studies show extreme heterogeneity in the genetic aetiology of ASD.

- Firstly, these researchers identified genes that are expressed differentially in the developing brains of males and females in the prenatal period.
- Secondly, they used whole exome sequencing and detected both – variants that were discordant between individuals with ASD and their healthy siblings, and variants that were shared between several siblings with ASD in the same family.
- Next they searched for overlaps between the exon clusters identified in the brains and the autism mutations, and identified 33 neurodevelopmentally ASD-segregating variants.
- Using two huge routine clinical datasets, they found that the blood lipid profiles of individuals with autism differ significantly from the physiological range.
- Having ASD and an abnormal lipid profile were found to be associated with epilepsy, sleep disorders and attention deficit hyperactivity disorder. This suggests that dyslipidemia might contribute to altered neurodevelopment. Among individuals with ASD and abnormal lipid levels, risks were also higher for hypothyroidism, anaemia and vitamin D deficiency.
- This novel ASD dyslipidemia-linked subtype is estimated to affect ∼7% of invidivuals with ASD.

The notion that lipid metabolism is important in neurodevelopment is supported by the features of Rett syndrome and Smith-Lemli-Opitz syndrome. The former is characterized by autism and a mutation in a cholesterol metabolism gene. The latter is an inborn error of cholesterol synthesis, whereby the vast majority of those affected also have autistic behaviours. The authors conclude that the ASD subtype characterized by dyslipidemia shows that genes involved in lipid metabolism are crucial during mammalian brain development. It will be interesting to investigate if treatment with lipid-lowering drugs in children with autism and dyslipidemia might improve their autism and other neurological traits.

13. Global Health for the Paediatric Endocrinologist

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Preface

Welcome to the 6th edition of this chapter on Global Health in Pediatric Endocrinology and Diabetes. As usual, the selected articles cover many aspects of our specialty. This year is important because we celebrate the 100th anniversary of the discovery of insulin by Sir Frederick Banting, Charles Best, John Macleod and James Collip at the University of Toronto in 1921. This discovery paved the way for the first administration of insulin to a 14-year-old boy with Type 1 diabetes, Leonard Thompson, in early 2022. Unfortunately, while insulin has saved the lives of millions of patients with Type 1 diabetes around the world, it remains out of reach for too many patients in low- and middle-income countries. On the endocrine side, it is encouraging to see many articles on various aspects of the management of CAH and other adrenal diseases.

History and Society

13.1. Population history and ecology, in addition to climate, influence human stature and body proportions

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doi: 10.1038/s41598-020-79501-w

- Variation in human stature and limb proportions reflects not only thermal adaptation but also environmental stressors
- The authors analysed global variation in stature and limb length using published anthropometric data from adult male and female populations across the world in relation to climate and net primary productivity, taking into account population history
- Net primary productivity was a consistent negative predictor of anthropometry, which may reflect the growth limiting effects of lower environmental resource accessibility and/or pathogen load

It is accepted that increasing distance from the equator is associated with higher body mass and shorter limbs relative to the trunk. Lower temperature, a variable that is directly related to increasing distance from the equator, is often cited as the main factor that explains these differences between populations. The authors attempted to tease out these widely cited associations between human phenotypic variation and climate, taking into account population history (broad pattern of human dispersal both within and outside of Africa). In addition to temperature, they considered other direct climate variables such as humidity, and indirect climate variables such as environmental productivity (which covaries with, e.g. nutrition, diet, pathogen load). Most models suggested that population history had an influence that was at least as important as the climate effect on limb length, sitting height and stature. After correcting for population history, temperature appeared as the main factor explaining variations between populations. However, humidity and indirect climate variables also played a role. The models yielded grossly similar results for males and females.

Overall, population history, temperature and other eco-geographical correlates (food availability, pathogen load) are all associated to stature and limb lengths (although causation was not demonstrated). This study is interesting, not only because it attempts to understand differences in anthropometric characteristics between populations, but also because it serves a reminder that our understanding of growth in children and adolescents needs to take context into consideration.

Diabetes

13.2. Worldwide differences in childhood type 1 diabetes: The SWEET experience

Saiyed M, Hasnani D, Alonso GT, Richmond E, Besançon S, Cotterill A, Ngwu U, Mazza C, Rottenbourg D, Lanzinger S and the SWEET study group.
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Pediatr Diabetes 2020;1–8.
doi: 10.1111/pedi.13137

- As part of the SWEET initiative, the authors analyzed the clinical characteristics of 26,726 individuals with type 1 diabetes (T1D) aged < 21 years living in Europe, Asia/Middle East/Africa, Australia/New Zealand, North America and South America
- Among patient care indicators, the percent of HbA1c < 7.5% ranged between regions from 18.1 to 46.3% and the percent of patients using pumps from 4.6 to 80.8%
- Overall, there was a significant heterogeneity in diabetes care between regions

The SWEET project (*www.sweet-project.org*) consists in a network of pediatric diabetes centers worldwide. Its goal is to identify best practices and harmonize care to optimize outcomes in children and adolescents with diabetes mellitus. In this study, which includes for the first time centers from low-income countries, the authors conducted a baseline comparison among five SWEET regions across the world to assess differences in pediatric T1D care. Overall, the indicators of the Asia/Middle East/Africa region reflected poorer diabetes control compared to the other regions: higher median HbA1c (8.9% compared to an overall median of 8.0%), lower frequency of blood glucose monitoring (2.0/day compared to 4.1/day), lower % of HbA1c < 7.5% (18.1% compared to 34.9%), lower number of severe hypoglycemias (1.1/year compared to 1.3 per year). Surprisingly, the Asia/Middle East/Africa region was also associated with a daily dose of insulin (0.9 U/kg/d) that was higher compared to the dose in high income countries (0.8 U/kg/d), where diabetes control is better. However, the dose of insulin reported in this study is the dose that was prescribed. It may not reflect the dose of insulin that was actually injected. Indeed, the cost of insulin remains a major barrier to diabetes care in countries where it is not covered by the government or in centers that do not receive free insulin from organisations such as Life for a Child (LFAC) or Changing Diabetes in Children (CDiC). The study also shows that the % of children using pumps or intensified conventional therapy is much lower in Asia/Middle East/Africa compared to other regions. This difference may not be the primary cause for the poorer control observed in the low-income countries of the Asia/Middle East/Africa region. It is important to remember an article published by Ogle et al (and highlighted in the 2019 edition of the Yearbook): 'intermediate care', characterized by human insulin in a basal bolus regimen, access to blood glucose monitoring and to point-of-care HbA1c testing, diabetes education, basic complications screening, and access to experienced doctors and nurses are the key components of diabetes care and should be available in less-resourced countries (1).

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13.3. Rapid increases in observed incidence and prevalence of Type 1 diabetes in children and youth in Mali, 2007–2016

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Pediatr Diabetes. 2021;1–7.
doi: 10.1111/pedi.13191

- The development of an awareness campaign and of a prospective registry in Mali enabled the collection of more accurate data on the incidence, prevalence and mortality of Type 1 diabetes (T1D) in children and youth <25 years
- T1D incidence rose from 0.12 in 2007 to 0.74 in 2016 and T1D prevalence from 0.43 in 2007 to 2.90 in 2016 (per 100 000 population/years)
- The registry improved the determination of true incidence and allowed for better care, reflected in the increase in prevalence

Type 1 diabetes (T1D) was once considered a rare disease in Sub Saharan African youth. It is now clear that T1D is not uncommon but that its diagnosis is often missed. Santé Diabète is an NGO that works in close partnership with the Malian Ministry of Health to ensure that medical care and diabetes education is provided, and with Life for a Child (LFAC) which supports the patients with free insulin, syringes, test strips, HbA1c testing and education resources. The 5-fold increase in the incidence and 7-fold increase in the prevalence of T1D observed in Mali between 2007 and 2016 show not only that prior to 2007, many cases of diabetes were misdiagnosed, but also that the registry leads to management of the newly diagnosed patients. This study also demonstrates the importance of collaboration between various stakeholders to ensure that diagnosis, management and access to insulin and to blood glucose monitoring become available even in Mali, one of the poorest countries in the world (Mali is a low-income country that ranked 170/192 in gross national income per capita in 2019).

13.4. Heat-stability study of various insulin types in tropical temperature conditions: New insights towards improving diabetes care

Kaufmann B, Boulle P, Berthou F, Fournier M, Beran D, Ciglenecki I, Townsend M, Schmidt G, Shah M, Cristofani S, Cavailler P, Foti M, Scapozza L
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PLoS ONE 2021;16: e0245372.
doi: 10.1371/journal.pone.0245372

- According to pharmacopeia, unopened insulin vials must be stored in a refrigerator (2–8 °C) while storage at ambient temperature (25–28 °C) is usually permitted for the 4-week usage period during treatment
- The authors investigated whether insulin remains stable and retains biological activity in oscillating tropical temperatures
- They found that the structure of insulin and its efficiency on insulin receptor and Akt phosphorylation in hepatic cells remain stable at temperatures oscillating between 25 and 37 °C over a 4-week period, which enables the barrier of cold storage to be removed

The existing recommendations represent a significant hurdle to insulin-treated patients in low resource settings where strict storage at 2–8 °C is usually unavailable in their homes. While several studies have reported significant degradations of insulin preparations under high isothermal conditions, the originality of the study reported by Kaufmann et al. consisted of studying the stability and the biological activity of different insulin preparations stored and used by patients under continuously fluctuating temperatures between 25 and 37 °C. These temperatures were chosen following daily measurements performed in a refugee camp in Northern

Kenya, run by MSF. Insulin potency was evaluated by high performance liquid chromatography, the 3-D structure by circular dichroism spectrometry and bioactivity by *in vitro* phosphorylation of the insulin receptor and the akt-signaling intracellular pathway of two different hepatocyte cell lines. The following insulin formulations used in the field by MSF in low resource countries were studied: rapid, NPH/isophane and premixed rapid/ NPH insulins. Measurements were performed at different time points up to 12 weeks on samples submitted to controlled temperature oscillations in the laboratory and on samples obtained up to 4 weeks from patients from the refugee camp and compared to formulations kept at 2–8 °C during the same time periods.

Under oscillating temperature conditions, the measured values of insulin quantification ranged between 98.3 and 99.9% of the initial concentrations and were all within the pharmacopeia acceptable range ($100 \pm 10\%$) after 4 weeks and even after 12 weeks for the preparations tested under laboratory conditions. In addition, the three dimensional conformation of the mixed insulins as well as their biological activities were conserved under the same fluctuating storage conditions when compared to control samples stored at 2–8 °C. Furthermore, no significant difference in stability was found between insulin conditioned in pen cartridges and 10 ml vials. The authors conclude that insulin preparations may be kept for at least 4 weeks under fluctuating temperatures in patients' homes, even in the absence of access to refrigeration, while retaining structural and efficacy integrity. These findings open new perspectives of diabetes care for tropical regions where refrigeration is not always available, affordable, or reliable. This does not preclude that the cold-chain of insulin prior to the period of use by the patient should be ensured as mentioned by the authors, although this remains an additional challenge for low resource countries.

(This commentary was prepared by Marc Maes, MD, PhD, Emeritus Professor of Pediatric Endocrinology and Diabetology, Cliniques Universitaires St Luc, 1200 Brussels, Belgium)

13.5. Access to insulin delivery devices and glycated haemoglobin in lower-income countries

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doi: https://dx.doi.org/10.4239/wjd.v11.i8.358

- To assess access to insulin delivery devices and HbA1c in 41 countries supported by the Life for a Child (LFAC) program
- Syringes (83.1%), insulin pens (16.7%) and pumps (0.2%) were used for insulin delivery
- If supplies were not donated, there was variable access to HbA1c testing within public health facilities and cost was usually covered by the patient

This article highlights how the high costs (extremely variable from country to country) and low availability of basic necessities (insulin, blood glucose monitoring and HbA1c) for a patient with Type 1 diabetes living in a low resource setting can lead to difficult or unsafe choices. The 2018 ISPAD guidelines (1) include a section on 'Limited Care Guidance' that makes suggestions for patients who need to prioritize several aspects of the care. Arguably, the priority is to ensure access to insulin and food. Blood glucose monitoring, while preferred, can be performed 3-4 times a day several days a week when possible, or substituted for urine glucose monitoring. HbA1c should be performed at least once a year in a reliable laboratory. Implementing such recommendations requires training of allied health professionals who can address diabetes management when there is no food security, understand how to manage a patient when only limited types of insulins (such as premix insulin) are available, be knowledgeable in the interpretation of urine and blood glucose monitoring and work with the local laboratory to ensure that HbA1c measurements are accurate. This information is not commonly found in textbooks published in high-income countries. Such courses are presently being developed in Africa through an initiative of ISPAD-LFAC and in Sudan through an initiative of the Sudanese Center for Diabetes in Children, in collaboration with Global Pediatric Endocrinology and Diabetes (GPED) and thanks to funding from the World Diabetes Foundation.

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Endocrinology

13.6. Tiered healthcare in South Africa exposes deficiencies in management and more patients with infectious etiology of primary adrenal insufficiency

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PLoS ONE 2020; 15: e0241845.
doi: 10.1371/journal.pone.0241845

- To determine the etiology, presentation, and available management strategies for primary adrenal insufficiency in South Africa
- A 23-question survey was sent to 23,321 physicians. It covered several domains including patient demographics, etiology, presentation, therapy, and barriers to diagnosis and treatment,
- 704 responses were received that highlighted significant disparities in the physicians' expertise, availability of diagnostic resources and management options between public versus private settings

This article highlights many key differences relevant to the management of primary adrenal insufficiency (PAI) in South Africa, an upper-middle income country characterized by the coexistence of public (accessed by 85% of the population) and private health systems. The study covered all ages, but included 48 children and adolescents $<$ 16 years. The prevalence of PAI derived from this survey was higher than previously reported, but remained about 10-fold lower than that reported in Western countries which is 136 per million. This lower number could be due to the study design (survey) but also more likely to the lack of timely diagnosis. Second, within South Africa, tuberculosis remains the leading cause of PAI among the poorest patients while autoimmune PAI is the leading cause in those accessing the private health system. This serves as a reminder that international speakers who teach in low-income countries should be familiar with the situation faced by the trainees in these settings. Third, even simple diagnostic tests were not always available in South Africa: electrolytes were deemed sometimes or never available in 32% of private practice physicians and in 67% of public practice physicians. For CT scan of the adrenals, the figures were 27% and 52%, respectively. Finally, while differences between the public and private systems were modest, overall access to corticosteroids and in particular to fludrocortisone remained poor. For instance, fludrocortisone (which is included in the WHO List of Essential Medicines since 2009), was sometimes or often unavailable for 52% of the patients in the public system and 57% of those in the private system.

Overall, this article is a reminder that optimal patient care requires well-trained physicians, access to diagnostic tools and access to appropriate medicines. Again, as demonstrated by Armstrong et al. in the following article (13.7), pediatric endocrinologists need to work with other stakeholders to improve global patient care.

13.7. We all have a role to play: redressing inequities for children living with CAH and other chronic health conditions of childhood in resource-poor settings

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Int. J. Neonatal Screen 2020; 6: 76.
doi: 10.3390/ijns6040076

- CLAN (Caring and Living as Neighbours) is an Australian non-governmental organisation (NGO) committed to a rights-based approach to optimizing quality of life for children and young people living with CAH and other chronic health conditions in resource-limited settings

– This paper used exploratory case study as a method to describe the seven phases of the action cycle in the Knowledge To Action (KTA) framework used by CLAN as it relates to congenital adrenal hyperplasia (CAH)
– It highlights the role of the community of patients with CAH

This very comprehensive paper describes the 7 stages of the KTA framework used by CLAN through cases studies in Vietnam, Philippines, Indonesia and Pakistan: 1. Identify the problem; 2. Adapt knowledge to local context; 3. Assess barriers to knowledge use; 4. Select, tailor and implement interventions; 5. Monitor knowledge use; 6. Evaluate outcomes and 7. Sustain knowledge use. You should look at this article as a 'How to' paper that guides pediatric endocrinologists who wish to enable better access to care for their patients. This article also highlights the need to involve all stakeholders: the community of patients but also the health professionals from low- and high-resource settings, the global and national pharmaceutical industry, and the health authorities. Importantly, in CLAN's framework, emphasis is put on the role of the community of children with CAH and their families, which plays a central role in improving access to optimal care in children with CAH. It Is suggested that one of the first steps towards this goal is to mobilize and engage patients. The power for change and successful advocacy of patient's organisations has been well demonstrated in high resource settings. Unfortunately, this is not commonly performed in low resource settings. This article illustrates how we, pediatric endocrinologists, can play a major role in driving this process.

13.8. Analysis of the screening results for congenital adrenal hyperplasia involving 7.85 million newborns in China: a systematic review and meta-analysis

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Front Endocrinol 2021; 12:624507.
doi: 10.3389/fendo.2021.624507

– This systematic review identified 41 Chinese studies totalling 7,853,756 newborns who underwent neonatal CAH screening
– The overall incidence of CAH in China was 1/23,024 (95% CI, 1/25,757 to 1/20,815)
– Among the CAH patients, the Male:Female ratio was 1.92:1, and 76% presented with salt wasting

The number of neonates screened is impressive (>7.8 million). This large number of tests provides a precise overall incidence for CAH in China of 1:23 024, lower than in Caucasian populations, and than in populations with high rates of consanguinity (CAH incidence was reported to be 1:7908 in Saudi Arabia and 1:9030 in the United Arab Emirates). However, this incidence should be interpreted in the light of the fact that the programs covered only $<20\%$ of the population, that richer regions are overrepresented and that there are large variations from region to region and several relatively small size programs (22% of programs screened $<$ 50 000 neonates).

The most surprising finding was a Male:Female ratio close to 2 for CAH. Such a difference has not been reported elsewhere and, as mentioned by the authors, is not easy to understand, considering that CAH is an autosomal recessive condition (a ratio close to 1 would be expected). Several points, not mutually exclusive, may explain this finding. First, the overall Male:Female sex ratio at birth is 1.1 in China. Second, it is possible that girls are diagnosed clinically early (due to virilization) and therefore do not undergo screening. Third, Chinese parents may pay more attention to boys, such that the recall rate of positive boys may be higher than that of girls. Finally, the authors highlight a study in the USA that found a higher sensitivity of the screening for boys than for girls.

13.9. Health-related quality of life of female patients with congenital adrenal hyperplasia in Malaysia

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Health Qual Life Outcomes 2020; 18: 258.
doi: 10.1186/s12955-020-01515-9

- The authors compared the quality of life (QOL) in 59 patients with CAH raised as females to 57 female patients with diabetes
- Patients with CAH included 12 children, 29 adolescents and 18 adults
- They found that the health-related QOL of female patients with CAH was similar to the QOL of patients with diabetes

This article adds to the recent interest in the QOL of patients with CAH in low-income settings. In the Yearbook 2020 (13.16, P 160), Musa et al. suggested that patients with CAH had a lower QOL compared to the general population (although the study did not include a control group). In this study, the authors compared QOL in children with two vastly different chronic conditions, CAH and diabetes. Another study by the same authors in the same population (see 13.10 below), reported that the majority of the participants with CAH had undergone female genitoplasty, usually at a young age. Although both patient groups were affected by a chronic condition, there was no healthy control group. Arguably, onset of the disease, management burden, perception by society and family differ vastly between CAH and diabetes raising questions about the meaningfulness of the comparison. Understanding the QOL of female patients with CAH in countries where discussion about sexuality is taboo, where fertility is very important and where treatment access often remains suboptimal for financial reasons is a key step towards changing attitudes. It is suggested that future studies should be designed carefully with an appropriate control group and with the goal of identifying steps that may ultimately improve QOL, should it be shown to be poor.

13.10. A multicenter cross-sectional study of Malaysian females with congenital adrenal hyperplasia: their body image and their perspectives on feminizing surgery

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J Pediatr Adolesc Gynecol 2020 Oct;33(5):477-483.
doi: 10.1016/j.jpag.2020.04.008

- Body image and perceptions around feminizing genitoplasty were assessed in 59 children, adolescents and adults with CAH (raised and identifying as females) and their parents
- Participants were more concerned with their overall appearance than with appearance of genitalia
- A younger age and a one step procedure for feminizing genitoplasty was preferred by both the participants and their parents

This article provides original information on the perceptions of body image and the age of feminizing genitoplasty in patients with CAH and their parents in Malaysia. The discussion section is interesting as the authors interpret the findings in the context of the Malaysian culture, which is typically a conservative society where intimate questions such as sexuality and the appearance of genitalia are not openly discussed. This is reflected in the high number of participants and their parents who did not answer questions on these topics despite agreeing to participate in the study. Indeed, participants have few opportunities to discuss these issues in

their day-to-day life, including with their friends, and have little access to information about the normal appearance of genitalia.

A limitation of the study is that the authors do not discuss the results on body image and genitalia appearance as a function of the age of the participants, which varies from prepubertal age (10–12 years) to adolescence and adulthood. Although the authors mentioned that only 1 patient was married and only 2 were sexually active, it should be emphasized that they included only 18 patients older than 18 years and that the median age of a bride in Malaysia is 27 years. I feel there are two take home messages. First, personal views on sexuality and appearance of genitalia are strongly influenced by the society our patients live in. Second, pediatric endocrinologists can play an important role in providing information to patients with CAH and their families, in particular in countries where they are unlikely to find it elsewhere. Proper information could lead to reassurance, informed decision making and improved satisfaction with body image.

13.11. Clinical characteristics of 46,XX males with congenital adrenal hyperplasia

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- The characteristics of 44 patients with 46,XX congenital adrenal hyperplasia (CAH) who were raised as males were described
- 15/44 (34%) were diagnosed before 2 years of age
- The median final height was 149.2 (range 133–172) cm

This paper discusses an important question that is not commonly raised in countries with a neonatal screening for congenital adrenal hyperplasia (CAH). Indeed, when CAH is diagnosed in the neonatal period, 46XX CAH babies are usually raised as females even when severe virilization is present. The situation is different when a disorder of sex development is not recognized at birth and when CAH diagnosis is performed later in life, as in the majority of the patients included in this study. On the one hand, long term studies of 46XX CAH raised as girls do not offer a reassuring picture of their future and poor outcomes are reported in terms of marriage, fertility and sexual life. In Turkey, similar to many countries in the world, being able to give birth is a key aspect of adult life for women and the consequences of infertility are usually much more severe than for men. Mete *et al*. (1) recently reported unhappiness, stress, financial difficulties and impaired social and family relationship in infertile women in Turkey. On the other hand, all 46XX CAH babies raised as males will be infertile and many will reach a short adult height. However, a literature review by Lee and Houk (2) concluded that extremely virilized 46,XX CAH patients who were reared as males demonstrated satisfactory levels of social and sexual function as adults when a healthy social support was present and that a male sex assignment should be considered in these circumstances. In the present study, Savaş-Erdeve et al. did no include quality of life data. There was also no mention of whether sex reassignment was ever considered. It will be important to address these questions in future studies.

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13.12. Trends of congenital hypothyroidism and inborn errors of metabolism in Pakistan

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- In Pakistan, there is presently no systematic neonatal screening for congenital hypothyroidism and other inborn errors of metabolism
- A review of published articles was performed to calculate a probable estimate of the disease burden in the Pakistani population

Mansoor highlights the fact that there are presently no national data on the prevalence or incidence of congenital hypothyroidism (CH) and other inborn errors of metabolism (IEM) in Pakistan. The author also emphasizes that most data come from a limited number of hospitals in the country. Focusing on CH, the most common congenital condition managed by pediatric endocrinologists, we can expect that a national neonatal screening program for would identify at least 2000 neonates with CH every year in Pakistan (assumptions: population of 216 000 000 in 2019, birth rate of 27.4 per 1000 people, incidence of CH 1/3000). As Pakistan has one of the highest consanguinity rates in the world, this number is likely to be higher. The author mentions that Pakistan has the capability of performing all required biochemical and genetic tests. There is no doubt about that. However, several issues must be carefully considered prior to initiating a national neonatal screening program for CH. First, although there has been a marked decrease in iodine deficiency in Pakistan over the last 20 years, several areas of low iodine intake are still present, which is likely to result in a high percentage of false positives for permanent CH (1). Second, home birth remains highly prevalent (ranging from 34% in the Sindh region to 74% in Baluchistan) (2), making the collection and shipping of samples to a reference laboratory a major logistics issue. Finally, ensuring timely recall of the positive neonates is also a key issue. Similar to other countries with a significant number of home births, pediatric endocrinologists will welcome TSH point of care testing when it becomes available.

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13.13. Aetiologies and clinical patterns of hypopituitarism in Sudanese children

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- This paper describes the characteristics of 156 children and adolescents with hypopituitarism from 2 hospitals in Sudan
- Congenital causes (86.5%) were more prevalent than acquired causes (13.5%)
- Consanguinity was found in 77.8% of patients overall and 91% of patients with congenital aetiologies. There were six family clusters with multiple pituitary hormone deficiencies and three families with isolated growth hormone deficiency

The population studied here presents unique characteristics that highlight the importance of considering the specificity of the environment (e.g. culture, society, access to medical care). First, among the 156 patients, consanguinity (1st or 2nd degree cousinship) was found in 112 (71.8%) (and in 91% of those with congenital

aetiologies). This is higher than the reported overall prevalence of consanguinity in Sudan (44.2 to 63.3%) (1), suggesting that consanguinity played a role in the disease. Second, patients were mostly adolescents, suggesting late diagnosis and onset of treatment. Indeed, the reasons for referral were primarily short stature and delayed puberty. This is also consistent with the finding of short stature, regardless on whether there was isolated GH deficiency or multiple hormone deficiencies (the height of the patients was between -2SD and -5SD in more than half of the cohort, and < -5 S.D. in more than 1/3 of the patients). It would be interesting to know whether the older patients originated from areas where specialist care is less likely to be available, such as in rural areas. Finally, the Male:Female ratio 1.8:1, contrasting with the expected ratio of 1. This may suggest societal factors, with more importance being given to short stature and delayed puberty in a boy compared to a girl. Understanding the characteristics of hypopituitarism in specific settings is an important step towards promoting appropriate training of health professionals.

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13.14. Effect on mortality of increasing the cutoff blood glucose concentration for initiating hypoglycaemia treatment in severely sick children aged 1 month to 5 years in Malawi (SugarFACT): a pragmatic, randomised controlled trial

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doi: 10.1016/S2214-109X(20)30388-0

- Hypoglycemia is associated with increased mortality in severely sick children. WHO recommends treatment of hypoglycemia for a blood glucose < 2.5 mmol/l
- The authors hypothesized that increasing the threshold for the treatment of hypoglycemia from 2.5 to 5 mmol/l would reduce all cause in-hospital mortality
- Analysis of the trial results after enrolment of 28% of the participants showed no difference in mortality between children with a baseline glycemia between 2.5 and 5 mmol/l receiving standard treatment and those receiving the tested hypoglycemia management

The cut off blood glucose concentration for the diagnosis and treatment of hypoglycaemia currently recommended by WHO (2·5 mmol/l) is not evidence-based. The authors previously showed that 'mortality in severely ill children with low blood glucose concentrations is higher than in those with normoglycaemia'. This observation served as a basis for their hypothesis that increasing the threshold for treatment of hypoglycemia from 2.5 to 5.0 mml/l might decrease mortality in very sick children. However, the results of this randomized, controlled trial performed in children aged 1 month to 5 years do not support this hypothesis. This strongly suggests that hypoglycemia between 2.5 and 5.0 mmol/l is merely a marker of severity of the disease but that it does not cause death. Low blood glucose concentrations may reflect chronically poor feeding, insufficient secretion of or resistance to counterregulatory hormones, low fat stores or other pathophysiological mechanisms. Understanding these determinants will be a first step in proposing novel approaches for the treatment of severely sick children in low resource settings.

13.15. Height and body-mass index trajectories of school-aged children and adolescents from 1985 to 2019 in 200 countries and territories: a pooled analysis of 2181 population-based studies with 65 million participants

NCD Risk Factor Collaboration (NCD-RisC). See article for list of members and their affiliations.
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- This study aimed to estimate trends in mean height and mean body-mass index (BMI) between 1985 to 2019 for children and adolescents aged 5–19 years
- There was a difference of $\geq$ 20 cm in mean height and of 9–10 kg/m^2 in mean BMI in 19-year-old adolescents between countries with the tallest/largest populations and the shortest/leanest populations
- The heterogeneity of trajectories between populations suggests variable nutritional quality and lifelong health benefits and risks

This study contrasts with most of the literature which focuses on the first five years of life and on the preconception period. It is important to remember that genetics plays a relatively small role in growth in height and BMI during the first 5 years of life. Indeed, the WHO Multicentre Growth Reference Study (1997 to 2003) that led to the design of the 0-5 years WHO growth charts demonstrated that under optimal social and nutritional conditions during preconception, pregnancy and the first 5 years of life, the variability in height and BMI between the six participating countries (Brazil, Ghana, India, Norway, Oman and USA) is minimal. A major limitation of the present study is that the causes underlying the observed heterogeneity in height and BMI changes between 5 and 19 years over several decades across most of the world's countries remains unclear. This knowledge will be necessary to optimize public health programs in particular in countries that are affected by the unhealthiest changes. Specifically, understanding the differences between countries, such as Vietnam, Azerbaijan (girls) or Montenegro (boys) where height increased proportionally more than BMI (changes perceived as healthy) and most countries of sub-Saharan Africa where BMI increased proportionally more than height (changes perceived as unhealthy), could inform public health policies. The respective roles of events that occurred in early life and between 5 and 19 years are also unclear. It is suggested that any changes in public health practices that may derive from the present data should not be limited to changes in children and adolescents aged 5 and 19 years but should also include early life experiences.

13.16. Maternal stature, maternal education and child growth in Pakistan: a cross-sectional study

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- Pakistan has a significantly higher prevalence of stunted children under 5 years old compared with other countries of the same income level. Stunting is more frequent in children of shorter compared to taller mothers
- The authors hypothesized that higher maternal education, a modifiable factor, is associated with decreased stunting in children of shorter mothers
- However, in this cross-sectional study, they found no significant difference in the marginal effect of maternal education on stunting among mothers with different statures

Malnutrition can lead to: (i) stunting (low-height-for-age); (ii) underweight (low-weight-for-age); (iii) wasting (low weight for height), and (iv) overweight (high weight-for-height). Stunting is a predictor of poor future human capital and of undesirable health outcomes. First, the authors found that mothers with short stature have a higher prevalence of stunting, wasted, underweight, and overweight children as compared to taller mothers. Second, they found that the higher prevalence of stunting, wasted, underweight, and overweight among under-

five children were associated with mothers of low education level, poor socioeconomic status, and short maternal stature. However, there was no significant interaction effect between maternal education and maternal stature on children's growth. This suggests that although maternal education has a positive effect on child growth, the role of education is similar across maternal statures. It is reassuring to see that, in a country where > 50% of mothers have no education (partly because 24.3% live below the poverty line), maternal education is an important factor in the growth trajectory of the children across the board, independently from maternal height. According to the Developmental Origins of Health and Disease (DOHaD) theory, adverse nutritional settings in pregnancy may lead to many diseases in adulthood. Therefore, correction of undernutrition (stunting and wasting) and over-nutrition (overweight and obesity) in childhood can prevent adult disease.

This study suggests that a global improvement of maternal education will have a positive effect on the prevention of adult diseases in their children but that this social measure should be implemented together with improved nutrition and with the development of health-promoting policies.

14. Medicine and Science

Ze'ev Hochberg, Ken Ong

14.1. Association of vitamin D levels, race/ethnicity, and clinical characteristics with COVID-19 test results

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This prospective observational cohort study ($n = 4638$) measured vitamin D level in the year before undergoing COVID-19 testing. The risk of a positive COVID-19 test in Black, but not White, individuals was 2.64-fold higher if vitamin D level was 30–40 ng/ml compared to $>$ 40 ng/ml.

Vitamin D may improve immune function and decrease inflammation. A meta-analysis of randomized clinical trials showed that daily or weekly vitamin D supplementation decreased risk of viral respiratory infections, especially in those who were deficient in vitamin D, but also in those with normal levels. Furthermore, vitamin D deficient levels have been associated with higher COVID-19 incidence and worse outcomes, especially in Black, Hispanic, and other non-White populations, who have also borne a disproportionately high burden of COVID-19. Both deficiency ($<$ 20 ng/ml) and insufficiency (20 to $<$30 ng/ml) of 25-hydroxyvitamin D are more common in Black individuals than White individuals. Almost half of the world's population has vitamin D levels $<$30 ng/ml.

From the very beginning of the COVID-19 epidemic, the question was raised whether vitamin D levels higher than the sufficient level (30 ng/mL) are associated with risk of COVID-19 infection. This research extends an earlier study showing that vitamin D deficiency ($<$20 ng/ml) is associated with higher risk of COVID-19 infection. All-cause mortality and other outcomes may be better with vitamin D levels 40–60 ng/ml, at least in some racial groups. It remains unknown whether increasing vitamin D level $>$40 ng/ml affects COVID-19 risk. That vitamin D levels are associated with COVID-19 infection, especially in individuals taking vitamin D supplements, supports the idea that supplementation might decrease COVID-19 risk by increasing vitamin D levels. The significant association only in Black individuals might reflect their higher COVID-19 risk, to which socioeconomic factors and structural inequities clearly contribute.

However, we should be aware that, beyond its traditional functions in calcium homeostasis and bone health, Vitamin D status has been associated with multiple health outcomes, ranging from metabolic syndrome traits to reproductive ageing, fetal growth and cancer risks. A major issue of studying circulating vitamin D in observational studies is that lower levels are strongly influenced by many potential confounding factors, such as higher BMI (due to storage in adipose tissue), exercise, household crowding and skin colour (which all affect sunlight exposure). The lack of confirmation of most of these benefits in trials have so far limited the translation of this evidence to public health policies. Hence, the authors will now recruit participants for two clinical trials to test the efficacy of vitamin D supplements to prevent COVID-19 and we eagerly await those results.

14.2. Lipocalin-2 is an anorexigenic signal in primates

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In the mouse, the osteoblast-derived hormone Lipocalin-2 (LCN2) suppresses food intake and acts as a satiety signal. This study now shows that a meal challenge increases serum LCN2 levels in humans with normal or

overweight, but not in those with obesity. Postprandial LCN2 serum levels correlate inversely with hunger sensation in challenged subjects. Brain PET scans show that injected radiolabelled recombinant human LCN2 (rh-LCN2) crosses the blood-brain barrier and localizes to the hypothalamus. Daily treatment of lean monkeys with rh-LCN2 decreased food intake by 21%, without overt side effects, suggesting that LCN2 may be a novel target for obesity treatment.

The lipocalins are a family of proteins which transport small hydrophobic molecules such as steroids, retinoids, and lipids. They share limited regions of sequence homology and a common tertiary structure architecture. LCN2 is mainly produced by bone cells of mice and humans and acts on the paraventricular nucleus (PVN) of the hypothalamus as a satiety signal that is upregulated after feeding in mice to limit food intake. Studies in mice have shown that it reduces food intake and prevents weight gain, without leading to a slow-down in their metabolism. The authors claim that LCN2 acts as a signal for satiety after a meal, leading mice to limit their food intake, and it does this by acting on the hypothalamus. In people with overweight or obesity, but not in people with normal weight, LCN2 levels increased after a meal. Non-responders, who showed no increase in LCN2 after a meal, tended to have a larger waist circumference and higher BMI, body fat, increased blood pressure and increased blood glucose. After gastric bypass surgery and weight loss they increased their LCN2, suggesting re-sensitization of these subjects.They also show that LCN2 can cross the blood-brain barrier. Monkeys treated with LCN2 for a week had a 28% decrease in food intake.

14.3. Decreasing body lengths in North Atlantic right whales

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The North Atlantic right whale (Narwhale, NARW) is a medium-sized toothed whale that lives year-round in the Arctic waters around Greenland, Canada, and Russia. It possesses a large 'tusk' from a protruding canine tooth. NARW born in recent years have experienced stunted growth, and over the same period have experienced increasing rates of entanglement in fishing gear. As a result, NARW appear to have less energy to devote to early growth. A portion of the estimated length reduction was directly attributable to entanglements.

These researchers evaluated changes in body lengths of NARW over a 20-year period. They combined age and length data collected from crewed aircraft in 2000–2002 and from remotely operated drones since 2016 to generate a growth model. The ages of measured whales ranged from <1 to 37 years old. They find that body lengths have been decreasing since 1981 and entanglements in fishing gear are associated with shorter whale lengths. Birth year had the greatest effect on the estimated length of NARW. Known energy-consuming entanglements of a whale with attached gear and also entanglements of its mother during nursing had negative effects on expected maximum length. The effect of entanglement as appeared to have a continuous effect, so that whales with longer entanglement durations had even greater reductions in length.

NARW have been declining in abundance since 2011 due primarily to vessel strikes. They are now protected from direct harvest but numbers remain far below their historical abundances. Climate-driven changes result directly in mortality. But it also impacts on life history, individual fitness, and population viability. Arrested growth may lead to reduced reproductive success and increased probability of lethal entanglements. These results show that sub-lethal stressors threaten the recoveries of vulnerable whale populations even in the absence of direct harvest.

14.4. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia

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In the Yearbook, we have been following the CRISPR story since its very beginning. Last year, Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry for discovering the CRISPR-Cas9 gene editing tool. Here, scientists have published the first successful treatment (as distinct from reports of using CRISPR-Cas9 to correct inherent mutations in patients), providing proof-of-principle that CRISPR-Cas9 gene editing is effective in human hematopoietic stem cells and capable of providing a cure for some of the most common and potentially fatal diseases in humans.

Patients with sickle cell disease (SCD) suffer from vaso-occlusive crises, while patients with transfusion-dependent thalassemia (TDT) exhibit chronic complications of iron overload, such as heart and liver failure, and several endocrinopathies. The only curative option for SCD and TDT is a stem cell transplant, which is available only for a minority of patients.

Here, Frangoul et al report a CRISPR-Cas9 approach that targets the BCL11A erythroid-specific enhancer, a transcription factor that represses expression of γ-globin (the structural subunit that is characterizes fetal hemoglobin) in erythroid cells and is responsible for the natural suppression of fetal hemoglobin beyond infancy. After myeloablation, two patients, one with SCD and one with TDT, received autologous (their own) CD34+ cells with genetically edited BCL11A. After > 1 year follow-up, both patients maintained high levels of edited alleles and several clinical improvements, including higher fetal hemoglobin levels, transfusion independence, and cessation of vaso-occlusive episodes.

These results represent an impressive proof-of-principle, opening the door not only for hemoglobinopathies but for a plethora of genetic diseases awaiting cure.

14.5. The role of the microbiota in human genetics

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https://bit.ly/3q47CTG

These authors review the human traits and genes that microbiota may have contributed or altered in response to changes in host diet, climate, or pathogen exposure.

It is now widely recognized that the microbiota of mammals is a product of coevolution. Nevertheless, humans are different in having a geographically specific microbiome and displays community compositions and a range of overlapping and redundant metabolic characteristics that can alter host physiology to become s combined host–microbiota entity. Microbiota evolve quickly and can provide capabilities that may be exchanged with other microbiotas, their hosts, and the environment. Microbes evolve faster than their host, which allows them to respond quickly to environmental change. They respond to changes in diet exposure to pathogens such as malaria parasites and changes in local climate.

Evolution of the microbiome, like all biological traits, arises randomly or is adaptive to the environment. Hosts may pass their microbiome to their offspring, which will affect the heritability and transmission modes of these microbes. The authors give the example of lactose digestion via microbial lactase activity in adults resulting from shifts in diet. The allelic variation of these genes also predicts compositional and functional variation of the gut microbiota.

Thus, host genes and the microbiota are linked and interact by dietary adaptations, where host and microbial enzymes metabolize the same dietary components (e.g., fatty acid and alcohol metabolism), through climate-related adaptations. Moreover, microbiota have the potential to affect host evolution by modifying the adaptive landscape.

A better understanding of reciprocal interactions between the host genome and microbiota in the context of adaptive evolution will add another dimension to our understanding of human evolution as we moved with our microbes through time and space.

14.6. Whole-genome sequencing of patients with rare diseases in a national health system

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The authors applied whole-genome sequencing (WGS) in 9,802 patients with a rare disease in a national health system to streamline diagnosis and to discover unknown aetiological variants in the coding and non-coding regions of the genome. WGS identified the genetic diagnosis in 1138/7065 extensively phenotyped participants. They identified 95 genes in which mutations were very likely to be the cause of a rare Mendelian disease.

The large majority of children born with rare diseases undergo a long journey until diagnosis, from the gradually emerging manifestation of the disease phenotype, through multiple health referrals and convoluted, repeated and invasive investigations. On average, it takes more than 2 years to reach a diagnosis. This paper describes a major milestone towards the use of genomics in routine clinical practice, especially in those patients who have a higher likelihood of a genetic disorder. They describe a number of cases for whom WGS findings informed treatment decisions (with a strong bias towards haematological disorders reflecting the interests of the investigators), including specific treatments for thrombocytopenia and early-onset dystonia. Other diagnoses informed prediction of high risk of disease progression, or stratification of patients into higher or lower risk of malignancy. Furthermore, they demonstrate the added value of WGS above gene centric sequencing (whole-exome sequencing), by identifying causal variants in gene-regulatory regions in the non-coding genome.

We look forward to the widespread use of genomics in paediatric endocrinology and diabetes, to shorten time to diagnosis and to direct personalised treatments and disease monitoring. Towards this aim there are still many challenges, discussed in this paper, ranging from laboratory protocols and quality control, development of fast and accurate data processing and statistical methodology, patient information and consent with potential ethical issues for the wider family, and complementary studies of WGS in large populations to ascertain the contribution of mutations to the extreme tails of normal phenotypic distributions.

14.7. Inherent mosaicism and extensive mutation of human placentas

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These authors performed whole-genome sequencing (WGS) of 86 bulk placental samples and of 106 microdissections of placental tissue in order to reconstruct the development of human placental cells from data on somatic mutations. They found that the placenta comprises of multiple very large, genetically distinct, clonal expansions (i.e. tissues derived from a single cell line) and that the cells carry a huge mutation burden, similar to that found in childhood cancers.

Abnormalities in placental development underlie the major disorders of pregnancy, preeclampsia, fetal growth restriction and stillbirth (1). Hence, understanding this process and the possible errors has important clinical relevance. Using a large scale WGS approach, the authors show that the placenta is an extreme outlier in terms of its high frequency of somatic mutations, compared to other healthy human tissues. Furthermore, these mutations do not appear to correlate with any functional segregation, as seen in other tissues (e.g. colonic crypt are clonal

expansions within the gut). Rather, the pattern of mutations reflects the placental 'tree-like' growth and anatomical structure, with each branch descending from a single cell with its own distinct somatic mutation fingerprint. The authors conjecture that the very rapid growth rate of the placenta compared to other tissues, increasing 3-fold between 8 and 12 weeks gestation, and its temporary existence, mean that these cells bypass the key mechanisms that typically protect against and remove DNA replication errors. We learn from these findings that, in the search for the placental changes that underlie major pregnancy disorders, genetic aberrations will not be uniformly distributed throughout the placenta, and this may explain inconsistencies in the findings of previous more limited studies.

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14.8. Mesenchyme-derived IGF2 is a major paracrine regulator of pancreatic growth and function

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The authors used mouse conditional gene knock-out models to investigate the role of Insulin-like growth factor-2 (Igf2) in pancreatic growth and function. When Igf2 was deleted specifically in mesenchyme-derived cells (but not when deleted in exocrine and endocrine cells), the entire pancreas was smaller and showed reduced exocrine and endocrine functions. These mice showed postnatal whole-body growth restriction and the female mice developed glucose intolerance when they became pregnant. Conversely, increased IGF2 levels in the mesenchyme-derived cells increased pancreatic size.

There is a fascinating specificity in the regulation of some key tissues and organs. First, Igf2 is a member of a small number of 'imprinted genes', which are active only on one parent-specific inherited copy. Hence, in this study, the consequences of Igf2 genetic engineering were only seen when they disrupted the function of the paternally-inherited gene - for some reason, the maternally-inherited Igf2 gene is naturally silenced. Second, the key findings of this study show that, within the pancreas, Igf2 is needed only in mesenchyme-derived cells. This understanding was made possible by the recent availability of mesenchyme-specific gene silencing tools. It is intriguing to find that, although Igf2 is clearly needed for normal exocrine and endocrine cell function, its expression is redundant within those cells. Instead, Igf2 is crucially active in the surrounding mesenchyme-derived stromal cells.

'Stroma' derives from Greek, meaning a layer or bed cover, and stromal cells typically contribute to connective tissues and organ structure. However, we are increasingly learning about other key functions of stromal cells, beyond their supportive anatomical characteristics. Other examples include the crucial roles of glial cells in neuronal cell function, and ovarian granulosa cells that surround oocytes. Notably, these authors also found that the expression of other key growth-related imprinted genes, such as Plagl1/Zac1, were highly enriched in the mesenchyme, and they hypothesize that genomic imprinting may have evolved a particular role in mesenchymal-specific function.

14.9. How to read numbers: a guide to statistics in the news (and knowing when to trust them)

Tom Chivers, David Chivers

Publisher: W&N (18 Mar. 2021).
ISBN-10: 1474619967

The authors describe this book as 'a short, practical, timely guide to the tools you need to understand the numbers we read in the news everyday - and how we often get them wrong'.

One of the societal consequences of the COVID-19 pandemic has been the rising public interest in numbers and statistics. 'Armchair experts' in statistics and epidemiology are no longer limited to academic settings, but few have a secure mathematical training. It is therefore concerning that, with this rising interest also comes the worrying explosion of confusing use of statistics and dubious interpretations of data on COVID infections, R numbers, excess deaths, vaccine effectiveness and vaccine side-effects. Public understanding of science needs clear and careful guidance to improve numerical literacy.

David Chivers is an assistant professor of economics and Tom Chivers is a freelance science writer. This combination is welcome because a person's interpretations of a 1 in 100 risk, versus 1 in 10 000, and 1 in a million, is not simply quantitative. To some, these all seem remote chances, but to others they are all worth taking significant steps to avoid. The authors helpfully suggest to describe other more commonly understood risks for comparison, such as the risks involved in driving or other forms of transport.

Some sections delve into deeper statistical understanding, such as the Poisson distribution and even Bayes' theorem. Most clinical researcher follow the 'frequentist' theory of statistical interpretation, in which probability is based on observed frequencies and proportions, and they look doubtfully on the alternative Bayesian approach, which also takes into account of (often broadly estimated) 'prior probabilities'. However, when we operate in the real world, the Bayesian approach actually does seem the sensible option. Consider this question: *what is the likelihood that you have a COVID-19 infection if your swab test is positive?* Even if we know the true false positive rate, the answer varies enormously depending on factors that alter the prior probabilities, i.e. the reason for taking the test (routine surveillance or because you have symptoms) and also on the prevalence of COVID-19 infections in your neighbourhood and workplace.

14.10. Corticosterone inhibits GAS6 to govern hair follicle stem-cell quiescence

Sekyu Choi, Bing Zhang, Sai Ma, Meryem Gonzalez-Celeiro, Daniel Stein, Xin Jin, Seung Tea Kim, Yuan-Lin Kang, Antoine Besnard, Amelie Rezza, Laura Grisanti, Jason D Buenrostro, Michael Rendl, Matthias Nahrendorf, Amar Sahay, Ya-Chieh Hsu

Nature 2021; 592: 428–432

https://www.nature.com/articles/s41586-021-03417-2

These authors report that in mice the stress hormone corticosterone (the rodent equivalent of cortisol in humans) acts on dermal papillae to suppress expression of Gas6 (encoding growth arrest specific 6) and thereby cause hair follicle stem cell (HFSC) quiescence and reduced hair growth. They show that in chronic stress, high levels of corticosterone prolong HFSC quiescence and maintain hair follicles in an extended resting phase.

There is much anectodal 'evidence' that chronic stress can lead to hair loss. Here, these researchers from Harvard University confirmed such a link and identified that the underlying mechanism involves our endocrinology. Hair follicles naturally cycle between growth and rest, reflecting whether HFSCs are in an active phase or in quiescence, when growth is slowed and hairs are easily shed. The authors show that these states are determined by corticosterone levels acting on the dermal papilla cells, which lie just beneath the HSFCs. By understanding the downstream mechanisms they show that, without reducing the high corticosterone levels induced by chronic stress, restoring Gas6 expression from dermal papilla cells is sufficient to overcome the stress-induced inhibition and drives HFSCs into frequent regeneration cycles, with no observable long-term adverse effects. The same team also recently identified a similar process whereby chronic stress activates the sympathetic nervous system and depletes melanocyte stem cells, leading to premature hair graying.

These novel findings have potential broad relevance for understanding how chronic stress may accelerate biological ageing and reduce lifespan, possibly also by stimulating glucocorticoid secretion and the sympathetic nervous system to inhibit stem cell activity.

14.11. Ageing hallmarks exhibit organ-specific temporal signatures

Nicholas Schaum, Benoit Lehallier, Oliver Hahn, Róbert Pálovics, Shayan Hosseinzadeh, Song E Lee, Rene Sit, Davis P Lee, Patricia Morán Losada, Macy E Zardeneta, Tobias Fehlmann, James T Webber, Aaron McGeever, Kruti Calcuttawala, Hui Zhang, Daniela Berdnik, Vidhu Mathur, Weilun Tan, Alexander Zee, Michelle Tan, The Tabula Muris Consortium, Angela Oliveira Pisco, Jim Karkanias, Norma F Neff, Andreas Keller, Spyros Darmanis, Stephen R Quake, Tony Wyss-Coray

Nature 2020; 583: 596–602
https://www.nature.com/articles/s41586-020-2499-y

In order to understand the cellular processes that underlie ageing, the authors performed plasma proteomics at 10 different ages across the lifespan of the mouse. They integrated these data with a parallel large study published alongside this paper in the same edition (1), which describes the 'Mouse Ageing Cell Atlas', a single-cell transcriptomic atlas that characterizes changes in gene expression with age across 23 tissues. Together, the data reveal clustered patterns of changes ('trajectory groups') in gene expression and protein levels, consistent with coherent biological functions, including extracellular matrix regulation, unfolded protein binding, mitochondrial function, circadian rhythm, and inflammatory and immune response.

One of these clustered ageing trajectories is an extensive immune cell activation, which is first detectable in white adipose tissue depots during middle age, and involves the accumulation of T cells and B cells in adipose tissue. Notably, many of these trajectory groups showed similar expression patterns across tissues, although with some differences in amplitude and timing, and these changes could be correlated with protein levels in plasma. Hence, potentially future plasma proteomic profiling could give insights into disordered ageing across several tissues.

The authors conclude that these data identify a coordinated yet often asynchronous inter- and intra-organ ageing process. The findings highlight biological processes that deserve renewed focus as mechanisms of ageing, such as immune cell activation, circadian rhythm disruption, and adipose tissue decline. The study also provides rich data on biomarkers to monitor the effects of rejuvenation strategies under current investigation, such as the removal of senescent cells and reducing nutrient sensing (e.g. using rapamycin and metformin).

Reference

1. The Tabula Muris Consortium. A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. *Nature* 2020; 583: 590–595.

14.12. Associations between maternal antenatal corticosteroid treatment and mental and behavioral disorders in children

Katri Räikkönen, Mika Gissler, Eero Kajantie

JAMA. 2020; 323(19): 1924-1933.
doi:10.1001/jama.2020.3937
https://jamanetwork.com/journals/jama/fullarticle/2766162

The authors assembled a retrospective population-based cohort using nationwide registries of births and public specialized medical care settings in Finland in order to study whether antenatal corticosteroid treatment is associated with mental and behavioral disorders in children. Antenatal corticosteroid treatment exposure was associated with higher risk of any mental and behavioral disorder (12.0% vs 6.4%; adjusted hazard ratio, 1.33 [95% CI, 1.26–1.41]). Findings were stronger in term born children, and also in a within-sibpair comparison of steroid-exposed vs. non-exposed term born siblings.

A significant proportion of infants are exposed to antenatal corticosteroid treatment, which is given to prevent neonatal comorbidities of prematurity. Current US guidelines recommend this treatment for pregnant women up to 36 weeks 6 days who are at risk for preterm delivery. In these Finnish national data, 14 868 of 674 877 (2.2%) singleton infants were exposed to antenatal corticosteroid treatment, of whom 6730 (45%) were born at term and 8138 (55%) were born preterm. However, corticosteroids cross the blood-brain barrier and may harm fetal brain development.

These findings show a robust association within term born children in a very large dataset. In particular, the within-sibpair comparison is a powerful design to control for potential familial and socioeconomic confounders.

However, it is unclear whether the findings extend to preterm born children, who experience a higher cumulative incidence of mental and behavioral disorders. Among preterm born children, it is possible that the opposing benefits of antenatal corticosteroid exposure on fetal peripheral tissue maturation versus disbenefits on disrupted fetal brain development balance each other out. The relevance of these findings to fetuses with suspected CAH, who are exposed to antenatal corticosteroids from much earlier in gestation, should be determined.

14.13. Past extinctions of homo species coincided with increased vulnerability to climatic change

Pasquale Raia, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Josè A F Diniz-Filho, Thiago F Rangel, Philip B Holden, Francesco Carotenuto, Neil R Edwards, Matheus S Lima-Ribeiro, Antonio Profico, Luigi Maiorano, Silvia Castiglione, Carmela Serio, Lorenzo Rook

One Earth, Volume 3, Issue 4, 23 October 2020, Pages 480-490

https://bit.ly/3vFcRue

By integrating past climate and fossil databases, these authors suggest that climate change was the primary factor in the extinction of Homo species.

Homo erectus, H. heidelbergensis and *H. neanderthalensis* all became extinct. Why? And are we going that way too? The authors claim that climate change drove those species to extinction. Climate change is happening again, with possible drastic consequences to the modern human race.

The authors used a recently implemented past climate emulator (a statistical modelling approach) and an extensive fossil database spanning 2754 archaeological records to model climatic niche evolution in Homo species. *Homo erectus* lived about 2 million years ago in Africa, throughout Eurasia, the Iberian Peninsula and Java, and became extinct ∼117 000–108 000 years ago. *H. heidelbergensis* lived in China, Spain and Zambia and became extinct 200 000 years ago. *H. neanderthalensis* disappeared from Europe ∼40 000 years ago. All three Homo species lost their climatic niche space just before extinction. These extinctions coincided with increased vulnerability to climate change. In the case of Neanderthals, this pressure added the effect of competition with *H. sapiens* even though they formed complex social networks, domesticated the fire, refined stone tools to make spear points, fitted clothes and inter-hanged with *H. sapiens*. Climate change and the incompetence to face it was such a major player that it overrode all the other evolutionary pressures.

14.14. The impact of sex on gene expression across human tissues

Meritxell Oliva, Manuel Muñoz-Aguirre, Sarah Kim-Hellmuth, Valentin Wucher, Ariel DH Gewirtz, Daniel J Cotter, Princy Parsana, Silva Kasela, Brunilda Balliu, Ana Viñuela, Stephane E Castel, Pejman Mohammadi, François Aguet, Yuxin Zou, Ekaterina A Khramtsova, Andrew D Skol, Diego Garrido-Martín, Ferran Reverter, Andrew Brown, Patrick Evans, Eric R Gamazon, Anthony Payne, Rodrigo Bonazzola, Alvaro N Barbeira, Andrew R Hamel, Angel Martinez-Perez, Jose Manuel Soria, GTEx Consortium, Brandon L Pierce, Matthew Stephens, Eleazar Eskin, Emmanouil T Dermitzakis, Ayellet V Segrè, Hae Kyung Im, Barbara E Engelhardt, Kristin G Ardlie, Stephen B Montgomery, Alexis J Battle, Tuuli Lappalainen, Roderic Guigó, Barbara E Stranger

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https://bit.ly/3wMzM8x

By integrating sex-specific Genotype-Tissue Expression (GTEx) data with gene function and transcription factor binding annotations, these authors describe mechanisms contributing to sex differences in the human transcriptome.

Many complex human traits and diseases exhibit sex-specific characteristics. These sex differences have been variously attributed to hormones, sex chromosomes, genotype × sex effects, differences in behavior, and differences in environmental exposures. The GTEx project provides an opportunity to investigate the prevalence and genetic mechanisms of sex differences in the human transcriptome (RNA expression levels) by surveying many tissues that had not previously been characterized in this manner.

Using GTEx data, the authors generated a catalogue of sex differences in gene expression across 44 human tissues, analyzed 16 245 RNA-sequenced samples and genotypes of 838 adults. They discovered sex-specific effects on gene expression in 13 294 genes across all tissues. These are involved in drug and hormone response, embryonic development and tissue morphogenesis, fertilization, sexual reproduction and spermatogenesis, fat metabolism, cancer, and immune response. They suggest hormone-related transcription factor regulation and sex-differentiated distribution of epigenetic marks.

Sex differences in the autosomal regulation of gene expression were much less common (369 sex-biased eQTLs across all tissues) and were highly tissue-specific. They identified 58 gene-trait associations driven by genetic regulation of gene expression in a single sex. These include loci where sex-differentiated cell type abundances mediate genotype-phenotype associations, as well as loci where sex may play a more direct role in the underlying molecular mechanism of the association. For example, they identified a female-specific locus in the liver for the hexokinase HKDC1 that influences glucose metabolism in pregnant females, and subsequently impacts on birth weight of the offspring.

These are tissue-specific and tissue-shared drivers and mechanisms contributing to sex differences in the human transcriptome. Some are multiple sex-specific genetic effects on gene expression that colocalize with complex trait genetic associations.

15. Editors' Choice

Ken Ong, Ze'ev Hochberg

15.1. Analysis of overlapping genetic association in type 1 and type 2 diabetes

Jamie RJ Inshaw, Carlo Sidore, Francesco Cucca, M Irina Stefana, Daniel JM Crouch, Mark I McCarthy, Anubha Mahajan, John A Todd

Diabetologia. 2021 Jun;64(6):1342–1347.
doi: 10.1007/s00125-021-05428-0.
https://pubmed.ncbi.nlm.nih.gov/33830302/

By studying data from very large-scale genome-wide association studies (GWAS) in European ancestry individuals, the authors compared genetic signals that confer risk of type 1 (T1DM) and type 2 diabetes (T2DM). Only 5 signals were associated with both T1DM and T2DM ('colocalised signals'). At 4 of these signals, variants that increased risk of T1DM decreased the risk of T2DM: (1) chromosome 16q23.1, near the CTRB1/BCAR1 genes; (2) 11p15.5, near Insulin and IGF2; (3) 4p16.3, near TMEM129 and (4) 1p31.3, near PGM1. Only one signal showed directionally concordant effects, increasing the risks of both T1DM and T2DM: 9p24.2, near GLIS3.

It is remarkable that, despite the knowledge of ~60 genomic regions associated with T1DM and >200 associated with T2DM, there is so little overlap in the genetic risk factors for these two forms of diabetes. Some have hoped that established treatments for T2DM (e.g. metformin) might also have obvious benefits for patients with T1DM. It was even hypothesised that both forms of diabetes shared a common genetic make-up but were exposed to different in utero and postnatal environments. With that in mind, it seems even more striking that, of the handful of cases of genetic overlap identified, most of the risk variants have directionally opposing effects on T1DM and T2DM. These findings suggest that biological studies of one condition could still shed some insights onto the other. However and unfortunately, the findings cast doubt on the effectiveness of developing common treatments that would benefit both diseases.

15.2. Interpreting type 1 diabetes risk with genetics and single-cell epigenomics

Joshua Chiou, Ryan J Geusz, Mei-Lin Okino, Jee Yun Han, Michael Miller, Rebecca Melton, Elisha Beebe, Paola Benaglio, Serina Huang, Katha Korgaonkar, Sandra Heller, Alexander Kleger, Sebastian Preissl, David U Gorkin, Maike Sander, Kyle J Gaulton

Nature. 2021 volume 594, 398–402
https://www.nature.com/articles/s41586-021-03552-w

The authors report a large genome-wide association study (GWAS) of type 1 diabetes (T1D) in 18 942 cases and 501 638 controls, finding 92 T1D-associated genomic loci (59 known and 33 novel). Furthermore they analyse DNA chromatin patterns in pancreas and peripheral white blood cells to help identify the underlying genes. T1D-associated loci were linked to genes that are active in T cells, but also in the acinar and ductal cells of the exocrine pancreas.

As well as performing the largest GWAS for T1D to date and finding 50% more risk loci for this disease, the major novel and eventually highly informative approach here was the integration of GWAS data with single-cell epigenomics data in very specific cell types. This approach relies on the understanding that certain DNA chromatin patterns indicate whether or not a gene is expressed (active) in a specific cell or tissue type. It involved analysing data on chromatin accessibility profiles from 131,554 individual cells. It is well known that T1D is an autoimmune condition related to T cell function. So the findings here that implicate genes active in T cells, and in

insulin expressing pancreatic beta cells, are confirmatory and as 'positive controls', they provide confidence in the novel approach.

The more unexpected finding was the involvement of several genes that are active in the acinar and ductal cells of the exocrine pancreas. For example, at the CFTR gene locus (which is mutated in cystic fibrosis), the T1D risk variant appears to reduce transcription factor binding, enhancer activity and CFTR expression in ductal cells, and increases the risks for acute and chronic pancreatitis. The authors note that the onset of T1D has been associated with exocrine pancreas abnormalities (1), and speculate that factors that increase pancreatic exocrine cell inflammation may contribute to intra-islet inflammation and immune infiltration and thus promote T1D.

Reference

1. Virostko J *et al*. Pancreas volume declines during the first year after diagnosis of type 1 diabetes and exhibits altered diffusion at disease onset. *Diabetes Care* 42, 248–257 (2019)

15.3. Metabolic effects of late dinner in healthy volunteers – a randomized crossover clinical trial

Chenjuan Gu, Nga Brereton, Amy Schweitzer, Matthew Cotter, Daisy Duan, Elisabet Børsheim, Robert R Wolfe, Luu V Pham, Vsevolod Y Polotsky, Jonathan C Jun

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https://doi.org/10.1210/clinem/dgaa354

The authors performed a randomized crossover trial of Late Dinner (22:00 hours) vs Routine Dinner (18:00) in a laboratory setting in 20 healthy adult volunteers. Meal contents and sleep period (23:00-07:00) were kept the same in both arms and sleep patterns were unaffected. Late Dinner increased postprandial glucose levels, lowered FFA and dietary fatty acid oxidation, and delayed the triglyceride peak.

Beyond the 'simple balance' of energy in vs. energy out, there is increasing interest in lifestyle patterns that alter our risk of excess weight gain possibly by altering the physiological processes that handle nutrient metabolism. Other studies have suggested adverse effects of skipping breakfast, balance of calories between morning and evening, and periods of intermittent fasting despite isocaloric conditions. A particular advantage of the current study is that they could control for sleep time and sleep architecture by collecting data via movement sensors and polysomnography.

The findings support the existence of circadian mechanisms in the control of metabolism, which deserves further study. The authors also warn that the metabolic pattern induced by Late Dinners, i.e. higher overnight glucose intolerance and lower fat mobilization and oxidation, may increase the risks of obesity and the metabolic syndrome.

15.4. Ketoacidosis in children and adolescents with newly diagnosed type 1 diabetes during the COVID-19 pandemic in Germany

Clemens Kamrath, Kirsten Mönkemöller, Torben Biester, Tilman R Rohrer, Katharina Warncke, Johanna Hammersen, Reinhard W Holl

JAMA. 2020;324(8):801–804.
https://jamanetwork.com/journals/jama/fullarticle/2768716

The authors analysed data from the German Diabetes Prospective Follow-up Registry (DPV) of children and adolescents with Type 1 diabetes (T1D) between March 13 to May 13, 2020, when most kindergartens and schools in Germany were closed due to COVID-19 restrictions. Compared to the previous 2 years, the risk of diabetic ketoacidosis (DKA) at T1D onset was nearly double during COVID-19 lockdown (adjusted relative risk: 1.84; 95% CI, 1.54-2.21) and the increased risk was highest in young children age $<$ 6 years (2.75; 95% CI, 1.88-4.02).

These are very robust data, collected from 216 of 217 eligible diabetes centers and involving 532 children and adolescents (median age 9.9 years) with newly diagnosed T1D during the COVID-19 lockdown period. DKA was present in 238 patients (44.7%) and severe DKA in 103 patients (19.4%), which is higher than in the previous 2 years (24.5% and 13.9%, respectively, in 2019). Children < 6 years had the highest rates of DKA (51.9%) and severe DKA (24.4%) during COVID-19 lockdown, and much higher than in 2019 (18.4% and 12.2%, respectively).

The authors discuss that the underlying reasons are likely to be multifactorial, including reduced access to medical services, fear of COVID-19 exposure, and other psychosocial factors. These same factors have also likely affected patients with diverse other acute and chronic health conditions across all Paediatric specialties and beyond. Few of those other clinics have such good systems for data collection and analysis as the DPV. So our ongoing challenge is to identify those services and families that have been adversely affected by the wide repercussions of COVID-19 and implement remedial actions.

15.5. Effect of school-based body mass index reporting in California public schools: a randomized clinical trial

Kristine A Madsen, Hannah R Thompson, Jennifer Linchey, Lorrene D Ritchie, Shalika Gupta, Dianne Neumark-Sztainer, Patricia B Crawford, Charles E McCulloch, Ana Ibarra-Castro

JAMA Pediatr. 2021;175(3):251–259.
doi:10.1001/jamapediatrics.2020.4768
https://jamanetwork.com/journals/jamapediatrics/article-abstract/2773004

The authors report a cluster randomized clinical trial in 79 California schools, involving 28,641 students in grades 3 to 8. The intervention comprised school measurements of BMI and sending the results to parents, but this had no effect on BMI Z-scores after 1 year or 2 years of follow-up. There were mixed effects on adverse outcomes: the intervention increased weight dissatisfaction and peer weight talk, but the risk of concerning weight control behaviors was reduced.

Public health approaches to avoid and reduce childhood obesity are widely implemented in schools, often through nationwide statutory activities. For example, the UK National Child Measurement Programme follows a very similar approach to that tested in the current trial. However, few of these interventions are evaluated with the rigour applied by these authors. Not only did they show a lack of evidence that the programme is effective in reducing BMI, but they provided intriguing data on the potential harms. A thoughtful editorial on this paper discusses the pros and cons of BMI screening in schools, including the potential adverse impacts of weight stigma and body dissatisfaction (1). Also, an astute online comment on this paper by Edward Schor at Stanford University reminds us that all screening programmes carry potential for harm, and should be offset by clearly beneficial actions and resources for those who screen positive.

I would add that there must surely be some degree of weight dissatisfaction to motivate the lifestyle efforts needed to achieve weight management - but too much may impair mental health. Hence it is reassuring to see the reduction in concerning weight control behaviors in this trial. However, deeper understanding is needed on the overall impacts on any preventive programme on health and wellbeing.

Reference

1. Tracy K. Richmond, Idia B. Thurston, Kendrin R. Sonneville. Weight-Focused Public Health Interventions—No Benefit, Some Harm. *JAMA Pediatrics*. 2021;175(3):238–239.

15.6. The gut–brain axis mediates sugar preference

Hwei-Ee Tan, Alexander C Sisti, Hao Jin, Martin Vignovich, Miguel Villavicencio, Katherine S Tsang, Yossef Goffer , Charles S Zuker

Nature, 2020. 580, 511–516.
https://www.nature.com/articles/s41586-020-2199-7

The authors identify in mice a population of neurons in the vagal ganglia and brainstem that are activated by the direct delivery of sugar but not artificial sweeteners to the gut. They genetically engineered changes in this gut-

to-brain circuit. Silencing of synaptic activity in this circuit prevented the behavioural preference for sugar. Conversely, by chemogenetic activation of this circuit, they could create preferences to alternative stimuli.

Sugar, the simple carbohydrate, provides our most immediate source of dietary energy and most species have evolved to recognise and favour its consumption. Specific sweet taste receptors in our mouths provide an immediate hard-wired signaling route to our brains. But our responses to sugar are more complex, including longer-term effects on brain centres for reward, pleasure and preferences. In mice, artificial sweeteners are less effective than sugars in generating long-term preferences, despite their stronger binding affinity to sweet taste receptors and similar short-term appeal (1). Furthermore, taste receptor-knockout mice retain a preference for sugars, indicating other post-ingestive mechanisms.

Here the authors identify a fascinating new circuit that links post-ingestive sugar-sensing to the brain via the vagal pathway. They identified sodium–glucose-linked transporter-1 (SGLT1) as a key link in activating this circuit. SGLT1 is the principal glucose transporter in the gut, corresponding to the role of SGLT2 in the renal proximal tubule. It seems conceivable that SGLT1 inhibitors could have health benefits, similar to the exciting evidence that is currently being generated on SGLT2 inhibitors. However, losing our euphoric buzz to sugars may not be an appealing prospect for many people. So alternatively, the authors suggest that new artificial sweeteners might be developed that can activate both our tongues and our gut sensors.

Reference

1. Sclafani A, Zukerman S & Ackroff K. Postoral glucose sensing, not caloric content, determines sugar reward in C57BL/6J mice. *Chem. Senses* 40, 245–258 (2015).

15.7. A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis

Philip N Newsome, Kristine Buchholtz, Kenneth Cusi, Martin Linder, Takeshi Okanoue, Vlad Ratziu, Arun J Sanyal, Anne-Sophie Sejling, Stephen A Harrison, for the NN9931-4296 Investigators

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DOI: 10.1056/NEJMoa2028395
https://www.nejm.org/doi/full/10.1056/NEJMoa2028395

The authors report a 72-week, double-blind placebo-controlled trial in 320 adult patients with biopsy-confirmed nonalcoholic steatohepatitis (NASH) and liver fibrosis (stage F1, F2, or F3). Patients were randomly assigned to once-daily subcutaneous semaglutide 0.1, 0.2, or 0.4 mg or placebo. NASH resolution was significantly higher in patients on 0.1-mg (40%), 0.2-mg (36%) and 0.4-mg (59%), than on placebo (17%). Mean percent weight loss was also much higher on 0.4-mg (13%) than on placebo (1%). However, there was no significant effect on improvement in fibrosis stage (43% on 0.4-mg vs. 33% on placebo; $P = 0.48$).

There are few effective treatments for NASH, other than lifestyle changes to produce weight loss.
By contrast, there is increasing evidence for the efficacy of GLP-1 agonists, exenatide, liraglutide, and semaglutide, in treating patients with Type 2 diabetes and/or obesity. They act by increasing beta cell insulin secretion, reducing glucagon secretion and delaying gastric emptying. Possible other mechanisms of GLP-1 agonists include central promotion of satiety and peripheral enhancement of insulin sensitivity in fat, muscle and liver.

This paper shows that semaglutide is clearly effective in treating NASH: odds ratio for NASH resolution (OR) = 3.36 in the 0.1 mg group, OR = 2.71 in the 0.2 mg group, and OR = 6.87 in the 0.4 mg group, compared to placebo. It is unclear whether or not these benefits were dependent on its effects on weight loss, which were also impressive. However, the lack of benefit of semaglutide on liver fibrosis stage may indicate that longer duration of treatment is needed, or that patients should start treatment earlier, before liver fibrosis is apparent or advanced. In this regard, a recent systematic review and meta analysis identified clear benefits of GLP-1 agonists in children and adolescents with Type 2 diabetes or obesity, and importantly these benefits seem to be as large as those in adults (1). Health service funding remains a major barrier to the wider use of GLP-1 agonists in children.

Reference
1. Chadda KR, *et al*. GLP-1 agonists for obesity and type 2 diabetes in children: Systematic review and meta-analysis. *Obesity Reviews*. 2021 Jun;22(6):e13177. doi: 10.1111/obr.13177.

15.8. Anti-Müllerian hormone levels and risk of type 2 diabetes in women

Renée MG Verdiesen, N Charlotte Onland-Moret, Carla H van Gils, Rebecca K Stellato, Annemieke MW Spijkerman, H Susan J Picavet, Frank JM Broekmans, WM Monique Verschuren, Yvonne T van der Schouw
Diabetologia, 2021; 64, 375–384.
https://link.springer.com/article/10.1007/s00125-020-05302-5#Abs1

The authors measured plasma anti-Müllerian hormone (AMH) levels over 2x 5-year intervals in a prospective cohort study of 3293 healthy women aged 20–59 years at baseline. Lower baseline age-specific AMH levels were associated with a higher risk of Type 2 diabetes (T2D) after a median 20 years follow-up. When AMH trajectories were predicted in each individual, lower plasma AMH levels at younger ages were seen in women who subsequently developed T2D.

AMH is produced by ovarian follicles in the earlier stages of development (small pre-antral to small antral stage follicle). Circulating AMH levels correspond to the size of the total ovarian follicle pool and declining AMH levels represent a marker of reproductive ageing. Therefore, the current findings widen the evidence linking reproductive and metabolic ageing. The authors adjusted the risk models for several key covariates and the results were not confounded by BMI or polycystic ovary syndrome. Women with lower age-specific AMH levels were more likely to be post-menopausal and the T2D association weakened on adjustment for use of hormone replacement therapy, which suggests that the underlying mechanisms involve sex hormone exposures. Estrogens are thought to be protective for T2D and cardiovascular disease, whereas there is recent evidence that testosterone increases the risk of T2D in women, but has directionally opposite effects on T2D risk in men (1). Alternatively, the link between reproductive and metabolic ageing may involve intrinsic cellular ageing processes that are shared across tissues.

Reference
1. Ruth KS, *et al*. Using human genetics to understand the disease impacts of testosterone in men and women. *Nature Medicine*. 2020 Feb;26(2):252–258.

15.9. Oxytocin administration increases proactive control in men with overweight or obesity: a randomized, double-blind, placebo-controlled crossover study

Franziska Plessow, Dean A Marengi, Sylvia K Perry, Elizabeth A Lawson
Obesity (Silver Spring) 2021 Jan;29(1):56–61.
https://bit.ly/2Sz4SBz

Oxytocin was investigated in this small experimental study for its potential benefits on obesity. To test oxytocin for suppression of behavioural impulses, 10 men with overweight or obesity were subjected to a task assessing ability and strategy to suppress behavioural impulses. After receiving intranasal oxytocin, compared with placebo, participants showed increased reaction times in suppressing snacking in a satiety state.

Recent advances in nutrition suggest that the hypothalamic neuropeptide oxytocin acts as a critical central-nervous-system factor in mediating food intake and weight. Oxytocin is produced in the paraventricular and supraoptic nuclei of the hypothalamus, it decreases food intake in rodent, primate, and humans, and induces weight loss with minimal side effects. Consequently, oxytocin is under investigation as a potential new neurohormonal treatment for obesity. In humans, oxytocin reduces food intake without altering subjective appetite.

Cognitive control regulates impulses, habits, and decision-making, as well as its effect on controlling hedonic food intake. Individuals with obesity show impaired ability to exercise cognitive control on impulses and habits

and more risky decision-making. Individuals with obesity show an increased responsiveness to food reward that renders them more susceptible to overeating, poor dietary choices, and ultimately weight gain. Intranasal administration of oxytocin reduces caloric intake but does not affect subjective appetite. The mechanism for this effect was unknown. Oxytocin affected hedonic eating (snacking in a satiety state) more than homeostatic food intake. These findings suggest that oxytocin exerts its effect on food intake through altering eating behaviour rather than by altering hunger signals.

These results open a new direction for investigation on cognitive control and its moderation in human obesity. Oxytocin becomes a new strategy in weight loss treatment as cognitive control can reduce the behavioural aspect of the urges to eat. Although the sample size of this pilot investigation is small, the overall pattern was consistent in 8/10 participants, suggesting that the reported finding reflects a robust and generalisable effect of oxytocin. The authors conclude that oxytocin triggers increased cognitive control over behaviour as the mechanism for anorexigenic effects of oxytocin in human obesity.

15.10. Maternal occupational exposure to endocrine-disrupting chemicals during pregnancy and semen parameters in adulthood: results of a nationwide cross-sectional study among Swiss conscripts

M Istvan, R Rahban, B Dananche, A Senn, E Stettler, L Multigner, S Nef, R Garlantézec
Human Reproduction, 2021; 36(7): 1948–1958
https://bit.ly/3vtcByF

This cross-sectional study shows that men who had been exposed in utero to endocrine disruptors (EDC) are twice more likely to have a low sperm count, below the reference values per ejaculation set by the World Health Organisation.

Male reproductive function is known to be highly sensitive to a number of chemical compounds generated by industrial and agricultural activities. There is convincing evidence that exposure to chemical agents present in certain occupational activities and environments during adulthood can affect testicular function and male fertility. Furthermore, exposure during antenatal development is suspected to influence male reproductive health later in life, including fertility.

Here, the authors studied Swiss conscripts aged 18 to 22. Maternal occupational exposure to potential EDC categories was defined using a job-exposure matrix (JEM). Overall, 2,326 conscripts (with gestation in Switzerland) provided a completed questionnaire and semen sample. Probability of maternal occupational exposure was assigned by three occupational hygienists from the JEM comprising 353 job titles into three levels of exposure: ‘unlikely’, ‘possible and ‘probable’. 14.0% of mothers were classified as exposed during pregnancy to at least one EDC category, by working in subsistence agriculture (71.2%) or as beauticians/hairdressers (21.9%). The most frequently affected semen parameters were the seminal volume and total sperm count per ejaculation, rather than sperm motility or morphology, regardless of the EDC category.

The findings indicate an adverse effect of fetal exposure to pesticides, phthalates, and heavy metals on later fertility in men. The authors argue for the need to inform pregnant women of the potential hazards of such occupational exposure during pregnancy. We now need to learn more about the possible impact of fetal exposure to EDCs on the female reproductive system.

15.11. Prediction of adult height by machine learning technique

Michael Shmoish, Alina German, Nurit Devir, Anna Hecht, Gary Butler, Aimon Niklasson, Kerstin Albertsson-Wikland, Ze’ev Hochberg
J Clin Endocrinol Metab. 2021; 16;106(7):e2700–e2710.
PMID: 33606028 DOI: 10.1210/clinem/dgab093

This paper illustrates the power of machine learning to successfully predict adult height using growth measurements before age 6 years, without the need for bone age.

Computers beat us in games of predictions, such as chess. They beat us also in the exercise of predicting adult height. This is because machine learning, after necessary pre-processing, is particularly suited to predictions based on existing data, such as anthropometric data. They are powered by the identification of strong, but theory-free, patterns in the data, capturing complex, nonlinear relationships that may markedly improve prediction accuracy over conventional regression models. Prediction of adult height is commonly used in clinical settings, using the Tanner–Whitehouse (TW2) or Bayley–Pinneau (BP) methods, which utilize data on current height and bone age. Whereas TW2 and BP were derived from on data from research studies that were carefully constructed to mitigate bias, routine clinical data sources are typically less clean.

This study aimed to use machine learning to predict adult height based on height and weight measurements up to age 6 years in 2282 children from a community-based, observational longitudinal growth study, the GrowUp 1974 Gothenburg cohort. Two additional validation cohorts were used to assess the accuracy of the algorithm – a second Swedish cohort, and the Edinburgh Longitudinal Growth Study. The winning model was the 'Random Forest', with 51 regression trees, and the best predictor variables were sex and height at age 3.4-6.0 years. Observed and predicted adult height were 173.9 ± 8.9 cm and 173.9 ± 7.7 cm, respectively, with prediction average error only -0.4 ± 4.0 cm. This models was more accurate than any other model with or without bone age assessment. Accuracy remained remarkably stable in the Swedish and Edinburgh validation cohorts, despite their use of different measurement protocols.

These findings show the utility of machine learning to predict adult height. The uneducated machine, employed by students of computer science, provided predictions that are as good, or even better, than methods developed by experts over decades. Yet, these new sophisticated algorithms are only moderately superior to the 19th century target height. The 4-5 cm prediction errors in all methods are not a methodological fault, but rather reflect the fact that additional environmental cues impact growth beyond age 6 years with a magnitude of 4-5 cm, whatever method is used.

15.12. Hormone seasonality in medical records suggests circannual endocrine circuits

Avichai Tendler, Alon Bar, Netta Mendelsohn-Cohen, Omer Karin, Yael Korem Kohanim, Lior Maimon, Tomer Milo, Moriya Raz, Avi Mayo, Amos Tana, Alon Uri
Proc Natl Acad Sci USA. 2021; 118 (7) e2003926118
https://bit.ly/35huXba

By analysing data from Israeli health records, including millions of hormone blood tests, the authors find peaks during winter – spring in the circulating levels of all primary hormones involved in human growth, stress, metabolism and reproduction. By contrast, the pituitary-derived regulatory hormones peak in summer. This circannual clock impacts on function; the endocrine gland masses grow with a timescale of months due to trophic effects of the pituitary hormones, generating a feedback circuit with a natural frequency of about a year according to seasons.

Animals show seasonal changes in both pituitary and primary effector hormones that govern seasonality in reproduction, activity, growth, pigmentation, morphology, and migration. This adaptive physiology results in seasonal changes in body composition, organ size, and function. Hormone seasonality is thought to be a dominant regulator of physiological and behavioral traits in animals. These researchers now show that a circannual rhythm is a general principle for hormones. Endocrine seasonality indicates that, like other animals, humans may have a physiological peak season for basic biological functions.

The classical model predicts that changes in pituitary hormones should coincide with their regulated primary hormones, and thus share the same seasonal peaks and troughs. Instead, the authors suggest a 'functional-mass model', that the observed phase shifts arise from trophic effects of pituitary hormones that peak in winter-spring to stimulate endocrine gland secretion that peaks in summer. The model predicts that the amplitude of seasonal variations should increase with latitude, due to greater photoperiod variation with the seasons. To test this, they compared cortisol profiles in studies from Australia (30°S), the United Kingdom (51°N), and Sweden (58°N). Indeed, the amplitude of cortisol seasonality rose with latitude, in agreement with the model predictions. The

functional-mass model makes another testable prediction: The masses of the glands that secrete the hormones should vary with the seasons with specific phases. They confirmed this by analyzing data on MRI brain scans and computed the volume of the pituitary.

The authors hypothesis that, because of the coordinated peaks in all hormone axes during the same winter/spring season and the widespread effects of hormones on metabolic and behavioral systems, even small changes from hormone baseline levels may have a selectable impact on organism fitness.

15.13. Socioeconomic status is related to pubertal development in a German cohort

Oelkers L, Vogel M, Kalenda A, Surup HC, Körner A, Kratzsch J, Kiess W
Hormone Research in Paediatrics 2020;93:548–557
https://bit.ly/2ToJvmv

The authors studied 2,657 German children aged 5-18 years to look for social patterning in the age at puberty timing and circulating gonadotrophin levels. Lower socio-economic status (SES) was associated with earlier thelarche, longer duration of puberty, and overweight in girls, but not age at menarche. In boys, lower SES showed a trend to earlier puberty onset. The paper also provides cut-off levels for serum LH (0.56 IU/L) and FSH (1.74 IU/L) as biochemical indicators of gonadarche in boys.

Puberty as a critical time of growth and development is influenced by a range of genetic, environmental, and lifestyle factors and may affect health risks. Early puberty might lead to adverse outcomes such as increased substance use, earlier sexual activity, and increased risk of breast cancer.
There is clear evidence that there has been a decline in the age of pubertal onset and that, besides weight status, pubertal onset and duration are influenced by socioeconomic context.

Possible explanations for the link between lower SES and earlier puberty timing include social patterning in BMI, diet, physical activity and psychological stress (1). In addition, the authors argue that the findings are in agreement with John Bowlby's theory of attachment and control of evolutionary strategy of puberty and fecundity. According to this theory, attachment in infants is primarily a process of proximity seeking to an identified attachment figure in situations of perceived distress or alarm for the purpose of survival. Infants become attached to adults who are sensitive and responsive in social interactions, and who remain as consistent caregivers for some months during the period from ages ~6 months to 2 years. Parental responses lead to the development of patterns of attachment, which in turn lead to "internal working models", which will guide the individual's feelings, thoughts and expectations in later relationships.

Reference

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15.14. First-in-class humanized FSH blocking antibody targets bone and fat

Gera S, Sant D, Haider S, Korkmaz F, Kuo TC, Mathew M, Perez-Pen H, Xie H, Chen H, Batista R, Ma K
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https://bit.ly/2RQiZlz

The authors report the generation, structure and function of a fully humanized, humanized antibody that profoundly inhibits follicle-stimulating hormone (FSH) actions in cell-based assays. Administration of the antibody to ovariectomized mice prevented bone loss and also accumulation of adipose tissue when fed a high-fat diet (HFD).

In women, serum levels of FSH increase markedly starting from the 2–3 years before menopause and remain high for many years, due to the lack of ovarian sex hormone feedback inhibition. There are increasing suggestions that, instead of being a passive marker of oocyte depletion, high FSH levels may contribute to the bone loss and visceral fat gains that coincide with these reproductive ageing changes.

To explore this question, the authors targeted a 13-amino-acid sequence in the β-subunit of FSH to produce an antibody with Kd (dissociation constant) 7 nM. Protein thermal shift, molecular dynamics and fine mapping of the FSH–FSH receptor interface confirmed stable binding of the Fab domain to 2 of 5 receptor-interacting residues of the FSH β subunit, which is sufficient to block the interaction of FSH with the FSH receptor. After 2 weeks of administration of the FSH antibody to mice, they observed mitochondrial biogenesis (activation) in brown adipose tissue, and beiging or visceral and subcutaneous white adipose tissue. To build on these findings, they fed male and female mice HFD or normal chow.

These findings show that humanized blocking antibodies to FSH have the potential to prevent and treat obesity, osteoporosis and hypercholesterolemia, particularly when these changes occur at around the menopause. This paradigm-shifting discovery provides the framework for preclinical studies in humans and subsequent clinical trials.

15.15. The cellular basis of distinct thirst modalities

Allan-Hermann Pool, Tongtong Wang, David A Stafford, Rebecca K Chance, Sangjun Lee, John Ngai, Yuki Oka
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https://go.nature.com/3go7UjO

Thirst is sensed by two distinct types of stimuli – osmotic and hypovolaemic. The authors show that in mice these two stimuli act via separate mechanisms and neuron types, and lead to distinct drinking behavioural responses. High blood osmolality induces osmotic thirst that drives water consumption. By contrast, hypovolaemia drives intake of both water and salt-containing solutions.

The authors induced acute osmotic stress in mice by intraperitoneal injection of hypertonic solutes – this stimulus triggered selective consumption of H2O over NaCl solution. Alternatively, they induced acute hypovolaemia by administration of polyethylene glycol or furosemide – this increased intake of both H2O and NaCl solution.

They then used a ‘stimulus-to-cell-type mapping’ optogenetics approach together with single-cell RNA sequencing to identify the cellular substrates that underlie these distinct types of thirst. Circumventricular organs (CVO) in the lamina terminalis, part of the brain that lacks a blood–brain barrier, are critical sites for sensing both types of thirst-inducing stimuli. However, within these CVOs are separate neuron subpopulations, characterised by distinct single cell transcriptomic profiles, which are activated by either osmotic or hypovolaemic stimuli. In turn, these act via separate downstream brain targets to achieve one or the other behavioural response.

So, we have two types of thirst, but only one word for the two related but different sensations. Fortunately, our bodies know this and make us reach for either water or a sports drink.

15.16. The effects of 20-kDa human placental GH in male and female GH-deficient mice: an improved human GH?

Edward O List, Darlene E Berryman, Reetobrata Basu, Mathew Buchman, Kevin Funk, Prateek Kulkarni, Silvana Duran-Ortiz, Yanrong Qian, Elizabeth A Jensen, Jonathan A Young, Gozde Yildirim, Shoshana Yakar, John J Kopchick
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https://bit.ly/3zqg6c5

This study, in a GH-deficient mouse model, shows that a 20-kDa variant of placental GH (20k-GH-V) retains the growth-promoting effects of normal pituitary-derived GH (GH-N), but with the advantages of having no diabetogenic or prolactin receptor (PRLR)-mediated tumour-promoting effects.

One of the first described actions of GH is its diabetogenic or anti-insulin activity. This diabetogenic activity was demonstrated by the Nobel Laureate, Bernardo Houssay, who showed that surgical removal of the pituitary in dogs and toads increased insulin sensitivity.
Placental GH (GH-V) differs from pituitary-secreted GH-N in 13 of the 191 amino acid residues, but the common isoforms of both share the same overall 22-kDa size. GH-V and GH-N also share the same growth

promoting and insulin inhibiting effects, but lacks PRLR binding activity. In 1998, Cesar Boguszewski discovered a rare smaller 20kDa isoform of GH-V in human placenta (its mRNA lacks 45-bp in exon 3). At that time, this 20k-GH-V was shown to lack all the usual functions of GH, including the inability to generate IGF-1 and increase length in normal mice. The key finding of the current study is that this rare placental variant, 20k-GH-V, does indeed stimulate IGF-1 and length growth to a similar extent to that of GH-N, but only in GH-deficient mice, and not in normal mice. They also confirm that, regardless of underlying GH status, 20k-GH-V has no insulin inhibiting effects and did not alter proliferation of human cancer cell lines that highly exhibit PRLR.

Both GH-N and 20k-GH-V also increased circulating FSH levels. Since basal and stimulated plasma FSH levels are attenuated in GH-immunoneutralized, GH-deficient, and GH-resistant animals, and exogenous GH has been shown to increases release of FSH from rodent pituitary glands, the increase in FSH following GH treatment in GH-/- mice is expected.

GH-V is expressed in the syncytiotrophoblast and extravillous cytotrophoblast layers of the human placenta. Its levels increase throughout pregnancy and eventually replaces GH-N in the maternal circulation. Studies suggest that GH-V is an important regulator of fetal growth and development, with reduced maternal circulating concentrations in pregnancies complicated by fetal growth restriction.[1] Unlike the pulsatile secretion pattern of GH-N, GH-V is secreted by the placenta in a tonic fashion with constant 24-hour circulating levels[1]. Interestingly, the 20-kDa variant of pituitary GH-N has also been reported to have diminished lactogenic effects *in vitro* compared with 22-kDa GH-N.[2]

In summary, 20k-GH-V is a potent stimulator of body growth, as indicated by increased IGF-1, femur length, body length, body weight, and lean body mass, and also reduces body fat mass similar to GH-N administration in a mouse model of GH deficiency. The authors argue that 20k-GH-V may allow an improved form of GH therapy, especially for GH deficient patients at risk for metabolic syndrome or PRLR-positive cancers.

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