Yearbook of Paediatric Endocrinology 2020

Editors
Ken Ong
Ze'ev Hochberg

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Paediatric Endocrinology 2020

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Editors

Ken Ong
Ze'ev Hochberg

Associate Editors

Anne-Simone Parent
Carla Bizzarri
Christa Flück
Evangelia Charmandari
Gabor Szinnai
Jean-Pierre Chanoine
Khalid Hussain
Martin Wabitsch
Ola Nilsson
Orit Pinhas-Hamiel
Stefano Cianfarani
Taneli Raivio
Wieland Kiess

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Editors

Ken Ong
Medical Research Council Epidemiology Unit & Department of Paediatrics
Wellcome Trust-MRC Institute of Metabolic Science
University of Cambridge, Cambridge CB2 0QQ, UK
ken.ong@mrc-epid.cam.ac.uk

Ze'ev Hochberg
Technion-Israel Institute of Technology
IL-31096 Haifa, Israel
rprzeev@technion.ac.il

Associate Editors

Anne-Simone Parent
Department of Pediatrics
University Hospital Liège, Belgium
Neuroendocrinology Unit
GIGA-Neurosciences, University of Liège, Belgium
asparent@ulg.ac.be

Carla Bizzarri
Unit of Endocrinology
Bambino Gesù Children's Hospital, Rome, Italy
carla.bizzarri@opbg.net

Christa Flück
Pediatric Endocrinology, Diabetology, and Metabolism
University Children's Hospital, 3010 Bern, Switzerland
Department of Biomedical Research
University of Bern, 3008 Bern, Switzerland
christa.flueck@dbmr.unibe.ch

Evangelia Charmandari
Division of Endocrinology, Metabolism and Diabetes
First Department of Pediatrics
National and Kapodistrian University of Athens Medical School
'Aghia Sophia' Children's Hospital, and
Division of Endocrinology and Metabolism
Center of Clinical, Experimental Surgery and Translational Research
Biomedical Research Foundation of the Academy of Athens
GR- 11527, Athens, Greece
evangelia.charmandari@gmail.com

Gabor Szinnai
Pediatric Endocrinology and Diabetology
University of Basel Children's Hospital (UKBB) and
Department of Clinical Research
University of Basel, Basel, Switzerland
gabor.szinnai@unibas.ch

Jean-Pierre Chanoine
Endocrinology and Diabetes Unit
British Columbia Children's Hospital and University of British Columbia
Vancouver, Canada
jchanoine@cw.bc.ca

Khalid Hussain
Department of Paediatric Medicine, Division of Endocrinology
Sidra Medical & Research Center
OPC, C6-337, PO Box 26999, Doha, Qatar
khussain@sidra.org

Martin Wabitsch
Division of Pediatric Endocrinology and Diabetes and Endocrine Research Laboratory
Department of Pediatrics and Adolescent Medicine
University of Ulm, Eythstrasse 24, DE-89075 Germany
Martin.wabitsch@uniklinik-ulm.de

Ola Nilsson
School of Medical Sciences
Örebro University and University Hospital, Örebro, Sweden
Divison of Pediatric Endocrinology and Center for Molecular Medicine
Karolinska Institutet and University Hospital, Stockholm, Sweden
Ola.Nilsson@ki.se

Orit Pinhas-Hamiel
Pediatric Endocrine and Diabetes Unit
Edmond and Lily Safra Children's Hospital
Sheba Medical Center, Ramat-Gan, and
Juvenile Diabetes Center, Maccabi Health Care Services
Tel-Aviv University, Sackler School of Medicine, IL-52621, Israel
Orit.hamiel@sheba.health.gov.il

Stefano Cianfarani
D.P.U.O. 'Bambino Gesù' Children's Hospital
University of Rome 'Tor Vergata'
P.zza S. Onofrio, 4, IT-00165, Rome, Italy and
Department of Women's and Children's Health
Karolinska Institutet, Stockholm, Sweden
stefano.cianfarani@uniroma2.it

Taneli Raivio
New Children's Hospital, Pediatric Research Center
Helsinki University Hospital, Helsinki
Finland Stem Cells and Metabolism Research Program
Research Programs Unit, Faculty of Medicine
University of Helsinki, Helsinki, Finland
taneli.raivio@helsinki.fi

Wieland Kiess
Hospital for Children and Adolescents
Centre for Pediatric Research
University of Leipzig, Leipzig, Germany
Wieland.Kiess@medizin.uni-leipzig.de

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Preface

This Yearbook highlights prominent Paediatric Endocrinology-related papers published from June 2019 to May 2020. For the third year, we are online at www.espeyearbook.org/. Those who prefer to read a hard copy can purchase the 2020 and 2019 volumes via Amazon for a nominal fee. This was the year of the COVID-19 pandemic caused by SARS-CoV-2. Among the many arising changes, the timing of this publication has been delayed to coincide with the ESPE CONNECT online conference, where the chapters will be presented by their Associate Editors.

Highlights of this edition include: new genes for hypopituitarism, hyperphagic obesity, anorexia nervosa, and same-sex sexual behavior; and use of next generation DNA sequencing in short stature and in DSD. New treatments include: an anti-CD3 antibody to prevent T1DM; levoketoconazole in Cushing's Syndrome; thyroid hormone receptor-β agonist in obesity-associated steatohepatitis; once-weekly GH for GHD; different treatment thresholds for neonatal hypoglycemia; time-restricted eating for the metabolic syndrome; as well as new agents for achondroplasia, Graves' orbitopathy, and MTC8 deficiency; and new evidence on liraglutide in adolescents with obesity and children with T2DM, aromatase inhibitors for constitutional delay of growth and puberty, and effects of gender-affirming hormone therapies on mental and cardio-metabolic health.

We also highlight papers that describe: guidelines for management of T1DM during Ramadan fasting in children; how neonatal macrosomia interferes with newborn biochemical screening; arginine-stimulated copeptin measurements to diagnose diabetes insipidus; hyperthyroidism after radiotherapy for childhood cancer; corruption in global health; and the roles of dietary cereal, gluten and fibre, congenital infections and rotavirus infection on risk of T1DM.

More widely, the US FDA approved Rybelsus (semaglutide) to reduce blood glucose in adults with Type 2 diabetes; this is the first oral GLP-1 receptor protein treatment. While some countries have yet to assess its cost-effectiveness, a WHO task force reported that drugs are expensive not due to lack of competition between pharmaceutical companies, but because of lack of competition in the regulatory drug approval process. They suggest reformation of the drug approval process by privatization and competition.

In our highlight on achievements 100 years ago: in October 1920, Frederick Banting gave a talk to his Physiology students at Western University, London, Ontario. He described his idea to ligate the pancreatic ducts in dogs until the acini degenerate to leave islets, and then isolate their secretions to relieve glycosuria. The next month, Banting met John Macleod, from the University of Toronto, and asked him to carry out this project. Despite little prior experience of this topic, Macleod accepted Banting's request and was joined by Charles Best, then a student assistant. The rest is history.

We thank our thirteen Associate Editors and their coauthors who have done enormous work to identify, describe and comment on this year's highlights. We thank Nicolas de Roux for his excellent contributions to the chapter on Neuroendocrinology ever since the Yearbook launched in 2004. We welcome Taneli Raivio who takes on this chapter.

We are grateful to ESPE for their continuing endorsement and support of the Yearbook series and to Bioscientifica for their highly professional production.

Ze'ev Hochberg (Haifa) and Ken Ong (Cambridge)
Editors
rprzeev@technion.ac.il
ken.ong@mrc-epid.cam.ac.uk

1. Pituitary and Neuroendocrinology

Matti Hero[1], Yafei Wang[2], Päivi J Miettinen[1,2], Taneli Raivio[1,2]

[1]New Children's Hospital, Pediatric Research Center, Helsinki University Hospital, Helsinki, Finland;
[2]Stem Cells and Metabolism Research Program, Research Programs Unit, Faculty of Medicine, University of Helsinki, Helsinki, Finland

Update on the Genetics of Hypopituitarism

1.1. Impaired EIF2S3 function associated with a novel phenotype of X-linked hypopituitarism with glucose dysregulation

Gregory LC, Ferreira CB, Young-Baird SK, Williams HJ, Harakalova M, van Haaften G, Rahman SA, Gaston-Massuet C, Kelberman D, GO Sgene, Qasim W, Camper SA, Dever TE, Shah P, Robinson ICAF, Dattani MT
EBioMedicine. 2019 Apr;42:470–480.
doi: 10.1016/j.ebiom.2019.03.013. Epub 2019 Mar 14. PMID: 30878599.

Is everyone familiar with the MEHMO syndrome (OMIM #300148)? MEHMO is an acronym for mental retardation, epileptic seizures, hypogonadism and hypogenitalism, microcephaly, and obesity. Previous studies show that patients with MEHMO carrying hemizygous severe loss-of-function mutations in *EIF2S3*, a gene encoding eIF2γ which is an important component of translation machinery, exhibit severe phenotypes such as microcephaly, epilepsy/seizures and pituitary hormone deficiencies and craniofacial phenotypes.

The current study reports a pedigree with three affected boys (twins and their maternal male cousin) with a missense *EIF2S3* mutation and shows that the mutation causes only moderate loss of eIF2γ function and was associated with a less severe phenotype. Importantly, the authors show that the neonatal hypoglycemia in these patients is due to hyperinsulinism, and is followed by an unusual form of diabetes in the second decade of life.

1.2. Mutations in MAGEL2 and L1CAM are associated with congenital hypopituitarism and arthrogryposis

Gregory LC, Shah P, Sanner JRF, Arancibia M, Hurst J, Jones WD, Spoudeas H, Le Quesne Stabej P, Williams HJ, Ocaka LA, Loureiro C, Martinez-Aguayo A, Dattani MT
J Clin Endocrinol Metab. 2019 Dec 1;104(12):5737–5750.
doi: 10.1210/jc.2019-00631. PMID: 31504653.

This paper describes two genes and three syndromes that clinicians would probably like to know when treating patients with a syndromic form of panhypopituitarism. Heterozygous mutation in a maternally imprinted gene, *MAGEL2*, was described in four patients with variable phenotypes. Mutations in this same gene, located in the Prader-Willi syndrome region, have been previously implicated in complex Schaaf-Young and Chitayat-Hall syndromes, and the authors speculate that the two syndromes may in fact represent one entity with variable expressivity. Another gene implicated in pituitary hormone deficiency was *L1CAM*, which is a cell adhesion molecule that plays a role in neuronal migration and has been previously implicated in L1 syndrome. The bottom line of all this is quite simple and important: patients carrying mutations in either of these two genes display intriguing phenotypic similarities, namely pituitary hormone deficiencies (most commonly GHD), arthrogryposis (joint contractures), intellectual disability, and dysmorphic features. The authors conclude that patients with hypopituitarism and arthrogryposis should be assessed for mutations in *MAGEL2* and *L1* before proceeding to whole exome or whole genome sequencing.

1.3. Mutations in *LAMB2* are associated with albuminuria and optic nerve hypoplasia with hypopituitarism

Mona Tahoun, Jennifer C Chandler, Emma Ashton, Scott Haston, Athia Hannan, Ji Soo Kim, Felipe D'Arco, D Bockenhauer, G Anderson, Meei-Hua Lin, Salah Marzouk, Marwa H Saied, Jeffrey H Miner, Mehul T Dattani, Aoife M Waters
J Clin Endocrinol Metab. 2020 Mar 1;105(3). pii: dgz216.
doi: 10.1210/clinem/dgz216. PMID: 31769495.

Septo-optic dysplasia (SOD) involves a combination of midline brain defects, pituitary hormone deficiency, and optical nerve hypoplasia. The etiology of SOD is multifaceted; genetic factors are known to play a role, yet the vast majority of SOD patients remain without molecular genetic diagnosis. Laminins are heterotrimeric basement membrane proteins that have numerous cellular functions such as the regulation of cell differentiation and migration, adhesion and proliferation. Pierson syndrome is caused by autosomal recessive mutations in *LAMB2* encoding laminin β2 and comprises of congenital nephrotic syndrome, ocular and neurologic abnormalities.

Herein, the authors describe a boy with compound heterozygous mutations in *LAMB2* due to Pierson syndrome accompanied by septo-optic dysplasia (SOD) and growth hormone deficiency. The authors complement these findings by showing that *lamb2*-deficient mice exhibit abnormal cellular clusters in the pituitary region. Taken together, this report adds a new gene to the rare genetic causes of SOD.

1.4. Loss-of-function variants in *TBC1D32* underlie syndromic hypopituitarism

Hietamäki J, Gregory LC, Ayoub S, Iivonen AP, Vaaralahti K, Liu X, Brandstack N, Buckton AJ, Laine T, Känsäkoski J, Hero M, Miettinen PJ, Varjosalo M, Wakeling E, Dattani MT, Raivio T
J Clin Endocrinol Metab. 2020 Feb 15. pii: dgaa078.
doi: 10.1210/clinem/dgaa078. PMID: 32060556.

Just another gene implicated in hypopituitarism? Yes, but it is a newish cilopathy gene in the hedgehog pathway. Hedgehog family of polypeptides (Sonic (Shh), Indian (Ihh) and desert (Dhh) hedgehog) are signaling molecules that are needed for many cellular events and play a role in both embryogenesis and adult physiology. Mutations in these genes result in subsequent deficiency of the downstream signaling pathways and in defects in brain, limb, and vertebral development, and, more specifically, they have been implicated in e.g. holoprosencephaly and Simpson-Golami-Behmel syndrome and in cilopathies. The mouse homologue of TBC1D32, Bromi is needed for the appropriate activation of Shh signaling and as TBC1D32 is part of the non-motile cilium, the loss-of function of TBC1D32 phenotype of hypopituitarism and craniofacial dysmorphism is a cilopathy. A new review of hedgehog signalling and genetic disorders was recently published (Sasai N *et al.*, *Front Genet.* 2019 Nov 8;10:1103. doi: 10.3389/fgene.2019.01103. eCollection 2019).

Hypopituitarism: Disease Modeling and New Discoveries

1.5. Hypothalamic contribution to pituitary functions is recapitulated in vitro using 3D-cultured human iPS cells

Kasai T, Suga H, Sakakibara , Ozone C, Matsumoto R, Kano M, Mitsumoto K, Ogawa K, Kodani Y, Nagasaki H, Inoshita N, Sugiyama M, Onoue T, Tsunekawa T, Ito Y, Takagi H, Hagiwara D, Iwama S, Goto M, Banno R, Takahashi, Arima H
Cell Rep. 2020 Jan 7;30(1):18–24.e5.
doi: 10.1016/j.celrep.2019.12.009. PMID: 31914385.

The hypothalamic-pituitary axis influences many body organ systems and its dysfunction can be fatal. A proper *in vitro* model is important for understanding the disease mechanisms affecting pituitary function. Based on their previous anterior pituitary modelling with human embryonic stem cells (ESCs) (Ozone *et al*. *Nat Commun*, 2016), the authors investigated here whether hypothalamic-pituitary functional units can also be differentiated from human induced pluripotent stem cell (iPSC) lines. Indeed, using a long-term 3D culture,

hypothalamic precursor-like cells were observed adjacent to the anterior pituitary tissue in the same cellular (hybrid) aggregates. They even showed that secretion of ACTH in cell aggregates was under the control of the hypothalamic CRH. These results provide fascinating possibilities for future human disease modeling of genes affecting the ontogeny and function of the hypothalamic-pituitary axis.

1.6. Congenital pituitary hypoplasia model demonstrates hypothalamic OTX2 regulation of pituitary progenitor cells

Matsumoto R, Suga H, Aoi T, Bando H, Fukuoka H, Iguchi G, Narumi S, Hasegawa T, Muguruma K, Ogawa W, Takahashi Y
J Clin Invest. 2020 Feb 3;130(2):641–654.
doi: 10.1172/JCI127378. PMID: 31845906.

Congenital pituitary hypoplasia (CPH) is a multifactorial disorder, in which the pituitary has not developed correctly. The underlying mechanisms are mostly unknown, and studies are limited by the lack of appropriate disease models. This work modelled the effect of a novel *OTX2* mutation (R127W), encountered in a CPH patient, by using induced pluripotent stem cells (iPSCs) derived from the patient's leucocytes. Quite intriguingly, the *OTX2*^{mut}-iPSCs had an impaired capacity to differentiate into anterior pituitary. This was attributed to deficient capability of hypothalamic *OTX2*^{mut} cells to stimulate *LHX3* expression in the oral ectoderm cells (giving rise to pituitary progenitor cells) via FGF signaling, which in turn enhanced apoptosis. When the *OTX2* mutation was corrected by CRISPR/Cas9 genome editing, the repaired-iPSC again exhibited pituitary differentiation capacity. This study shows that the pituitary and hypothalamus self-organization model in combination with patient-derived iPSCs can be used to analyze the molecular mechanisms of CPH.

1.7. Single cell transcriptome analysis of developing arcuate nucleus neurons uncovers their key developmental regulators

Huisman C, Cho H, Brock O, Lim SJ, Youn SM, Park Y, Kim S, Lee SK, Delogu A, Lee JW
Nat Commun. 2019 Aug 16;10(1):3696.
doi: 10.1038/s41467-019-11667-y. PMID: 31420539.

The hypothalamic arcuate nucleus (ARC) controls crucial physiological processes, such as growth, reproduction and energy homeostasis. However, it is unknown how ARC neurons gain their unique cellular identities and what are their developmental relationships. Here, the authors investigated ARC development by analyzing mouse embryonic ARCs with single cell RNA sequencing. When compared with a previous dataset on adult ARC neuronal types, they found that one cluster of embryonic ARC cells represented a pool of multiple types of developing ARC neurons. On closer analysis, the authors provided new markers required for systematically defining the developmental trajectories of ARC neurons, as well as key transcriptional regulators for fate determination and differentiation of ARC neurons. Furthermore, to validate two newly identified transcription factors implicated in specific cell types in the developing ARC, the authors knocked them out in mice; deletion of *Foxp2* (enriched in developing Ghrh neurons) and *Sox14* (enriched in developing kisspeptin neurons) led to reduced number of Ghrh neurons and complete infertility, respectively. Overall, this report greatly advances our understanding on how ARC neurons develop during embryogenesis and offers important clues to human diseases.

Clinical Highlights

1.8. Should we assess pituitary function in children after a mild traumatic brain injury? A prospective study

Claire Briet, Karine Braun, Michel Lefranc, Patrick Toussaint, Bernard Boudailliez, Hélène Bony
Front Endocrinol (Lausanne). 2019 Mar 19;10:149.
doi: 10.3389/fendo.2019.00149. eCollection 2019. PMID: 30941101.

It has known that severe traumatic brain injury (TBI) can cause permanent pituitary dysfunction with variable rates, but what about mild TBI?

Briet *et al.* studied 109 children with a traumatic brain injury and Glasgow come scale (GCS) >12. Growth hormone deficiency (defined by stringent criteria: GH peak <20 mU/l in two tests, plus IGF-1 <-1SDS, plus delta height <0 SDS) was detected in only 2 out of 96 patients (~2%) during the first year post-TBI. A further 5 patients had low prolactin levels. No clinical or biochemical parameters at the time of injury could predict the development of pituitary insufficiency.

The findings do not support the routine assessment of pituitary function following mild traumatic brain injury. However, since growth hormone deficiency occurred even in patients with GCS of 15, growth should be monitored carefully for at least 1 year post-mild TBI.

1.9. Autoimmune phenomena involving the pituitary gland in children: New developing data about diagnosis and treatment

Alberto Romanoa, Donato Rigantea, Clelia Cipolla
Autoimmun Rev. 2019 Oct;18(10):102363.
doi: 10.1016/j.autrev.2019.102363. Epub 2019 Aug 8. Review. PMID: 31401342.

Autoimmune hypophysitis is rare in children (fewer than 100 reported cases). Here, Romanoa *et al.* review the literature on this fairly unknown topic. They teach us that hypophysitis can be divided into adeno-, infundibuloneuro- and panhypophysitis. It can be primary or secondary depending on the presence of a triggering condition (immune checkpoint inhibitor therapy, trauma, neoplasm, infection, or other autoimmune disorders, such as APECED). Germinoma is listed as a secondary cause of hypophysitis and can be diagnosed a few years after presentation of hypophysitis. As diabetes insipidus is commonly presented in both diseases, careful follow-up is needed for pediatric hypophysitis. A biopsy should be taken to exclude malignant etiology, but, as it is a risky procedure, its pros and cons need to be carefully weighed. Overall, the etiology and diagnostic lab work need more research. See also a good review by Gubbi *et al.* 2018 (Reviews in Endocrine and Metabolic Disorders 19: 335–347).

1.10. Significant benefits of AIP testing and clinical screening in familial isolated and young-onset pituitary tumors

Pedro Marques, Francisca Caimari, Laura C. Hernández-Ramírez, David Collier, Donato Iacovazzo, Amy Ronaldson, Kesson Magid, Chung Thong Lim, Karen Stals, Sian Ellard, Ashley B. Grossman, Márta Korbonits (on behalf of The International FIPA Consortium)
J Clin Endocrinol Metab. 2020 Jan 30. pii: dgaa040.
doi: 10.1210/clinem/dgaa040. PMID: 31996917.

The International FIPA Consortium recommend that aryl hydrocarbon receptor-interacting protein (*AIP*) gene mutations are worth screening for prospectively in family members of *AIPmut* patients, and the detected carriers should be clinically followed. AIP gene mutations account for one fifth of familial isolated pituitary adenoma patients, but they can also present sporadically particularly in young patients due to incomplete penetrance. When pituitary neuroendocrine tumors are diagnosed earlier, they are smaller, and patients have fewer pituitary hormone deficiencies and require fewer treatments. If you wish to learn more on the AIP saga, we recommend reading Chahal *et al.* 2011, NEJM, 364: 43–50.

1.11. Endocrinology and adolescence: Dealing with transition in young patients with pituitary disorders

Sbardella E, Pozza C, Isidori AM, Grossman AB
Eur J Endocrinol. 2019 Oct;181(4):R155–R171.
doi: 10.1530/EJE-19-0298. PMID: 31370006.

Transition is described as the planned process of transferring care from pediatric to adult healthcare services. Recent recommendations by various medical associations underline that preparation of adolescents and their parents, consideration of the adolescent's developmental phase, individualized timing, and long-lasting doctor-patient relationships are important for successful transition. In this review, Emilia Sbardella and colleagues

provide guidance on how these principles should be applied in the care of youth with panhypopituitarism, a condition that requires a demanding degree of self-management at this delicate period of life. A holistic and personalized approach is needed that takes into account the specific changes in pituitary function and interactions between pituitary hormones during puberty. As the authors point out, special transition clinics should be increasingly created to bridge care and to avoid the negative sequelae of inadvertent treatment discontinuation.

2. Antenatal and Neonatal Endocrinology

Khalid Hussain
Department of Paediatric Medicine, Division of Endocrinology Sidra Medical & Research Center OPC, C6-337, PO Box 26999, Doha, Qatar khussain@sidra.org

Neonatal Hypoglycaemia

2.1. Clinical and genetic characterization of 153 patients with persistent or transient congenital hyperinsulinism

Männistö JME, Maria M, Raivo J, Kuulasmaa T, Otonkoski T, Huopio H, Laakso
J Clin Endocrinol Metab. 2020 Apr 1;105(4).
PMID: 32170320

In congenital hyperinsulinism (CHI) there is dysregulation of insulin secretion that leads to persistent hypoglycaemia. Mutations in the *ABCC8/KCNJ11* genes which encode the pancreatic K_{ATP} channels proteins (SUR1/KIR6.2 respectively) are the most common causes of CHI. Mutations in these genes typically lead to diazoxide unresponsive CHI. In this study, the authors looked at the clinical characteristics and genetic causes of hypoglycaemia in 153 patients with persistent and transient CHI. They undertook sequencing of the 10 known genes associated with CHI and also selected another 104 genes associated with abnormalities in glucose metabolism (presumably looking for novel mechanisms of CHI). Pathogenic variants were found in the genes *ABCC8, KCNJ11, GLUD1, GCK, HNF4A, and SLC16A1* (all known CHI genes) in patients with persistent CHI, but no genetic mutations were found in patients with transient CHI. No mutations were identified in the 104 genes which were selected as potentially novel genes for CHI. The transient CHI group included infants who had risk factors for secondary hyperinsulinaemic hypoglycaemia.

These results agree with two previously published studies (1, 2) which analyzed the genotype and phenotype characteristics of a large cohort of CHI patients. The majority of patients (~90%) with persistent CHI, who are typically unresponsive to diazoxide therapy, have an underlying genetic cause detected (typically in *ABCC8*or *KCNJ11*). In contrast, an underlying genetic cause is not found in the majority of patients with either transient or diazoxide responsive CHI. This study and the previous two studies (1, 2) suggest that genetic testing for CHI should focus on those patients with persistent CHI and are unresponsive to therapy with diazoxide. However, it would be interesting to know the mechanisms of transient and diazoxide responsive CHI as these will provide new insights into the physiology of insulin secretion and glucose metabolism. Thus, whole genome analysis, epigenetic studies and given the role of miRNA in insulin secretion, these avenues should be explored in this unique group of patients with transient CHI.

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2. Snider KE, Becker S, Boyajian L, *et al*. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. *J Clin Endocrinol Metab*. 2013;98(2):E355–E363.

2.2. Diazoxide-induced pulmonary hypertension in hyperinsulinaemic hypoglycaemia: Recommendations from a multi-centre study in the United Kingdom

Chen SC, Dastamani A, Pintus D, Yau D, Aftab S, Bath L, Swinburne C, Hunter L, Giardini A, Christov G, Senniappan S, Banerjee I, Shaikh MG, Shah P
Clin Endocrinol (Oxf). 2019 Dec;91(6):770–775.
PMID: 31520536

Diazoxide is the first line treatment for patients with hyperinsulinaemic hypoglycaemia (HH). The vast majority of patients tolerate diazoxide well without any major complications. However, diazoxide is known to cause several side effects including hypertrichosis, neutropaenia, thrombocytopaenia, hyperuricaemia and fluid retention. Pulmonary hypertension (PH) is a rare side effect of diazoxide and has been documented in several previous studies in addition to this study. The US Food and Drug Administration (FDA) issued a safety alert in September 2015 cautioning that diazoxide could lead to PH, which is characterized by an elevation of the pulmonary arterial pressure that consequently increases right ventricular afterload and results in right heart failure. Interestingly there are genetic causes of primary pulmonary hypertension due to defects in one of the genes (*ABCC8*) which also leads to congenital hyperinsulinism (1). The underlying risk factors and mechanisms which trigger PH in patients who are treated with diazoxide are not known. Potential risk factors based on a previous study include being born premature, respiratory failure and congenital heart disease (2).

This current study from four HH Centers in the UK supports the fact that having underlying congenital heart disease (ventricular and atrial septal defects in particular) seems to be an important risk factor for developing PH and therefore recommends that patients should have a baseline echocardiogram prior to commencing treatment with diazoxide. In addition, this study suggests that the total fluid volume before commencing diazoxide therapy might also be a potential risk factor. Although PH has been reported in association with diazoxide treatment, it is difficult to directly link diazoxide with PH. Diazoxide is a known vasodilator of vascular smooth muscle and has been used to treat hypertension in adults, in whom there are no reports of PH on diazoxide. This suggests that the mechanism/s of PH in HH patients treated with diazoxide are probably multifactorial and not just due to diazoxide per se. As diazoxide is known to cause fluid retention, as a general recommendation any newborn started on diazoxide must have careful monitoring of fluid balance. The mechanisms that lead to PH due to diazoxide need further investigations.

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2. Herrera A, Vajravelu ME, Givler S, *et al*. Prevalence of Adverse Events in Children With Congenital Hyperinsulinism Treated With Diazoxide. *J Clin Endocrinol Metab*. 2018;103(12):4365–4372.

2.3. Lower versus traditional treatment threshold for neonatal hypoglycemia

van Kempen AAMW, Eskes PF, Nuytemans DHGM1, van der Lee JH, Dijksman LM, van Veenendaal NR, van der Hulst FJPCM, Moonen RMJ, Zimmermann LJI, van't Verlaat EP, van Dongen-van Baal M, Semmekrot BA, Stas HG, van Beek RHT, Vlietman JJ, Dijk PH, Termote JUM, de Jonge RCJ, de Mol AC, Huysman MWA, Kok JH, Offringa M, Boluyt N; HypoEXIT Study Group

N Engl J Med. 2020 Feb 6;382(6):534–544.
PMID: 32023373

Neonatal hypoglycaemia is one of the most common biochemical findings in the newborn period and is an important cause of brain injury. However, despite being so common there is no consensus regarding the glucose threshold concentration at which treatment for asymptomatic neonatal hypoglycemia should be initiated. Neurodevelopmental outcome studies in infants after neonatal hypoglycaemia have been mostly observational, comparing newborns with and without hypoglycaemia, and these studies have reported inconsistent results. Due to the lack of evidence and consensus, there is no consensus screening or treatment protocol, and this leads to both overtreatment and undertreatment with adverse effects on newborn health, bonding of mother–child, and health care costs. Therefore, well-designed clinical trials are required to devise the appropriate treatment strategies.

This multicenter, randomized, non-inferiority trial involved 689 otherwise healthy newborns born at 35 weeks of gestation or later and identified as being 'at risk' for hypoglycemia. The authors compared two glucose threshold values (2 mmol/l and 2.6 mmol/l) for treatment of asymptomatic moderate hypoglycemia. They found no difference between groups with respect to the psychomotor development at age 18 months, assessed by Bayley Scales of Infant and Toddler Development. Based on these observations, the authors suggest that a glucose value of 2 mmol/l is non-inferior to the standard value of 2.6 mmol/l for intervention.

The strengths of this study its multicenter, randomized control trial design involving a large number of newborns. However, follow up was only until 18 months of age. It is known that the neurodevelopment impact

of neonatal hypoglycaemia does not manifest until the age of about 4 years (1, 2), so the conclusions should be interpreted with caution. Also, newborns with severe hypoglycaemia were excluded, so the conclusions cannot be applied to that group. Finally, there is a potential for bias, as the caregivers were aware of the treatment group for each newborn. It will be interesting to know the neurodevelopment outcome of all these newborns at age ~4 years, so they should have long-term follow up. The issue question 'which blood glucose level is safe for brain function and neurodevelopment' still remains to be answered.

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2.4. Targeting glucose control in preterm infants: Pilot studies of continuous glucose monitoring

Thomson L, Elleri D, Bond S, Howlett J, Dunger DB, Beardsall K
Arch Dis Child Fetal Neonatal Ed. 2019 Jul;104(4):F353–F359.
PMID:30232094.

In preterm and in the very low birthweight infants, hyperglycaemia is associated with increased risks of mortality, brain injury, retinopathy of prematurity and worse neurodevelopmental outcomes (1). Treatment of hyperglycaemia with continuous insulin infusion leads to increased episodes of hypoglycaemia which in itself can potentially lead to brain damage. In addition, glycaemic variability is also associated with impaired long-term outcomes. Therefore, the use of continuous (interstitial) glucose monitoring (CGM) in very preterm, low birth weight infants has the potential to minimise the incidence and severity of hypoglycaemia and hyperglycaemia. This would allow glycaemic stability during critical developmental periods and provide new opportunities to improve long-term neurocognitive outcomes in these children.

The results of this this study are consistent with other studies showing that the CGM sensor was well tolerated, was acceptable to staff caring for the infants and, when combined with an algorithm informing clinical decisions based on real time-CGM data, allowed clinicians to keep glucose concentrations in these preterm infants within a narrower range. This last finding, combined with the ability of CGM to uncover episodes of occult hypoglycaemia, is an important reason why the use of CGM in the care of the very low weight preterm infant has been considered.

However, before GCM can be used widely in the neonatal period (and especially in the preterm very low birth weight baby) there are several important points that need to be considered. The current CGM algorithms used to derive interstitial glucose concentrations are based on interstitial glucose–blood glucose kinetics in adults and these have not been fully tested in the neonatal period. Further advances in CGM technology should focus on detecting low (0–2.6 mmol/l) blood glucose levels as these are more common in the late preterm and very low birthweight infants in the first a few days after birth. As CGM technology improves, this should allow more accurate assessment of blood glucose levels in the preterm and very low birthweight and improve outcome.

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2.5. Glucose profiles in healthy term infants in the first 5 days: the glucose in well babies (GLOW) study

Harris DJ, Weston PJ, Gamble GD, Harding JE
J Pediatr. 2020 May 4. pii: S0022-3476(20)30295-X.
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Understanding the typical patterns of blood glucose profiles in healthy term infants is needed to inform guidelines for the management of hypoglycaemia. Typically blood glucose levels fall rapidly after birth (30–

90 min), then stabilize at 48 h, and eventually reach adult values after ~4 days. Almost all previous studies assessing the changes in blood glucose after birth have been undertaken on infants in a hospital setting with intermittent blood or plasma glucose measurements, sampled at varying intervals, and therefore changes in glucose concentrations between sampling could not be captured.

Here, the authors measured blood glucose (heel prick) and also interstitial glucose levels (CGM started 2 h after birth) to understand the blood glucose profiles from birth to 5 days in a cohort of term healthy newborns. These newborns were born either in hospital, at a birthing center or at home, and were breast or bottle fed. There were are 2 distinct patterns in glucose concentrations, the first over the initial 18 h and the second after 48 h, reaching a new plateau by 96 h at concentrations similar to adult concentrations. These glucose profiles suggests that healthy infants complete their metabolic transition by day 4. Despite this overall pattern, blood glucose levels varied widely in individual patients, and infants appeared to take varying periods of time to stabilize their glucose concentrations, suggesting immature regulatory mechanisms during this transitional phase. More importantly, many healthy infants had glucose concentrations below the international recommended thresholds for treatment in at-risk infants. 39% of infants had episodes of low plasma glucose and 73% had episodes of low interstitial glucose concentrations during the first 48 h. As CGM technology becomes used more widely in the newborn this will allow a more detailed understanding of blood glucose profiles over time and will then inform decisions about the management of hypoglycaemia.

Neonatal Diabetes Mellitus

2.6. Intellectual disability in KATP channel neonatal diabetes

Svalastoga P, Sulen Å, Fehn JR, Aukland SM, Irgens H, Sirnes E, Fevang SKE, Valen E, Elgen IB, Njølstad PR
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doi: 10.2337/dc19-1013. Epub 2020 Jan 13. PMID:31932458

Neonatal diabetes mellitus (NDM) is defined as diabetes that develops in the first 6 months of age. In Western countries, the most common causes of NDM are activating mutations in the *KCNJ11/ABCC8* genes. Neurological dysfunction is also common (up to 30%) in patients with NDM and includes: developmental delay, muscle hypotonia, attention deficit hyperactivity disorder and in some cases epilepsy (DEND syndrome, developmental delay and epilepsy). Some mutations in these genes (in particular p.V59M genotype in *KCNJ11*)are linked to abnormal neurological development including the DEND syndrome. It is currently unclear if early treatment for NDM with oral sulphonylureas can delay, improve or prevent the onset of abnormal neurological development in patients with NDM.

This study found a strong genotype-phenotype correlation; the p.V59M genotype in *KCNJ11* showed the strongest association with substantial intellectual disability. Interestingly, there was no significant correlation with age at initiation of sulfonylurea therapy. Consistent with previous studies, other genotypes were associated with minor cognitive impairment. In all patients (except one) the brain MRI scan showed normal anatomy, suggesting that the *KCNJ11* mutation does not affect brain development.

Thus, early treatment with oral sulphonylurea does not improve neurocognition in patients with p.V59M genotype in *KCNJ11*. The reasons for this are unclear but could be due to the fact that sulphonylureas no not cross the blood brain barrier and may even be actively transported out of the brain (1). Another possibility is that overactivity of K_{ATP} channels at an early developmental stage results in permanent neurological changes

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2.7. De novo mutations in EIF2B1 affecting eif2 signaling cause neonatal/early-onset diabetes and transient hepatic dysfunction

De Franco E, Caswell R, Johnson MB, Wakeling MN, Zung A, Dung VC, Bích Ngoc CT, Goonetilleke R, Vivanco Jury M, El-Khateeb M, Ellard S, Flanagan SE, Ron D, Hattersley AT
Diabetes. 2020 Mar;69(3):477–483.
doi: 10.2337/db19-1029. Epub 2019 Dec 27. PMID: 31882561

Endoplasmic recticulum (ER) stress plays an important role in the etiology of several forms of diabetes mellitus. There is evidence that ER stress plays a role in both type 1 and type 2 diabetes. More importantly molecular defects in the ER stress pathway are linked to monogenic and syndromic forms of diabetes mellitus. ER stress is defined as an imbalance between the protein folding capacity of the cell and the functional demand that is placed on it. Any imbalance will lead to the accumulation of unfolded or misfolded proteins in the ER lumen. In order to restore ER homeostasis, cells trigger the ER stress response, also known as the unfolded protein response (UPR). The proteins synthesized in the ER comprise all secreted and membrane expressed proteins and any mutation leading to misfolding of these proteins in the ER can theoretically cause ER stress and beta-cell damage and diabetes.

This paper describes a novel disorder of permanent neonatal/early onset diabetes with transient hepatic dysfunction due to mutations in the gene *EIF2B1*. This gene encodes for the alpha subunit of a complex in the ER which regulates and initiates the ER stress response. The phenotype of the five patients described overlaps with another disorder linked to ER stress called Wolcott–Rallison syndrome (WRS) and is in the same pathway as the *EIF2B1*. However, the clinical spectrum of WRS is more severe with most patients dying from hepatic dysfunction. All of the mutations in the five patients were de-novo and in heterozygote state. In-silico studies showed that these mutations impaired phosphorylation of the alpha subunit. Interestingly, homozygous mutations in *EIF2B1* lead to a severe fatal neurological disorder characterized by leukoencephalopathy with vanishing white matter (VWM). Only one of the five patients described had a mild learning disability. These observations expand our knowledge about the ER stress pathways and suggest that genetic testing for *EIF2B1* should be undertaken in any patient with early onset diabetes and hepatic dysfunction, even if only transient.

2.8. Patterns of post-meal insulin secretion in individuals with sulfonylurea-treated KCNJ11 neonatal diabetes show predominance of non-K_{ATP}-channel pathways

Bowman P, McDonald TJ, Knight BA, Flanagan SE, Leveridge M, Spaull SR, Shields BM, Hammersley S, Shepherd MH, Andrews RC, Patel KA, Hattersley AT
*BMJ Open Diabetes Research and*Care2019; 7:e000721.
PMID: 31908791

Understanding of the molecular mechanisms underlying neonatal diabetes mellitus (NDM) has helped to transform the clinical management of some patients. Those with NDM due to mutations in the *KCNJ11/ABCC8*genes can now be switched to oral sulphonylurea treatment and their daily insulin injections stopped. Despite use of high doses of sulphonylureas, severe hypoglycemia is rare in patients with NDM due to *KCNJ11* mutations. However anecdotal reports from patients with NDM due to *KCNJ11* mutations have suggested that they have mild to moderate hypoglycemia after meals rich in protein/fat and lacking carbohydrate, or after meals smaller than usual in size (1).

This study of a small number of adult patients with two *KCNJ11* mutations provides potential insights into the mechanism of hypoglycaemia. Patients with NDM due to *KCNJ11* mutations had higher blood glucose levels after a carbohydrate meal compared to after a protein/fat meal. However, their plasma insulin levels were the same for both groups. This observation suggests that the insulin level might be 'inappropriately high' for these patients when they eat a protein/fat meal and this may then lead to post-prandial hypoglycaemia. As amino acids (and fatty acids) are known to trigger insulin secretion via alternative pathways (non K_{ATP}channel), this could be one potential mechanism for the dysregulated insulin secretion in response to a protein/fat meal. These observations have important clinical implications for those patients who are on sulphonylurea therapy. These patients should have sufficient carbohydrates in their diet and should not miss any meal, as this will increase the risk of hypoglycaemia.

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2.9. Long-term metabolic and socio educational outcomes of transient neonatal diabetes: A longitudinal and cross-sectional study

Le Bourgeois F, Beltrand J Baz B, Julla JB Riveline JP, Simon A, Flechtner I, Ait Djoudi M, Fauret-Amsellem AL, Vial Y, Scharfmann R, Sommet J, Boudou P, Cavé H, Polak M, Gautier JF, Busiah KTNDM Long-Term Follow-Up Study Group
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TransientNeonatalDiabetes (TNDM) develops in the first six months of life, and then remits only to relapse again during adolescence and adulthood. The three main genetic causes of TNDM are: 1) 6q24 abnormalities, 2) activating mutations in genes encoding the ATP-sensitive potassium (KATP) channel subunits *KCNJ11*(KIR6.2) and *ABCC8* (SUR1) and 3) mutations in the pre-proinsulin (INS) gene. No study has assessed the long-term metabolic profile and educational outcomes in patients with TNDM.

Therefore, this study was undertaken to assess the long-term metabolic and socio-educational outcomes of individuals with TNDM history, and also their relatives carrying the same genetic abnormalities. Although the number of patients in the three genetic sub-groups (6q24, ABCC8 and KCNJ11 mutations) was low, relapse rates were high in all groups. The study found that the underlying genetic defect did not predict relapse of diabetes in TNDM participants, nor the occurrence of diabetes in relatives. The underlying genetic defects were not associated with insulin responses to glucose or to arginine stimuli in adulthood. TNDM participants had learning difficulties and a lower educational attainment than the general population. They were significantly more often outside the labor market but did not differ for other socioeconomic status parameters.

These observations have important clinical implications for the management and follow up of TNDM patients. As the relapse rate was high in all subgroups, all patients with TNDM should have regular HBA1c (2 yearly in childhood and yearly in adolescence) as well as an oral glucose tolerance test in adolescence. During childhood, close attention should be directed to education and neurodevelopmental milestones in TNDM patients with or without later diabetes.

2.10. The clinical and genetic characteristics of permanent neonatal diabetes (PNDM) in the State of Qatar

Al-Khawaga S, Mohammed I, Saraswathi S, Haris B, Hasnah R, Saeed A, Almabrazi H, Syed N, Jithesh P, El Awwa A, Khalifa A, AlKhalaf F, Petrovski G, Abdelalim EM, Hussain K
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The unique population in the State of Qatar comprises over 2.6 million people who derived primarily from the Middle East and North Africa (MENA) and South Asia regions. Around 15% are indigenous Qataris of Arabian Peninsula ancestries, who have also immigrated to the State in the past decades. Native Qataris represent a population with a combination of Bedouin, South Asian and African descent, which contributes to their observed diversity at the genome level. Specifically, the Bedouin subpopulation continues to practice within?tribal marriages leading to high levels of homozygosity compared with other populations. The clinical and genetic analysis of neonatal diabetes mellitus (NDM) in The State of Qatar has not been studied before.

In this study 100% of NDM patients had an identifiable genetic etiology. RecessivePTF1A (encoding a transcription factor involved in pancreatic development)distal enhancer deletions mutations were the most frequent cause of NDM in this population, followed byINS (insulin) gene mutations. All NDM patients with a PTF1Adistal enhancer deletion mutations had pancreatic hypoplasia/agenesis, failure to thrive and short stature. No mutations were found in the *ABCC8/KCNJ11* genes (which are the most common causes of NDM in Western populations). The incidence of NDM during the period 2001–2016 was 1:22 938 live births among the

indigenous Qatari population, which is at least 10 times higher than worldwide estimates. This is one of the highest incidences in the world. The principal cause for the high incidence of NDM is attributed to a combination of consanguinity and low birth rates in Qatar. This study confirms that the causes of NDM in the MENA region are different from those in Western countries.

Updates on the Genetics of Neonatal Diabetes Mellitus, Congenital Hyperinsulinism and Glucose Disorders

2.11. Update of variants identified in the pancreatic β-cell K_{ATP}channel genes KCNJ11 and ABCC8 in individuals with congenital hyperinsulinism and diabetes

De Franco E, Saint-Martin C, Brusgaard K, Knight Johnson AE, Aguilar-Bryan L, Bowman P, Arnoux JB, Larsen AR, May S, Greeley Saw, Calzada-León R, Harman B, Houghton JAL, Nishimura-Meguro , Laver TW, Ellard S, Del Gaudio D, Christesen HT, Bellanné-Chantelot C, Flanagan SE

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Pancreatic K_{ATP}channels play a key role in regulating insulin secretion. These channels are composed of four subunits of SUR1 and four subunits of KIR6.2 encoded by the *ABCC8* and *KNCJ11* genes, respectively. Inactivating mutations in these two genes lead to unregulated insulin secretion and severe hypoglycaemia (congenital hyperinsulinism CHI) whereas activating mutations lead to decreased insulin secretion and cause either permanent or transient neonatal diabetes mellitus (NDM). Since the discovery that mutations in these two genes leads to either CHI or NDM, a significant number of variants have been reported. In this update review the authors report a total of 748 ABCC8 and 205 KCNJ11pathogenic or likely pathogenic variants identified in individuals with CHI or NDM. Founder mutations have been identified in many populations, with the best recognized example being the ABCC8 p.(Phe1388del) and c.3992–9G>A mutations present in >90% of cases from the Ashkenazi Jewish population. In addition, 368 benign/likely benign variants and variants of uncertain significance were found in both genes. Just like any genetic disease, predicting variant pathogenicity can be challenging and just finding a missense variant may not be sufficient proof of disease causality. In these cases, the authors recommend using the guidelines by the American College of Medical Genetics to help predict pathogenicity. In addition, the use of large variant databases such as GnomAD and LOVD can also aid in variant interpretation and allow for reclassification of variants. Based on the information from these databases, some variants in *ABCC8/KCNJ11* that were previously reported as being pathogenic are now found to be too common to be disease causing; these are now reclassified as a variant of uncertain significance or a benign variant. The spectrum of mutations in *ABCC8/KCNJ11* causing CHI and NDM continues to expand.

2.12. Ion Transporters, channelopathies, and glucose disorders

Demirbilek H, Galcheva S, Vuralli D, Al-Khawaga S, Hussain K

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Ion channels and transporters play essential roles in excitable cells, including cardiac, skeletal and smooth muscle cells, neurons, and endocrine cells. In pancreatic beta-cells, KATPchannels link the metabolic signals generated inside the cell to changes in the beta-cell membrane potential and ultimately, insulin secretion. In addition, ion channels and transporters have roles in non-excitable tissues such as the liver and skeletal muscle. Mutations in the genes encoding the ion transporter and channel proteins lead to several diseases related to glucose physiology. For example, mutations in the genes *ABCC8/KCNJ11* which encode for the pancreatic K_{ATP} channels lead to different forms of congenital hyperinsulinism (CHI), neonatal diabetes mellitus (NDM) and in some cases Maturity Onset Diabetes of the Young (MODY). The voltage-gated potassium channel (Kv11.2) and the Kv7.1 channel have also been linked to possible episodes of hypoglycaemia. In addition, defects in some calcium channels (Calcium Voltage-Gated Channel Subunit Alpha1 D (*CACNA1D*)) also lead to hyperinsulinaemic hypoglycaemia (HH). Genetic defects in the membrane transporter (MCT1) lead to

exercise induced HH. Mutations in the glucose transporter GLUT2 as associated with Fanconi-Bickel syndrome which can present with dysglycaemia (post-prandial hyperglycaemia, fasting hypoglycaemia and neonatal diabetes mellitus). Mutations in *SLC19A2*ion transporter lead to Thiamine-responsive megaloblastic anaemia (TRMA), also known as Roger's Syndrome, an autosomal recessive disorder characterized by early-onset non-autoimmune DM, megaloblastic anaemia, and sensorineural deafness. Finally, Genome-Wide Association Studies (GWAS) have identified variants or polymorphisms in channel and ion transporter genes associated with type 2 diabetes mellitus (T2DM). For example, the non-synonymous E23K variant in theKCNJ11was the first robustly replicating signal to emerge as a link to T2DM. Thus, genetic defects in ion channels and transporters genes are associated with a large number of diseases related to glucose physiology.

2.13. New insights into K_{ATP} channel gene mutations and neonatal diabetes mellitus

Pipatpolkai T, Usher S, Stansfeld PJ, Ashcroft FM

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ATP-sensitive potassium (K_{ATP}) channels are composed of a pore-forming Kir6.2 potassium channel and a regulatory ABC transporter sulfonylurea receptor 1 (SUR1). This channel has a hetero-octameric structure compromising of four SUR1 subunits and four Kir6.2 units. Metabolic signals generated in the beta-cells in the form of nucleotides (such as ADP and ATP) regulate the function of this channel. This regulation of the activity of the K_{ATP} channel by adenine nucleotides is central to its ability to control insulin secretion. Alterations in the levels of ATP and ADP close the channel and magnesium nucleotides (MgATP and MgADP) open the channel. Inactivating mutations in these channels lead to congenital hyperinsulinism (CHI) and activating mutations cause neonatal diabetes mellitus (NDM).

This 'state of the art' review provides an in-depth understanding of how mutations in the genes encoding these channels lead to NDM and the mechanisms of action of sulphonylurea therapy in patients with NDM. Novel techniques such as cryo-electron microscopy (similar to X-ray crystallography) have aided understanding of the atomic level organization of the K_{ATP} channel complex and identification of the binding sites for nucleotides and sulphonylurea drugs. This has informed understanding of how mutations cause NDM and their functional effects. Gain of function mutations that lead to NDM result in K_{ATP} channels with reduced sensitivity to inhibition by ATP and therefore remain in the open state despite hyperglycaemia. There is some correlation between the ability of a given mutation to reduce the ATP sensitivity of the channel and the clinical phenotype, with the more severe mutations leading to NDM and neurological features.

Analysis of cryo- electron microscopy structures has shown that sulfonylureas and glinides bind within the same pocket, which lies within the transmembrane domains of SUR1. Most NDM patients can be transferred onto oral sulphonylurea, but not every patient responds to sulphonylurea. Whether a patient responds to oral sulphonylurea is determined by their specific mutation and duration of diabetes (1). Some mutations in the channel genes can affect the efficacy of sulphonylurea. Understanding the structure and functional aspects of K_{ATP} channels has provided unique insights into beta-cell physiology and disease mechanism but more importantly improved patient care and management.

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2.14. Gestational diabetes adversely affects pancreatic islet architecture and function in the male rat offspring

Agarwal P, Brar N, Morriseau TS, Kereliuk SM, Fonseca MA, Cole LK, Jha A, Xiang B, Hunt KL, Seshadri N, Hatch GM, Doucette CA, Dolinsky VW

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PMID: 31237608

Gestational diabetes (GDM) is associated with impaired beta-cell function in the offspring and this leads to increased risk of type 2 diabetes mellitus (1). However, the underlying mechanisms are not well understood. GDM exposure may alter islet gene expression and function in the fetus. Impaired beta-cell function is apparent in the offspring of women with gestational diabetes by age 7 years and in adulthood (2). Beta-cells undergo major structural and functional changes during gestation and early postnatal life. Evidence from animal models shows that maternal hyperglycemia and overnutrition can trigger maladaptive beta-cell growth (3).

This study used a rat model of high fat diet to determine how GDM may program islet structure and function in infancy (day 1) and then in young adulthood (15 weeks of age). The study also examined how the maternal environment interacts with the postnatal diet to affect islet structure and function. Islet size was increased in the newborn pups of GDM dams but there was no change in islet size in young adult rats. The offspring of GDM dams who were fed a high fat diet postnatally also showed no increase in islet size suggesting that GDM exposure impaired the ability of their islets to increase in beta-cell numbers/mass. Mechanistically, GDM leads to defects in the secretion of insulin and glucagon and this might be mediated by altered expression patterns of genes that regulate insulin and glucagon secretion. Consistent with this, unbiased analysis of RNA-seq data revealed significant changes in genes in several categories, including inflammation, mitochondrial function/oxidative stress resistance, and ribosomal proteins. Further studies will be required to understand the function of these genes and how maternal gestational hyperglycaemia impacts offspring islet function.

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2.15. Efficacy of fish oil and/or probiotic intervention on the incidence of gestational diabetes mellitus in an at-risk group of overweight and obese women: A randomized, placebo-controlled, double-blind clinical trial

Pellonperä O, Mokkala K, Houttu N, Vahlberg T, Koivuniemi E, Tertti K, Rönnemaa T, Laitinen K

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PMID: 30967436

Gestational diabetes mellitus (GDM) is increasing in incidence, affecting approximately 14% of pregnancies. Preventing GDM may significantly improve outcome for both mothers and offspring. The pathogenesis of GDM involves insulin resistance, pancreatic beta-cell failure and an increase in inflammatory markers. Both probiotics and the n-3 long-chain polyunsaturated fatty acids (LC-PUFA) present in fish oil have been demonstrated to possess anti-inflammatory properties and a capability to reduce insulin resistance. Furthermore, a combination of these two active components might exert synergistic immunoregulatory effects.

To test a novel way to prevent GDM, the authors conducted a randomized, placebo-controlled intervention trial of a dietary supplementation. The hypothesis was that fish oil and probiotic supplements, either individually or in combination, could improve blood glucose control during pregnancy and prevent GDM by reducing insulin resistance and inflammation. 439 women ‘at risk for’ GDM were randomized into four intervention groups: fish

oil + placebo, probiotics + placebo, fish oil + probiotics, and placebo + placebo. Although the fish oil and probiotics were well tolerated with no side effects, they found no difference in any maternal and neonatal outcome; primarily the various diets conferred no benefit in lowering the risk of GDM.

This was the first study to use a combination of fish oil and probiotics but found no benefit in lowering the risk of GDM. The findings are consistent with a previous study reporting no benefit of fish oil supplementation (1) but a recent meta-analysis of 7 clinical trials found that supplementation with Omega-3 fatty acids has substantial benefits on glycemic control and inflammatory responses (2). The putative mechanisms by which fish oil and probiotics might improve the outcome in GDM include improvements in intestinal barrier integrity and reduction in the risk of metabolic endotoxemia and subsequent low-grade inflammation. It seems that there is still uncertainty whether probiotics and fish oils impact GDM outcomes and thus further studies are required.

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2.16. Lactational metformin exposure programs offspring white adipose tissue glucose homeostasis and resilience to metabolic stress in a sex-dependent manner

Carlson Z, Hafner H, Mulcahy M, Bullock K, Zhu A, Bridges D, Bernal-Mizrachi E, Gregg B

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doi: 10.1152/ajpendo.00473.2019. Epub 2020 Mar 10. PMID: 32154743

The window of lactation is a critical period during which nutritional and environmental exposures may impact lifelong risks of metabolic diseases (such as type 2 diabetes and obesity). Significant organ and tissue development, organ expansion and maturation of cellular functions occur during the lactation period, making this a vulnerable time during which transient insults may have lasting effects. This period also provides a window of opportunity for possible interventions to improve long-term metabolic health.

In this mouse study, the authors hypothesized that metformin given during the lactational period would improve the metabolic profile of offspring later in life. Metformin was administered to C57BL/6 dams (lactating mothers) from the day of birth to postnatal day 21. Maternal and offspring health were monitored and glucose homeostasis assessed, as well as pancreatic and adipocyte biopsies taken. Maternal exposure to metformin during lactation programmed offspring body composition and glucose homeostasis in a sex-dependent manner. Throughout life, both male and female offspring were leaner. Furthermore, males tended to have lower visceral adiposity with more smaller visceral white adipocytes and an improvement in glucose tolerance that was unrelated to changes in beta-cell mass or function. Females showed no change in adiposity. Both males and females were protected from metabolic impairment when subjected to a high fat diet but this differed between males and females. Lactational metformin had no effect on beta-cell mass or insulin secretion and changes in insulin sensitivity were observed only in the setting of a high fat diet.

How metformin may be mediating its effects is not clear but suggested mechanisms include a direct effect on the gut, on the liver, and by altering maternal milk composition. These data demonstrate long-term metabolic programming in offspring by maternal exposure to metformin during lactation – at least in mice. It will be interesting to understand if similar findings can be replicated in humans.

2.17. Children exposed to maternal obesity or gestational diabetes mellitus during early fetal development have hypothalamic alterations that predict future weight gain

Page KA, Luo S, Wang X, Chow T, Alves J, Buchanan TA, Xiang AH
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PMID: 31332028

Maternal obesity and gestational diabetes (GDM) increase the risk of offspring developing childhood obesity and type 2 diabetes. The underlying mechanisms and possible neuronal pathways are unknown but might involve changes in the hypothalamic structure and function. Novel non-invasive imaging techniques (such as pulsed arterial spin labeling (PASL) a type of functional MRI scan) allow the assessment of hypothalamic function in response to glucose by measuring changes in cerebral blood flow. These have been used in children and adults with reproducible results.

In this study, infants who had been exposed to maternal obesity and GDM (diagnosed at $<$26 week's gestation) had significantly higher hypothalamic responses to glucose at age 7–11 years. On further follow up 1 year later, higher hypothalamic responses to glucose were predictive of greater gains in child's adiposity. Maternal obesity was also was associated with child's adiposity at 7–11 years of age and with increases in adiposity 1 year later. It is unclear why children aged 7–11 years were studied; presumably they were able to undergo the PASL assessments.

These observations of higher blood flow in the children's hypothalamus in response to glucose represent a possible mechanism by which exposure to maternal metabolic disorders during fetal development increases the risk for obesity later in life. However, the imaging techniques used here provide indirect measures of neuronal activity and cannot distinguish between activation of hypothalamic areas involved in hunger versus areas involved in satiety. Therefore, a casual inference cannot be made regarding the role of altered brain pathways on the development of obesity in children. Furthermore, childhood obesity is a multifactorial disease process, and associations between maternal GDM and obesity and higher offspring obesity risk may be conveyed by many biological and behavioural mechanisms. However, the concept of assessing hypothalamic function in response to glucose by measuring changes in cerebral blood flow is interesting and exciting for future studies.

2.18. Exposure to maternal obesity programs sex differences in pancreatic islets of the offspring in mice

Nicholas LM, Nagao M, Kusinski LC, Fernandez-Twinn DS, Eliasson L, Ozanne SE
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PMID:31773193

Maternal obesity is a risk factor for the development of type 2 diabetes in the offspring. Exposure to maternal obesity alters pancreatic islet structure and function, including reduced beta-cell mass and impaired glucose stimulated insulin secretion, which both increase susceptibility to type 2 diabetes. However, most previous studies have not assessed if these changes in pancreatic islet structure and function are explained by other confounding factors, such as offspring obesity, offspring high fat diets or ageing.

This mouse study was undertaken to understand the potential sex-specific changes in islet function in offspring born to obese dams, and whether these are independent of offspring obesity, glucose intolerance and ageing. Female C57BL/6J mice were fed ad libitum either chow or obesogenic diet prior to and throughout pregnancy and lactation. The offspring were weaned onto a chow diet and remained on this diet until the end of the study. They found sex differences in type 2 diabetes susceptibility in offspring as a consequence of exposure to maternal obesity and this could potentially be driven by differences in islet function. Islets from female mice that were exposed to maternal obesity showed increased insulin secretion as well as improved mitochondrial function. In addition, these islets had increased expression of the estrogen receptor and a reduction in the markers of apoptosis. In contrast islets from exposed male mice had compromised mitochondrial function, with reduced expression of key calcium channel proteins that are required for insulin secretion, and defects in beta-

cell insulin granules. In male mice, islet function became compromised later in life following the onset of obesity and insulin resistance.

Understanding the maternal factors (such as for example hyperinsulinaemia, hyperglycaemia and hyperlipidaemia) and critical developmental periods that might underlie the programming of metabolic adversity and islet function across the life course of the offspring is an important research goal.

2.19. Prolonged prepregnant maternal high-fat feeding reduces fetal and neonatal blood glucose concentrations by enhancing fetal β-cell development in C57BL/6 mice

Qiao L, Wattez JS, Lim L, Rozance PJ, Hay WW Jr, Shao J
*Diabetes.*2019 Aug;68(8):1604–1613.
doi: 10.2337/db18-1308. Epub 2019 May 24. PMID: 31127056

Maternal obesity is an important risk factor for neonatal hypoglycaemia. Maternal BMI predicts the risk of neonatal hypoglycaemia independently from maternal obesity suggesting that the link between maternal adiposity and neonatal hypoglycaemia is complex. Identifying the underlying mechanisms of maternal obesity induced neonatal hypoglycaemia may help to predict which newborns are at high risk of hypoglycaemia and improve clinical management.

This mouse study aimed to understand the impact of pre-pregnant weight gain on fetal and neonatal blood glucose levels as well as beta-cell mass and function. Prolonged pre-pregnant high fat feeding (as opposed to high fat feeding during pregnancy) in C57BL/6 mice was found to lead to fetal and neonatal hypoglycaemia as well as increased pancreatic beta-cell mass and insulin secretion. Pre-pregnant high fat feeding also led to increased expression of genes regulating the placental supply of fatty acids. When the supply of these fatty acids was blocked (by placental-specific knockout of adipose triglyceride lipase) this then reduced beta-cell mass and improved low blood glucose levels in the perinatal period. By contrast, high fat feeding during pregnancy in the same mouse line increased maternal adiposity but not neonatal hypoglycaemia.

This is the first mouse study to show that pre-pregnant high fat feeding alters fetal and neonatal glucose levels as well as pancreatic beta-cell mass. High fat feeding in adult mice is known to increase beta-cells mass and dietary fatty acids might play a role in this. This study shows that placental fatty acids play an important role in fetal pancreatic beta-cell mass and function and that pre-pregnant maternal adiposity impacts fetal and neonatal glucose physiology.

Fetal and Neonatal Cortisol Physiology

2.20. Complications of antenatal corticosteroids in infants born by early term scheduled cesarean section

Gupta K, Rajagopal R, King F, Simmons D
Diabetes Care. 2020 Apr;43(4):906–908.
doi: 10.2337/dc19-2126. Epub 2020 Jan 23. PMID: 31974101

Antenatal corticosteroid administration prior to planned cesarean section in early labour reduces by >50% the risk of admission to the neonatal intensive care unit for respiratory distress syndrome and transient tachypnea of the newborn. However, among pregnant women with gestational diabetes mellitus (GDM) or pre-existing diabetes, corticosteroid administration results in unpredictable maternal hyperglycemia, which is associated with neonatal hypoglycaemia. Therefore, it is unknown whether antenatal corticosteroid administration before planned cesarean section for early labour is beneficial among women with diabetes in pregnancy.

In this study, the authors compared the rates of neonatal respiratory distress syndrome, transient tachypnea of the newborn and neonatal hypoglycemia before and after the introduction of a clinical policy to avoid the use of corticosteroids before elective early cesarean section for pregnant women with diabetes. They observed that

antenatal glucocorticoid administration was associated with higher risk of neonatal hypoglycemia, but no change in the rates of neonatal respiratory distress syndrome and transient tachypnea of the newborn. Based on these observations, the authors suggest that corticosteroids may be safely omitted in women with any form of diabetes in pregnancy undergoing early planned cesarean section. Such management could potentially significantly reduce admissions to the neonatal unit for neonatal hypoglycemia without increasing admissions for neonatal respiratory diseases. However, this single center study included a relatively small number of patients (90% with GDM). A larger randomized controlled trial would greatly strengthen this possibility.

Vitamin D Supplementation in Pregnancy and Fetal and Infant Growth

2.21. Cord blood Vitamin D status is associated with cord blood insulin and c-peptide in two cohorts of mother-newborn pairs

Switkowski KM, Camargo Jr. CA, Perron P, Rifas-Shiman SH, Oken E, Hivert MF
*J Clin Endocrinol Metab.*Vol 104, Issue 9, September 2019, Pages 3785–3794.
PMID: 31127822

Maternal vitamin D status during pregnancy has been associated with markers of fetal growth and development. The circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D], crosses the placenta, and the developing fetus relies on the mother for its vitamin D source. Maternal vitamin D insufficiency during pregnancy has been linked to impaired fetal growth, increased risk of gestational diabetes mellitus (GDM), and adverse postnatal growth patterns and insulin resistance in the offspring (1). Thus, maternal vitamin D status may contribute to prenatal programming of the insulin axis and glucose regulation. However, some observational studies have not found associations of maternal 25(OH)D concentrations during pregnancy with markers of fetal growth or fetal insulin production (2,3). Although adequate vitamin D status seems to influence glycemic control in adults, the association of vitamin D with insulin secretion during fetal life is unclear. As both vitamin D and adequate nutrient supply are important for fetal adiposity and weight gain, vitamin D may play a different role in insulin secretion and beta-cell function during the prenatal period than in childhood and adulthood.

This study examined the associations of measures of prenatal vitamin D (cord blood vitamin D) status with markers of fetal insulin secretion. The authors included two pre-birth cohorts of mother-newborn pairs based in high-latitude regions where there is high risk of 25(OH)D deficiency, which allowed the replication of findings in two similar but independent populations. Among mother-child pairs with high prevalence of vitamin D insufficiency, newborn cord blood 25(OH)D levels were positively associated with cord blood insulin and c-peptide concentrations, but maternal 25(OH) status itself showed a non-linear relationship with cord blood insulin and c-peptide. These results suggest that vitamin D may play a role in regulating fetal beta-cell function, with a potential long-term impact on post-natal glucose regulation and growth. The same results were obtained when adjusting for maternal pre-pregnancy BMI and gestational weight gain, which would also impact cord blood insulin and c-peptide levels. Thus, cord blood 25(OH)D concentration might be a more accurate measure of fetal vitamin D status and that fetal vitamin D status is directly related to fetal insulin secretion, whereas the relationship with maternal vitamin D status is more complex. Further studies should explore the role of cord blood 25(OH)D in fetal beta-cell function and glucose physiology.

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3. Leffelaar ER, Vrijkotte TGM, van Eijsden M. Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. *Br J Nutr.* 2010;104(1):108–117.

Neonatal Oxytocin and Brain Development

2.22. Expression of synaptic proteins in the hippocampus is modulated by neonatal oxytocin treatment

Filova B, Reichova A, Zatkova M, Srancikova A, Bukatova S, Bacova Z, Bakos J
Neurosci Lett. 2020 Apr 23;725:134912.
doi: 10.1016/j.neulet.2020.134912. Epub 2020 Mar 12. PMID: 32173625

Oxytocin is the main neuropeptide regulating sociality and is expressed in neurons exclusively localized in the hypothalamus. Abnormalities in oxytocin signaling are associated with neurodevelopmental disorders such as autism. Oxytocin plays a role in the early development of neurons and participates in synapse formation. Binding of oxytocin to its receptors in some neuronal cells triggers calcium related pathways leading to enhanced expression of cytoskeletal proteins associated with neurite elongation. Activation of oxytocin receptors regulates the expression of synaptic cell-adhesion molecules. Oxytocin-producing cells appear during the early phase of brain development and their maturation, particularly their ability to produce oxytocin, may influence the formation of neural circuits, and oxytocin receptors play a role in hippocampal neurogenesis. The developing brain undergoes major neurogenesis and synaptogenesis during the first postnatal week of life. The expression of the synaptic proteins during development coincides with periods of functional synapse maturation. Cell adhesion molecules – neurexins and neuroligins are important for the synapse formation.

This study examined the role of oxytocin in regulating the of expression of selected cell-adhesion molecules and scaffolding proteins in the hippocampus in early rat development. Oxytocin administration altered pre and post-synaptic proteins as well as proliferation of neuronal cells. Pre-synaptic proteins showed decreased expression of Synapsin I at the gene and protein levels in response to oxytocin. Synapsin I is one of the most abundant brain phosphoproteins localized at pre-synaptic terminals, cell bodies, and neuronal growth cones. Changes in post-synaptic proteins included the neuroligins as well as other synaptic scaffolding proteins and cell-adhesion molecules. In addition, oxytocin stimulated the proliferation of cells in regions of the hypothalamus. These observations add support to the key role of oxytocin in early brain developmental and synaptogenesis and potentially in the etiology of neurodevelopmental disorders such as autism.

Neonatal Macrosomia and Analytical Analysis

2.23. Neonatal macrosomia is an interfering factor for analytes on the Colorado state newborn screen

Wright EL, Baker PR II
J Clin Endocrinol Metab. Volume 105, Issue 4, April 2020, Pages e1561–e1568,
PMID: 32126138

Newborn screening techniques are being used to detect an increasing number of inborn errors of metabolism. The application of tandem mass spectroscopy has allowed for high-throughput simultaneous quantification of acylcarnitine species and amino acids. Established patterns of metabolites are characteristic of specific acylcarnitine and amino acid disorders. However, the way a specimen is collected and handled, the condition of the infant and mother, as well as any treatments, all lead to both false-positive and false-negative results. While macrosomia is an established complication in offspring of obese and diabetic mothers and both macrosomia and maternal obesity are independent risk factors for future obesity, there is little evidence on the possible influence of newborn macrosomia on the accuracy of newborn screening.

The authors accessed data on 139 702 newborns, including 7806 with macrosomia (birth weight 4000–8000 g) and 131 896 non-macrosomic newborns (2500–3999 g). Macrosomic infants were more likely to be male than female (consistent with the typical sex difference in birth weights). Macrosomic infants had higher levels of certain carnitine and acylcarnitine species which interfered with the accuracy of newborn screening test, giving more false positive results.

These observations are interesting and suggest subtle biological differences in the context of macrosomia but the underlying reasons are not clear. Some of these biomarkers might reflect mitochondrial dysfunction and incomplete beta-oxidation (how fatty acids are used in the cell) as observed in obesity-related diseases. A previous study (1) reported that large for gestational age infants have an acylcarnitine and carnitine profile which is characteristic of childhood and adult obesity. Thus, it would be of interest to follow up the macrosomic infants with false positive markers to see if this pattern predicts higher risks of obesity, type 2 diabetes and the metabolic syndrome.

Reference

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3. Thyroid

Gabor Szinnai
Pediatric Endocrinology and Diabetology, University Children's Hospital Basel, University of Basel, Basel Switzerland and Department of Clinical Research, University Hospital Basel, Basel, Switzerland

Preface

The highlight of the year in basic thyroidology is the description of the molecular architecture of the thyroglobulin dimer. For decades, small pieces of knowledge were accumulated that are now integrated into the complete picture of the thyroglobulin glycoprotein three-dimensional structure as well as the few key functional hormonogenic tyrosine residues which allow thyroxine and tri-iodothyronine synthesis. Genetic research revealed new and unexpected molecular mechanisms causing Pendred syndrome and autosomal dominant familial multinodular goiter. In clinical thyroidology, important trials showed the effectiveness of new therapeutic approaches for Graves' orbitopathy and for MTC8 deficiency but failed to provide evidence for a beneficial effect of thyroid hormones in early life on psychomotor development in euthyroid children with Down syndrome. Finally, preliminary results on the association of air pollution and mild maternal hypothyroxinemia during pregnancy were confirmed in a large study investigating 5 different cohorts. In summary, the year in thyroidology is again characterized by fascinating insights into thyroid biology and important clinical progress for diagnosis and treatment of thyroid diseases.

Mechanism of the year

3.1. The structure of human thyroglobulin

Coscia F, Taler-Verčič A, Chang VT, Sinn L, O'Reilly FJ, Izoré T, Renko M, Berger I, Rappsilber J, Turk D, Löwe J
Nature. 2020;578:627–630.

In this extensive work, Coscia *et al.* determined the molecular architecture of the full-length human thyroglobulin (TG) at high resolution by cryo-electron microscopy and identified the four hormonogenic tyrosine residues which act either as mono- or di-iodotyrosine acceptor and donor sites (A-D) and allow the ultimate step of thyroid hormone synthesis – coupling of two di- or one mono- and one di-iodotyrosine to T4 and T3. The authors validated their structural results by extensive site-directed mutagenesis of human TG, analyzing the hormone synthetic capacity of the mutated TG proteins *in vitro*. Although distributed in three domains the four donor acceptor sites share structural key features: acceptor donor residues are located in proximity in an antiparallel way and structurally positioned to be solvent-exposed for iodination. Finally, the authors tested the hormone production efficiency of the characterized TG reaction sites outside of the TG structure by transferring them to a bacterial protein by maintaining structural properties of the residues as in the TG monomer. The hormone production of the engineered construct containing the human TG acceptor-donor residues is comparable to that of human TG.

This fundamental, elegant and extensive study resolves the molecular basis of thyroglobulin function conserved in many species. It was well established that tyrosines within the TG protein play a central role for thyroid hormone synthesis [1]. Further, it was known that tyrosine residues either act as mono- or di-iodotyrosine donor or acceptor sites. The TG monomer contains 66 tyrosines of which only 33 are iodinated after secretion into the follicular lumen. But only a few of these 33 tyrosines have been identified so far as acceptor site for coupling of two di- or one mono- and one di-iodotyrosine. In summary, the authors identified all functional sites of hormone synthesis as well as their structural properties. This work represents a milestone in thyroid physiology

providing the full picture of TG structure and function and integrating and completing previous results of the last decades.

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Thyroid hormone action

3.2. Multiple mechanisms regulate H3 acetylation of enhancers in response to thyroid hormone

Præstholm SM, Siersbæk MS, Nielsen R, Zhu X, Hollenberg A, Cheng SY, Grøntved L
PLoS Genet. 2020;16:e1008770.

Præstholm *et al.* quantified by a genomics approach histone H3 acetylation – an epigenetic mark for DNase accessible chromatin – at thyroid hormone target gene enhancers to challenge the current model of genome binding action of T3 (type 1 action of thyroid hormone receptors (TR)) [1]. For this, they investigated genome-wide the liver of WT mice at hypothyroid and hyperthyroid state. First, they observed that less than 40% of genomic regions that became hyperacetylated in response to T3 contained T3 response elements (TRE). Second, comparing WT vs. TR inactive tissues, hyperacetylation in response to T3 only occurred in TRE negative regions in the presence of functional TR. Finally, they revealed that T3 hyperacetylated enhancers without TRs and TREs were activated indirectly by co-association with TR bound enhancers in a higher order organization of hyperacetylated enhancers either at short distance to super-enhancers, or at large distance to topological associated domains.

Until now, a bimodal switch model was generally accepted to describe TR activation of enhancers of thyroid hormone target genes by direct binding to the genome at TREs (type 1 action of TR) [1]. Based on the current results, the authors propose a new and more differentiated concept than the bimodal switch, describing T3 dependent enhancer activation mediated by H3 hyperacetylation. The concept integrates 3 mechanisms: A) poised enhancers hyperacetylated upon dissociation of co-repressors and recruitment of co-activators, B) dormant enhancers hyperacetylated upon T3 dependent TR and co-activator recruitment to chromatin, and C) Non TR containing enhancers hyperacetylated by T3 activation of TR occupied enhancers at distance in the context of higher order chromatin structure.

These important data add to current knowledge a more differentiated mechanistic concept of type 1 T3 induced target gene transcription. They show in detail how epigenetic mechanisms, such as acetylation, are involved in thyroid hormone action. Beyond thyroid hormones, these data highlight the complexity of hormone dependent gene regulation possibly also functional for estrogen or vitamin D.

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Thyroid development

3.3. *Glis3* as a critical regulator of thyroid primordium specification

Rurale G, Marelli F, Duminuco P, Persani L
Thyroid. 2020;30:277–289.

GLIS3 gene mutations are associated with a syndrome that combines neonatal diabetes and congenital hypothyroidism due to thyroid dysgenesis [1]. *Glis3* knockout mouse models showed functional deficits of

thyroid hormone synthesis but were not able to shed light on the role of *Glis3* during thyroid organogenesis resulting in thyroid dysgenesis. Rurale *et al.* close this gap by a systematic developmental study on transient *glis3* knockdown in zebrafish embryos during the critical time window of endodermal development and thyroid primordium specification. *glis3* knockdown embryos showed developmental defects characterizing thyroid dysgenesis: lower expression of thyroid enriched transcription factors in the endodermal thyroid area, lower number of functional follicles producing thyroxine and lower expression of thyroglobulin. Hypoplasia of the differentiated thyroid tissue was not due to lower rate of proliferation or higher rate of apoptosis in determined thyrocyte precursors. In conclusion, these results provide the first developmental evidence for the key role of *GLIS3* for normal thyroid primordium specification and *GLIS3* gene defect associated phenotype of thyroid dysgenesis.

Reference

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Thyroid and pregnancy

3.4. Association of exposure to ambient air pollution with thyroid function during pregnancy

Ghassabian A, Pierotti L, Basterrechea M, Chatzi L, Estarlich M, Fernández-Somoano A, Fleisch AF, Gold DR, Julvez J, Karakosta P, Lertxundi A, Lopez-Espinosa MJ, Mulder TA, Korevaar TIM, Oken E, Peeters RP, Rifas-Shiman S, Stephanou E, Tardón A, Tiemeier H, Vrijheid M, Vrijkotte TGM, Sunyer J, Guxens M

JAMA Netw Open. 2019;2:e1912902.

Air pollution has recently been associated with maternal thyroxine levels during mid gestation in a large single cohort study [1]. No data exist so far on the association of exposure to specific markers of residential air pollution during the first trimester, when neurodevelopment of the embryo is exclusively dependent on transplacental passage of maternal thyroxine.

Ghassabian *et al.* closed this knowledge gap by comparing 4 European and one US cohort, comprising a total of 9931 first trimester pregnant women, to study the association of exposure to particular matter (PM) of different size and nitrogen dioxide (NO_2) during the first trimester. They observed a 20% higher odds ratio of mild hypothyroxinemia with an increase of 5 g/m^3 in PM with an aerodynamic diameter of $\leq$2.5 micrometer. Importantly, this increase was observed at much lower levels of PM concentration compared to the original study that observed this association during midgestation [1].

In this large study, no data are available on long-term effects of maternal hypothyroxinemia on child development. Over the last years, Ghassabian *et al.* have published first data derived from their Generation R cohort suggesting negative impact of maternal hypothyroxinemia in general on offspring brain development. In a global health perspective, reduction of residential air pollution, especially of PM of $\leq$2.5 micrometer, might decrease risk of maternal hypothyroxinemia and putative consecutive adverse effects on offspring neurodevelopment. Future large prospective studies need to investigate the trimester- and dose-dependent pollution effects on neurodevelopment in the offspring mediated by maternal hypothyroxinemia.

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Follow-up paper - Graves disease

3.5. Teprotumumab for the treatment of active thyroid eye disease

Douglas RS, Kahaly GJ, Patel A, Sile S, Thompson EHZ, Perdok R, Fleming JC, Fowler BT, Marcocci C, Marinò M, Antonelli A, Dailey R, Harris GJ, Eckstein A, Schiffman J, Tang R, Nelson C, Salvi M, Wester S, Sherman JW, Vescio T, Holt RJ, Smith TJ
N Engl J Med. 2020;382:341–352.

Teprotumumab, an IGF-1R monoclonal antibody, showed promising results for the treatment of Graves' orbitopathy in a first phase 2 study reported in 2017 [1]. Here, this randomized double-masked, placebo controlled phase 3 multicenter study confirms those initial promising results in patients with moderate-to severe Graves' eye disease: The primary outcome of proptosis reduction by ≥ 2 mm after 24 weeks was reached by 83% on teprotumumab vs. 10% of controls. Also, all secondary outcomes, including objective measures of thyroid eye disease activity and subjective quality of life, were better in the teprotumumab group with clinically relevant differences vs. placebo. Adverse events were mostly mild-to-moderate, however one patient had to stop treatment due to infusion-related reaction.

In summary, this first phase 3 randomized controlled study of teprotumumab revealed a good treatment responses with a number needed to treat (NNT) of only 1.36 in the context of a low number of adverse events during the short observational period of 24 weeks. As no alternative specific treatment option exists for Graves' orbitopathy, these data are an important next step to clinical approval and routine use in adults. Although severe thyroid eye disease is less frequent in childhood, we as pediatric endocrinologists look forward to teprotumumab studies during adolescence when Graves' disease incidence is highest in the pediatric population.

Reference

1. Smith TJ, Kahaly GJ, Ezra DG, Fleming JC, Dailey RA, Tang RA, Harris GJ, Antonelli A, Salvi M, Goldberg RA, Gigantelli JW, Couch SM, Shriver EM, Hayek BR, Hink EM, Woodward RM, Gabriel K, Magni G, Douglas RS. Teprotumumab for Thyroid-Associated Ophthalmopathy. *N Engl J Med.* 2017;376:1748–1761.

Congenital hypothyroidism

3.6. Neonatal screening for congenital hypothyroidism: what can we learn from discordant twins?

Medda E, Vigone MC, Cassio A, Calaciura F, Costa P, Weber G, de Filippis T, Gelmini G, Di Frenna M, Caiulo S, Ortolano R, Rotondi D, Bartolucci M, Gelsomino R, De Angelis S, Gabbianelli M, Persani L, Olivieri A
J Clin Endocrinol Metab. 2019;104:5765–5779.

It is not clear whether retesting is needed for a healthy cotwin of a twin pair discordant for congenital hypothyroidism (CH) at the first neonatal screening. Medda *et al.* retrospectively analyzed a cohort of 47 twin pairs discordant for CH at the first neonatal screening. On follow-up, 7 (15%) of cotwins who were initially negatively screened then tested positive on a second screening test up to 1 month of age. Two (4%) further siblings were diagnosed with CH on longer-term follow-up beyond the neonatal period at 9 months and 12 years of age. All 9 patients received levothyroxine substitutive therapy from diagnosis.

In conclusion, this study provides evidence for a higher detection rate of CH of initially healthy cotwins by repeated neonatal screening. These data clearly argue for systematic retesting of all healthy cotwins within the first month of life, and importantly irrespective of zygosity. In the context of increasing numbers of twin births over the last decades, repeated testing of twins will close an important and yet unexpected gap in neonatal screening programs.

3.7. *DUOX2/DUOXA2* mutations frequently cause congenital hypothyroidism that evades detection on newborn screening in the UK

Peters C, Nicholas AK, Schoenmakers E, Lyons G, Langham S, Serra EG, Sebire NJ, Muzza M, Fugazzola L, Schoenmakers N
Thyroid. 2019;29:790–801.

Patients with mutations in the dual oxidase 2 (*DUOX2*) gene – encoding a NADPH oxidase that generates hydrogen peroxidase for iodide organification – have been repeatedly reported as not being detected by neonatal screening because it causes only mild hyperthyrotropinemia at birth. Here, Peters *et al.* determined the incidence of *DUOX2* and dual oxidase 2 maturation factor (*DUOXA2*) mutations in patients with borderline blood spot TSH values. In a retrospective genetic study in 52 patients with thyroid gland *in situ* and borderline TSH, they detected a surprisingly high incidence (50%) of *DUOX2* or *DUOXA2* mutations. The key characteristic of mutation positive vs. mutation negative patients during the neonatal period was a significantly higher TSH on venous confirmatory testing or on second blood spot screening than on initial screening and lower mean FT4 values despite mild hyperthyrotropinemia.

These data provide clinically important answers: by current TSH cutoffs, we need to be aware that we will miss genetic forms of CH. Thus, clinical suspicion of this entity in the context of mild signs of hypothyroidism in neonates or infants should prompt retesting. However, the data also raise new questions: should TSH cutoffs be further lowered to better detect this mild dyshormonogenetic form of CH? Or should we perform targeted sequencing for *DUOX2* and *DUOXA2* in these patients? Biochemical diagnosis and genetic confirmation of mild dyshormonogenetic CH would argue for levothyroxine treatment at least during the sensitive early life neurodevelopmental time window. But is there enough evidence that these patients will benefit from early diagnosis and treatment? This chapter is not closed.

3.8. Thyrocyte cell survival and adaptation to chronic endoplasmic reticulum stress due to misfolded thyroglobulin

Morishita Y, Kabil O, Young KZ, Kellogg AP, Chang A, Arvan P
J Biol Chem. 2020;295:6876–6887.

In this experimental study, Morishita *et al.* developed a CRISPR/Cas-9 mediated cell model to investigate the molecular effects of thyroglobulin (TG) accumulation in the endoplasmatic reticulum (ER) of thyrocytes, as is observed in the context of dyshormonogenic CH caused by *TG* gene mutations. TG accumulation in the ER is a result of *TG* mutations altering the three-dimensional structure of the large TG glycoprotein dimer. Under normal conditions TG is proteolyzed in lysosomes and does not accumulate in the ER. First, the authors showed, that thyrocytes survive and grow in the presence of chronic ER stress. Secondly, the authors revealed that thyrocytes adapted to chronic ER stress. Proteomic analysis of their chronic ER stress model provided important insights into several active molecular mechanisms. The major finding was up-regulated AMP-Kinase activity and down-regulated mTOR activity, which is a conserved cell survival mechanism promoting adaptation to ER stress. A second key mechanism to escape chronic ER stress was a less differentiated thyrocyte phenotype with decreased protein levels of thyrocyte differentiation markers such as FOXE1, PAX8 and TPO.

In summary, these extensive *in vitro* data are in accordance with the phenotype of patients with *TG* mutations and of mice models of TG accumulation and provide fascinating new insights into the molecular mechanisms associated with misfolded TG in human *TG* mutation associated CH. Whether mutations in the hormonogenic tyrosine residues in the TG dimer (see first paper of this chapter, [1]) cause similar effects remains to be shown.

Reference

1. Coscia F, Taler-Verčič A, Chang VT, Sinn L, O'Reilly FJ, Izoré T, Renko M, Berger I, Rappsilber J, Turk D, Löwe J. The structure of human thyroglobulin. *Nature.* 2020;578:627–630.

3.9. Digenic inheritance of mutations in EPHA2 and SLC26A4 in Pendred syndrome

Li M, Nishio SY, Naruse C, Riddell M, Sapski S, Katsuno T, Hikita T, Mizapourshafiyi F, Smith FM, Cooper LT, Lee MG, Asano M, Boettger T, Krueger M, Wietelmann A, Graumann J, Day BW, Boyd AW, Offermanns S, Kitajiri SI, Usami SI, Nakayama M
Nat Commun. 2020;11:1343.

Li *et al*. describe a new genetic mechanism causing Pendred syndrome and extend evidence for an oligogenic origin of congenital hypothyroidism (CH). Autosomal recessive mutations in Pendrin (*PDS/SCL26A4*) were described in 1997 to cause Pendred syndrome [1]. However, over the years patients with either only heterozygous or even no mutation in *PDS/SLC26A4* have been diagnosed with the full clinical picture of Pendred syndrome, including mild dyshormonogenetic hypothyroidism and severe deafness, suggesting alternative genetic origins.

In this elegant work, the authors identified mutations in the *EPHA2* gene in patients with heterozygous *PDS/SLC26A4* mutations. EPHA2 belongs to the Ephrin ligands involved in transmembrane protein localization processes and governing compartmentalization during epithelial development. By studying *Epha2* knockout mice, the authors revealed disrupted epithelial development in the inner ear and in the thyroid with mislocalization of Pendrin in thyrocytes. These data are intriguing, as the classical difference between thyroid dysgenesis and thyroid dyshormonogenesis is challenged by the digenic inheritance of Pendred syndrome combining a functional defect of *PDS/SLC26A4* with a structural defect of Pendrin localization caused by the second gene defect in *EPHA2*. It further extends the concept of digenic origin of CH to genes beyond transcription factors involved in thyroid development or enzymes crucial for thyroid hormone synthesis. Based on this concept and approach, further new genes are expected to be identified in CH patients.

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3.10. DGCR8 microprocessor defect characterizes familial multinodular goiter with schwannomatosis

Rivera B, Nadaf J, Fahiminiya S, Apellaniz-Ruiz M, Saskin A, Chong AS, Sharma S, Wagener R, Revil T, Condello V, Harra Z, Hamel N, Sabbaghian N, Muchantef K, Thomas C, de Kock L, Hébert-Blouin MN, Bassenden AV, Rabenstein H, Mete O, Paschke R, Pusztaszeri MP, Paulus W, Berghuis A, Ragoussis J, Nikiforov YE, Siebert R, Albrecht S, Turcotte R, Hasselblatt M, Fabian MR, Foulkes WD
J Clin Invest. 2020;130:1479–1490.

This detailed genetic and molecular analysis of a three-generation family reveals a new form of autosomal dominant tumor susceptibility syndrome entitled familial multinodular goiter (MNG) with schwannomatosis. Biallelic alterations of the *DGCR8* gene were found in all affected patients: First, all patients harbored the same heterozygous germline mutation p.E518K. Secondly, all thyroid nodules and all schwannomas analyzed from the patients showed somatic loss of the whole chromosome 22 in addition to the p.E518K germline mutation, resulting in loss of heterozygosity. DGCR8 is key element of the canonical microRNA production pathway by forming a trimeric nuclear complex called microprocessor. The biallelic alteration of *DGCR8* at the tumor tissue levels caused disrupted microprocessor function, resulting in reduced precursor and mature microRNA expression due to disrupted cleavage of precursor microRNA from primary RNA.

These data extend the spectrum of familial forms of MNG beside DICER1 syndrome, which was the first tumor susceptibility syndrome to include MNG [1–3], and reveal a striking mechanistic analogy between DGCR8 and DICER1 associated tumor syndromes: both genetic familial forms of MNG result from altered microRNA processing, however at different levels of the micro RNA production pathway. Whether disrupted microRNA biogenesis is involved in other MNG forms remains to be shown.

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Clinical trials for thyroid disease

3.11. Thyroid hormone and folinic acid in young children with Down syndrome: the phase 3 ACTHYF trial

Mircher C, Sacco S, Bouis C, Gallard J, Pichot A, Le Galloudec E, Cieuta , Marey I, Greiner-Mahler O, Dorison N, Gambarini A, Stora S, Durand S, Polak M, Baruchel A, Schlumberger E, Dewailly J, Azar-Kolakez A, Guéant-Rodriguez RM, Guéant JL, Borderie D, Bonnefont-Rousselot D, Blondiaux E, Ravel A, Sturtz FG

Genet Med. 2020;22:44–52.

This single center, randomized, double-blind, placebo-controlled phase 3 study investigated the effects of levothyroxine, folic acid, or both in combination over 12 months on global development in 143 infants with Down syndrome (DS). Over the last decades, treatment of DS associated co-morbidities has improved their life-expectancy. However, a therapy to improve mental development is still lacking. Folic acid as well as thyroxine are both crucial for normal neurodevelopment *in utero* and postnatally. Previously, two randomized controlled studies suggested beneficial effects of either folic acid especially in children treated with levothyroxine in an age group of 3–30 months [1] and levothyroxine alone started in the neonatal period [2], while a short-term cross-over study did not observe effects of levothyroxine on cognitive function in DS patients [3].

This new, important, well designed clinical trial with four highly homogenous patient groups showed no effect on any of the analyzed subclasses (locomotor, social skills, hearing and language, hand-eye coordination, general performance) of global development – neither of folic acid or levothyroxine alone nor in combination. In summary, neither folic acid nor levothyroxine if TSH is below 7 mU/l is recommended to improve neurologic development in DS infants, although these therapies are widely used since years without evidence of effect. Non-pharmacological approaches might open new avenues for research. One suggested by the authors could be prevention of sleep apnea.

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3.12. Effectiveness and safety of the tri-iodothyronine analogue Triac in children and adults with MCT8 deficiency: an international, single-arm, open-label, phase 2 trial

Groeneweg S, Peeters RP, Moran C, Stoupa A, Auriol F, Tonduti D, Dica A, Paone L, Rozenkova K, Malikova J, van der Walt A, de Coo IFM, McGowan A, Lyons G, Aarsen FK, Barca D, van Beynum IM, van der Knoop MM, Jansen J, Manshande M, Lunsing RJ, Nowak S, den Uil CA, Zillikens MC, Visser FE, Vrijmoeth P, de Wit MCY, Wolf NI, Zandstra A, Ambegaonkar G, Singh Y, de Rijke YB, Medici M, Bertini ES, Depoorter S, Lebl J, Cappa M, De Meirleir L, Krude H, Craiu D, Zibordi F, Oliver Petit I, Polak M, Chatterjee K, Visser TJ, Visser WE

Lancet Diabetes Endocrinol. 2019;7:695–706.

The hallmarks of MCT8 deficiency are severe psychomotor retardation due to intracellular hypothyroidism in neuronal tissues and peripheral T3 thyrotoxicosis associated low weight, muscle wasting, high normal or increased heart rate and systolic blood pressure. TRIAC – the T3 analogue 3,3',5-tri-iodothyroacetic acid – enters target cells in an MCT8 independent way, and therefore could potentially improve intracellular thyroid hormone deficiency in the brain. On the other hand, TRIAC has only weak thyromimetic activity in peripheral tissues, thus represents a bona fide candidate to improve the MCT8 deficiency associated imbalance between neuronal intracellular hypothyroidism and peripheral hyperthyroidism.

This single-arm, phase 2 clinical trial in 45 pediatric and adult patients with MCT8 deficiency revealed beneficial biochemical and clinical effects of TRIAC treatment during 12 months on peripheral T3 thyrotoxicosis: T3 levels, cardiovascular parameters, and bodyweight improved and these effects were maintained in 10 patients who continued on a treatment extension for a median of 40 months. A clear improvement in gross motor function was seen in 6 of 7 patients treated before age 4 years, but there was no change in patients aged 4 years and older.

These data are important and provide evidence of effectiveness and safety of TRIAC for alleviating peripheral hyperthyroidism in MCT8 deficient patients. Neurologic improvement was observed only in the youngest patients, advocating starting TRIAC treatment as early as possible. A next clinical trial is under way to investigate TRIAC effectiveness when started in childhood (Triac Trial II; ClinicalTrials.gov number NCT02396459).

3.13. Cognitive function in children with idiopathic subclinical hypothyroidism: Effects of 2 years of levothyroxine therapy

Capalbo D, Alfano S, Polizzi M, Di Mase R, Improda N, Esposito A, Bravaccio C, Salerno M

J Clin Endocrinol Metab. 2020;105:e774–e7781.

This prospective non-randomized clinical trial investigated the effect of two-years levothyroxine treatment in children with subclinical hypothyroidism (TSH 5.0–9.9 mU/l with normal FT4 for two years preceding the study) starting at a mean age of 9 years. Capalbo *et al.* observed no change in IQ scores in 20 children after two-years of levothyroxine. Further, they observed no differences in IQ between these 20 treated and a further 14 untreated patients after two years.

The consequences of mild subclinical hypothyroidism on cognitive function during childhood is debated. In analogy to adults, levothyroxine treatment in children with subclinical hypothyroidism and a TSH <10 mU/l without clinical signs or symptoms is controversial. These important data, although limited by a small sample size and lack of randomization, showed no benefit of levothyroxine on IQ and all relevant subclasses in childhood over the so far longest observation period. Whether more complex and specific cognitive testing would reveal significant effects remains open. In summary, current practice of non-treatment of asymptomatic patients with a TSH below 10 is strengthened.

Thyroid cancer

3.14. Preoperative metabolic classification of thyroid nodules using mass spectrometry imaging of fine-needle aspiration biopsies

DeHoog RJ, Zhang J, Alore E, Lin JQ, Yu W, Woody S, Almendariz C, Lin M, Engelsman AF, Sidhu SB, Tibshirani R, Suliburk J, Eberlin LS

Proc Natl Acad Sci U S A. 2019;116:21401–21408.

This prospective diagnostic study used desorption electrospray ionization mass spectrometry (DESI-MS) to classify thyroid nodules by their metabolic signature, and more precisely their lipid profile. DESI-MS is a technique using charged aerosols of microdroplets which are sprayed onto a surface (e.g. tissue) to desorb and ionize molecules which are then analyzed by MS [1]. Statistical classifiers to discriminate normal vs. papillary thyroid carcinoma (PTC) and normal vs. follicular thyroid carcinoma (FTC) were developed on a large training set of 178 validated tissue samples. Benign, PTC and FTC could be discriminated mainly based on their lipid profiles (e.g. phosphoinositol species). The two classifier models to detect benign vs. malignant tissues showed high sensitivity and specificity in 69 prospectively analyzed clinical samples. Further, in the subgroup of undetermined cytology on fine-needle aspiration (FNA), preoperative DESI-MS-predicted classification (benign or malignant) was confirmed by postoperative histology of surgical samples in 20 of 24 cases.

This fascinating feasibility study provides a first convincing data on a new innovative technique possibly improving preoperative diagnostic accuracy of standard FNA cytology for thyroid nodules. However, due to small sample size, these promising results need to be confirmed in larger series and need also to be tested against diagnostic tools based on gene expression analysis of FNA smears, which are now established in clinical use. Although providing fascinating insights into tumor biology, an important drawback of DESI-MS is its complex and expensive technology, which might prevent routine clinical use outside large expert centers. Finally, whether pediatric PTC and FTC show the same metabolic profile as adult carcinomas and can be detected with the same degree of accuracy needs to be tested in future.

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Medication associated thyroid disease

3.15. The effects of amiodarone on thyroid function in pediatric and young adult patients

Barrett B, Hawkes CP, Isaza A, Bauer AJ

J Clin Endocrinol Metab. 2019;104:5540–5546.

This single center, retrospective study analyzed the prevalence and natural history of amiodarone associated thyroid disease in 190 children and young adults with detailed thyroid function tests. Amiodarone induced hypothyroidism (AIH) and thyrotoxicosis (AIT) are well known entities due to direct thyroid toxicity. Further, subclinical hypothyroidism (SCH) is caused by amiodarone induced inhibition of T4-to-T3 conversion, thereby stimulating TSH, and resulting in high FT4 but low-normal T3, as summarized in a recent detailed review on drug effects on the thyroid [1]. In their cohort, Barrett *et al.* observed thyroid abnormalities in one third of patients (SCH 17%, AIH 14%, AIT 2%). Sixteen percent of all amiodarone exposed patients developed persistent thyroid disease (SCH 9/33, AIH 18/26, AIT 3/4). While AIT occurred within the first two weeks, AIH and SCH incidence plateaued at 5 weeks after start of therapy. Amiodarone therapy for longer than ten days increased the risk for SCH and AIH. A final important result was that, despite the single center design, only 40% of all amiodarone treated patients received thyroid function testing, while in a national study this proportion was reported to be as low as 12% [2].

This important paper describes the incidence and prognosis of amiodarone induced thyroid diseases in a large retrospective cohort. Based on their findings, the authors provide recommendations for systematic thyroid function testing in patients before and weekly for 5 weeks after treatment start to screen for SCH, AIH and AIT. To establish these recommendations, pediatric cardiologists will need to be informed about these new data and be involved in establishing standard operating procedures.

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4. Growth and Growth Factors

Elena Inzaghi[a], Danilo Fintini[a], Stefano Cianfarani[a,b]

[a]D.P.U.O. "Bambino Gesù" Children's Hospital – University of Rome 'Tor Vergata', Rome, Italy;
[b]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

(Address correspondence to: Stefano Cianfarani, Bambino Gesù Children's Hospital, P.zza S. Onofrio, 4, 00165, Roma, Italy. Tel. +39 06 6859 3074 (Hosp.) / +39 06 72596178 (Lab.); Fax. +39 06 6859 2508 (Hosp.)/ +39 06 72596172 (Lab.) E-mail: stefano.cianfarani@uniroma2.it)

Preface

The spread of next generation sequencing (NGS) together with the progressive reduction in its cost has led to a broader application of this approach in children with short stature. We have selected a number of papers describing new gene mutations associated with familial short stature, Silver-Russell-like syndrome and syndromic short stature. The roles of IGF-I in the work-up of short stature, GH dose titration, cognitive function, predicting final height, supporting the embryonic stem cell niche, and as cardiovascular disease risk marker are emphasized in well-designed studies. Finally, studies reporting the consequences of GH deficiency on bone geometry and gut microbioma, and the results of a phase 2 trial of a once-weekly GH preparation in GHD patients are considered to be highly relevant for our readers. In the last year, the vast majority of papers on growth and growth factors reported clinical rather than basic science research. Nevertheless, many clinical observations remain without a clear pathophysiological explanation, thus paving the way for further studies aimed at elucidating the underlying biological mechanisms.

Important for clinical practice

4.1. Diagnosis, genetics, and therapy of short stature in children: A growth hormone research society international perspective

Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK, Boguszewski MCS, Cheung PT, Choong CSY, Cohen LE, Cohen P, Dauber A, Deal CL, Gong C, Hasegawa Y, Hoffman AR, Hofman PL, Horikawa R, Jorge AAL, Juul A, Kamenický P, Khadilkar V, Kopchick JJ, Kriström B, Lopes MdLA, Luo X, Miller BS, Misra M, Netchine I, Radovick S, Ranke MB, Rogol AD, Rosenfeld RG, Saenger P, Wit JM, Woelfle J

Disciplina de Endocrinologia, Departamento de Medicina Interna, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

paulosolberg@yahoo.com

Horm Res Paediatr. 2019;92:1–14

In March 2019, 46 international experts from 14 countries across 5 continents attended a 3-day workshop organized by the Growth Hormone Research Society (GRS) and produced this perspective on the diagnosis, management and therapy in children with short stature. In this context, this expert panel tackled almost all aspects related to the management of children with short stature, providing recommendations for clinical practice.

Clinical evaluation: The panel underlined the importance of family and personal history, physical examination and the correct choice of growth charts. WHO growth charts can be utilized for children up to 2 years of age, whereas national growth charts are more appropriate for older children. A thorough work-up should be considered in a child with height below – 2 SDS, height deviating from their family background, or a significant decrease in height SDS over time (by at least 0.3 SDS/year).

Laboratory tests: should be guided by clinical evaluation rather than routinely applied in all short children. Use of sitting height, arm span and sitting/height ratio measurements may provide clues for genetic short stature (SHOX deficiency, FGFR3 mutations, etc). Bone age was confirmed to be helpful for driving to the right diagnosis although it needs to be critically considered within the clinical, laboratory and radiological context. IGF-I values play a key role in the evaluation of a child with growth failure, but IGF-I levels are physiologically low in children aged <3 years when there is an overlap in values between normal and GHD children. An IGF-I level >0 SDS at any age makes the diagnosis of GHD unlikely. Measurement of IGF binding protein (BP)-3 may be helpful in the work-up of GHD in very young children. GH provocative tests were confirmed to be the gold standard in GHD diagnosis and the values of insulin tolerance test (ITT), glucagon, arginine, clonidine, L-dopa and GH-releasing peptide-2 were considered similar. The majority of delegates suggested 7 ng/ml as the threshold to distinguish between normal and subnormal peak GH responses to stimulation. In neonates in first week of life, a random GH value <7 ng/ml should be considered diagnostic for GHD. Provocative tests were considered unnecessary for children with Turner syndrome, Noonan syndrome, Prader-Willi syndrome, SHOX deficiency, renal insufficiency, Silver-Russell syndrome and short children born small for gestational age (SGA). There was no consensus on the use of sex steroid priming prior to performing GH stimulation tests, except for adolescents with clinically ascertained delayed puberty.

Radiology: Pituitary Magnetic Resonance Imaging (MRI) should be performed in all patients diagnosed with GHD but is not a test for the diagnosis of GHD. If a midline defect, hypoglycemia or microphallus in newborns has been detected, GH provocative tests are not necessary as a routine. The presence of empty sella, ectopic posterior pituitary and/or absence of anterior pituitary, as such as pituitary stalk hypoplasia, are suggestive of GHD.

Genetics: Karyotype should be performed in all girls with short stature to exclude Turner syndrome. Genetic and epigenetic testing are not required for all children with short stature, but only in those with a phenotype suggestive of a genetic cause, or in severe GHD, severe short stature (<3 SDS), or signs of skeletal dysplasia. SNP array, whole genome sequencing and methylation analysis should be performed on the basis of phenotype.

GH therapy: The panel suggested that GH doses should be titrated on the basis of diagnosis (severe or partial GHD), auxological parameters (severe short stature), or genetic diagnosis (Turner syndrome, Prader Willi Syndrome, etc.). The suggested doses ranged from 25 to 43 μg/kg per day, and the panel recommend to titrate the initial dose by severity of GHD with lower doses in more severe deficiency. In non-GHD patients, the dose may be higher according to the approved indication. IGF-I levels during treatment may provide further information for dose adjustment and should be kept in the normal range, with the exception of specific conditions such as IGF-I resistance where a level above 2 SD could be accepted at least transiently. An inadequate response to GH therapy in patients with GHD is defined by one or more of the following criteria: increase of height velocity by <2 cm/year, height velocity <0 SDS, or increase in height by <0.3 SDS during the first 6–12 months of therapy. Long acting GH therapy was discussed as well as oral Ghrelin analogue in severe forms of GHD, and C-Natriuretric peptide analogues in achondroplasia.

The paper is extremely comprehensive and deals with all aspects relating to children with short stature. Although comprehensive, it is not exhaustive. The document is largely confirmatory and reasserts well established notions. In addition, the indications are based on expert views instead of a systematic review of the literature, meta-analysis and grading of the evidence. Therefore, these recommendations should be considered as suggestions rather than guidelines, which will require an evidence-based approach.

4.2. Genetic disorders in prenatal onset syndromic short stature identified by exome sequencing

Homma TK, Freire BL, Honjo Kawahira RS, Dauber A, Funari MFA, Lerario AM, Nishi MY, Albuquerque EV, Vasques GA, Collett-Solberg PF, Miura Sugayama SM, Bertola DR, Kim CA, Arnhold IJP, Malaquias AC, Jorge AAL
Genetic Endocrinology Unit, Laboratory of Cellular and Molecular Endocrinology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil
alexj@usp.br

J Pediatr. 2019 Dec;215:192–198.

Identifying the diagnosis in children with syndromic short stature and those with recognized genetic growth disorders is often challenging, as they may share many clinical features (1)(2). The candidate gene approach has many limitations in unveiling the genetic cause. Therefore, whole exome sequencing (WES) has been proposed to improve the diagnostic rate in children with short stature of unknown etiology, including both idiopathic short stature (ISS) and SGA (3). In this study, 44 children with the following inclusion criteria were selected for WES analysis: available DNA samples, consent by the patient and family, a syndromic condition without an initial clinical diagnosis and a negative result on candidate gene testing based on clinical suspicion. Among these 44 patients, 40 had already been investigated by a genetic test before enrollment, mainly chromosomal microarray analysis, multiplex ligation-dependent probe amplification for Silver-Russell syndrome, or targeted gene panel sequencing, including genes frequently associated with growth disorders.

Positive genetic results on WES were found in 15 of 44 patients (34%): 11 had pathogenic and 4 likely-pathogenic gene variants. All variants were not present in local and public genetic databases and the detected genes are involved in fundamental cellular process and pathways: *ACTG1*, *AFF4*, *ANKRD11*, *BCL11B*, *BRCA1*, *CDKN1C*, *GINS1*, *INPP5K*, *KIF11*, *KMT2A*, *POC1A*, *COLA2A1* and *SRCAP*. Although the majority of patients did not have a definite diagnosis even after WES, the authors concluded that the WES approach should be considered as the first line investigation in children with syndromic short stature without initial clinical, laboratory and radiological diagnosis. This WES based approach seems to be more cost-effective and less time consuming in patients selected on clinical grounds, instead of the conventional step-by-step diagnostic work-up. Genetic characterization is important not only for identifying the diagnosis and informing genetic counseling, but also to avoid potentially harmful growth promoting therapies in children who harbor cancer-susceptibility genetic mutations.

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4.3. IGF2 Mutations

Masunaga Y, Inoue T, Yamoto K, Fujisawa Y, Sato Y, Kawashima-Sonoyama Y, Morisada N, Iijima K, Ohata Y, Namba N, Suzumura H, Kuribayashi R, Yamaguchi Y, Yoshihashi H, Fukami M, Saitsu H, Kagami M, Ogata T
Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan
kagami-ms@ncchd.go.jp

J Clin Endocrinol Metab. 2020 Jan 1;105(1):dgz034.

Using different genetic approaches, the authors identified 5 novel pathogenic or likely pathogenic *IGF2* gene variants in Japanese patients who underwent genetic testing for the variable associations of multiple congenital anomalies such as mental retardation, Silver-Russell syndrome (SRS), disorders of sex development (DSD), ectrodactyly (split hand/foot malformation), fetal growth restriction (FGR) and extremely high serum insulin-like growth factor I (IGF-I). Four of the five patients had a Netchine-Harbison (N-H) score of 5+ out 6 for the clinical diagnosis of SRS. In comparison with *H19/IGF2*:IG-DMR epimutations, *IGF2* mutations were associated with low frequency of hemi-hypoplasia, high frequency of feeding difficulty and/or reduced body mass index, milder degree of relative macrocephaly, occasional development of severe limb malformations, high frequency of cardiovascular anomalies and developmental delay, and low serum IGF-II values.

The *IGF2* gene is located on an imprinted paternally-expressed region on chromosome 11p15 and encodes for IGF-II which is actively involved in the regulation of placental and fetal growth. *IGF2* up-regulating genetic and epigenetic mutations cause Beckwith-Wiedemann syndrome, characterized by overgrowth and increased risk of cancer, while down-regulating mutations cause Silver-Russell syndrome (SRS) characterized by intrauterine and postnatal growth retardation besides a wide range of dysmorphic features, severe feeding difficulties, body asymmetry, and neurodevelopmental delay (1)(2). The most frequent *IGF2* alteration in SRS

is hypomethylation of the paternally-inherited *H19/IGF2* intergenic-DMR, although copy number variants of the 11p15.5 region have also been reported (1). So far, few heterozygous paternally-inherited *IGF2* genetic mutations have been described in SRS patients (3)(4). A recent paper described a paternally-inherited *IGF2* nonsense mutation in four members of one family with a clinical phenotype suggestive of SRS (2).

This study suggests that, although rare, DNA sequence analysis of *IGF2* should be performed in patients with no demonstrable epigenetic cause of SRS. Furthermore, the results show a certain degree of phenotypic differences between patients with *H19/IGF2*:IG-DMR epimutations and those with *IGF2* genetic mutations.

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4.4. Cognitive profiles and brain volume are affected in patients with Silver–Russell syndrome

Patti G, De Mori L, Tortora D, Severino M, Calevo M, Russo S, Napoli F, Confalonieri L, Schiavone M, Thiabat HF, Casalini E, Morana G, Rossi A, Ramenghi LA, Maghnie M, Di Iorgi N
Department of Pediatrics, IRCCS Istituto Giannina Gaslini, Genova, Italy
giusypattis@gmail.com

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Silver–Russell syndrome (SRS) is a rare condition associated with pre and postnatal growth retardation. The most common causes of SRS are 11p15 ICR1 loss of methylation (LOM) and maternal uniparental disomy of chromosome 7 (mUPD7). Almost all patients with SRS have a history of intrauterine growth retardation (IUGR) and may be born small for gestational age (SGA). Children with SRS show typical features, including relative macrocephaly, prominent forehead, body asymmetry, feeding difficulties and cognitive delay (1)(2). SRS patients with 11p15 LOM show high IGF1 levels suggestive of IGF1 insensitivity. IGF1 is implicated in brain development, in particular with neural proliferation and cognitive function (2)(3).

The aim of this study was to assess cognitive function and brain volume in 38 patients with clinical and genetic diagnosis of SRS. 30 of 38 patients (15 with 11p15 LOM; 15 with mUPD7) underwent cognitive assessment and 23 of these 30 had a brain MRI. The control groups were 33 children tested for cognitive profile and 65 who had brain MRI. The outcome measures were the intelligence quotient, Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI), Processing Speed Index, and brain volume. Children with mUPD7 showed impaired cognitive profiles compared with both controls and 11p15 LOM children. Brain volumes at the frontal/temporal lobes and at the globi pallidi were reduced in all SRS patients.

Multiple factors other than SRS genetics may account for the cognitive deficit in patients with SRS, including intrauterine undernutrition, preterm birth, perinatal morbidity and neonatal hypoglycemia. MRI did not show any neuroanatomical abnormalities but gray matter volumes in temporal lobes and globi pallidi were reduced in SRS children, suggesting the brain sparing phenomenon may be regional and may play a role in neurological damage rather than protecting brain development. These findings need to be confirmed in larger SRS populations, in SRS patients with more homogenous characteristics at birth, and using more specific study designs (e.g. focused on patients with *IGF2* gene deletions).

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4.5. Growth and adult height in girls with Turner syndrome following IGF-I titrated growth hormone treatment

Wang AC, Hagen CP, Nedaeifard L, Juul A, Jensen RB
Department of Growth and Reproduction, University of Copenhagen, Rigshospitalet, Copenhagen, Demark
amanda.cleemann.wang.01@regionh.dk

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This retrospective study evaluated the long-term effects of GH treatment (median duration 6.7 years) in 63 girls with Turner syndrome (TS) whose GH doses were titrated to maintain IGF-I levels within the normal range. The median GH dose to maintain normal IGF-I levels was 33 (g/kg/day. Across all TS karyotypes, IGF-I titrated GH dosing led to a median adult height (AH) of 1.25 SDS by age-specific TS references, and a median gain in height (adult height minus baseline height SDS) of 0.50 SDS, corresponding to only 3.2 cm.

TS is characterized by X deficiency due to a complete or partial loss of one X chromosome (1). The clinical spectrum is wide, including short stature, ovarian dysgenesis with hypergonadotropic hypogonadism, cardiac malformations, metabolic disorders, osteoporosis and autoimmune disorders. A variable combination of prenatal growth failure, impaired childhood growth and the lack of a pubertal growth spurt leads to short adult height, approximately 20 cm shorter than the normal population. TS is a recognized indication for GH therapy which increases adult height by on average 7.22 cm in comparison with untreated TS girls (2)(3).

As short stature in TS females is due to haploinsufficiency of the short stature homeobox-containing (SHOX) gene, instead of anomalies in GH/IGF-I axis, GH administration often results in higher than normal circulating IGF-I levels. It is generally accepted that IGF-I levels should be monitored during GH treatment and maintained within the normal range for age and sex (4). According to the current study, the GH dose needed to maintain IGF-I within the normal range (33 (g/kg per day) is lower than that currently recommended for TS (45–50 μg/kg per day) (4) and the use of IGF-I titration for tuning GH dose reduces the overall height gain. This study emphasizes the difficulty of balancing the optimal height growth response with normal IGF-I levels for achieving the best clinical outcome for girls with TS without jeopardizing their safety.

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Clinical Trials

4.6. Once-weekly somapacitan vs daily GH in children with GH deficiency: results from a randomized phase 2 trial

Sävendahl L, Battelino T, Brod M, Rasmussen MH, Horikawa R, Juul RV, Saenger P REAL 3 study group
Department of Women's and Children's Health, Karolinska Institute and Pediatric Endocrinology Unit, Karolinska University Hospital, Stockholm, Sweden
lars.savendahl@ki.se

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The REAL 3 multicenter randomized, controlled, double-blind phase 2 study evaluated the efficacy and safety of different doses of once-weekly Somapacitan, compared to conventional once-daily GH in 59 prepubertal GHD children. The treatment period lasted 26 weeks and an additional extension to 52 weeks. At week 26, height velocity (HV) was 7.8, 10.9 and 13.1 cm/year, respectively, for the three increasing doses of Somapacitan (0.04, 0.08 and 0.16 mg/kg per week) compared to 11.4 cm/year on daily GH (0.034 mg/kg per day). The corresponding HV at 52 weeks were 7.5, 9.7 and 11.7 cm/year for Somapacitan, and 9.9 cm/year for daily GH. Similar effects of the different treatment regimens were observed on heights gain from baseline. The Somapacitan dose 0.16 mg/kg per week was considered to have an efficacy overlapping that of conventional daily GH. A PK/PD model used to derive individual IGF-I profiles and IGF-I average levels showed that Somapacitan 0.16 mg/kg per week provided IGF-I levels equivalent to daily GH. No major side effect was reported and the overall safety profile was not different among the treatment groups.

Recombinant human (rhGH) therapy, by daily subcutaneous injection, is currently used to improve linear growth and, ultimately, adult height, in a wide range of conditions associated with short stature. Clinical responses to GH therapy are variable, influenced by factors such as the underlying diagnosis, GH dose, age at start of treatment, parental heights, and adherence to therapy (1). Poor adherence to therapy may be a major issue, eventually affecting adult height. The prevalence of non-adherence to GH in childhood seems to be extremely variable, ranging from 5 to 82% in different populations (1) (2).

Long-acting (LA) GH formulations were developed to improve adherence to GH therapy and reduce the its burden to patients and families. Somapacitan is a once-weekly modified rhGH containing a fatty acid with non-covalent albumin-binding properties, which prolongs the half-life and thus its duration of action. This LAGH formulation has been shown to be well tolerated, safe and effective in GHD adults (3). The current trial confirms its efficacy and short-term safety in GHD children.

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New perspectives

4.7. Circulating insulin-like growth factor-1 is positively associated with growth and cognition in 6- to 9-year-old schoolchildren from Ghana

Grenov B, Larnkjær A, Lee R, Serena A, Mølgaard C, Michaelsen KF, Manary MJ
Department of Nutrition, Exercise, and Sports, University of Copenhagen, Frederiksberg C, Denmark
bgr@nexs.ku.dk

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There is increasing evidence that dairy milk positively affects linear growth, weight and body composition in children, particularly in low- and middle-income countries (1)(2). This effect of milk on growth has been proposed to be mediated by IGF-I (2), (3). The aim of this double-blinded, controlled trial was to evaluate the effect of milk supplements on circulating IGF-I and to assess IGF-I as a correlate of growth and cognition in children. The study enrolled 261 healthy children (median age 6–9 years) from 13 schools in Ghana. They were divided into 3 groups on the basis of milk intake (Milk8 = 8.8 g milk protein; Milk/Rice = 4.4 g milk + 4.4 g rice protein; Milk4 = 4.4 g milk protein) and control children who received a powder containing multiple micronutrients. Auxology, body composition (assessed by bioelectric impedance analysis), IGF-I and free amino acids (analyzed by dried blood spots at 3.5 and 8.5 months) and cognitive function (Cambridge Neuropsychological Test Automated Battery-CANTAB) were measured. Only Milk8 group showed a

statistically significant increase in IGF-I compared to control subjects (+15.3; 95% CI: 9.4, 31.2). During the intervention, IGF-I increased more in girls than in boys. The change in IGF-I levels was positively associated with changes in amino acids (valine, leucine, Amino-butyric acid, Threonine, Trytophan), height, weight, fat free mass and 4 of 5 CANTAB domains.

The authors concluded that milk intake improved growth and body composition through stimulation of IGF-I production. Furthermore, they speculate that IGF-I plays a key role in ameliorating cognitive function, an action consistent with the well-known role of IGF-I in brain development and maturation. The mechanisms underlying the observed effect of milk intake on IGF-I production and the gender related different response to milk remain to be elucidated.

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4.8. Deficits in bone geometry in growth hormone-deficient prepubertal boys revealed by high-resolution peripheral quantitative computed tomography

Tamar G. Baer, Sanchita Agarwal, Shaoxuan Chen, Codruta Chiuzan, Aviva B. Sopher, Rachel Tao, Abeer Hassoun, Elizabeth Shane, Ilene Fennoy, Sharon E. Oberfield, Patricia M. Vuguin
Department of Pediatrics, Columbia University Irving Medical Center, New York, NY, USA
tamgbaer@gmail.com

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Growth hormone deficiency (GHD) in childhood may influence bone accrual and bone peak mass, ultimately affecting bone density in adulthood (1). In prepubertal GHD children, there is no evidence of increased risk of fractures, but relative lower bone density measured by dual X ray absorptiometry (DXA) has been reported. Interpretation of DXA values in small children is biased by bone size, and furthermore is not an adequate tool to assess bone architecture (2). High resolution peripheral quantitative computed tomography (HR-pQCT) is able to assess trabecular and cortical bone, thus providing an indirect index of bone strength (2)(3).

In this cross-sectional study, 30 prepubertal GHD boys (age 5–11 years) were compared to 15 healthy children with normal height, by DXA and HR-pQCT. GHD boys were younger and shorter than controls ($P < 0.001$). In GHD boys, IGF-I levels correlated with DXA whole body BMD ($R = 0.73$; $P = 0.003$) and lean mass ($R = 0.76$; $P = 0.002$). Peak GH responses to stimulation test correlated negatively with visceral adipose tissue ($P = 0.01$) and fat mass ($P = 0.01$). At the radius, GHD boys had smaller HR-pQCT cortical perimeter and trabecular cross-sectional areas than controls. No differences were found in microarchitectural structure and bone strength between the two groups.

The authors concluded that prepubertal GHD boys had deficits in bone geometry, not visible on DXA, independent of age, height z score, lean body mass and vitamin D levels. This is the first study to apply this detailed HR-pQCT bone analysis in prepubertal GHD patients. Larger studies are warranted to confirm these preliminary results showing bone geometry alterations not detectable with DXA in GHD children.

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4.9. Growth hormone deficiency and excess alter the gut microbiome in adult male mice

Jensen EA, Young JA, Jackson Z, Busken J, List EO, Carroll RK, Kopchick JJ, Murphy ER, Berryman DE
Translational Biomedical Sciences Graduate Program, Graduate College, Ohio University, Athens, Ohio (berrymad@ohio.edu), USA

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The aim of this study was to examine the role of growth hormone (GH) on gut microbiome and intestinal phenotype by using two animal models characterized by an opposite GH status: GH gene-disrupted (GH-/-) mice, characterized by GH deficiency; and bovine GH transgenic (bGH) mice with chronic GH excess. The abundance of common bacterial genera, such as Parasutterella, Ruminococcaceae NK4A214, Rikenellaceae, and Lactobacillus, differed between the GH deficient and GH excess mouse lines. Similarly, metabolic pathways that are regulated in opposite directions between the 2 mouse lines were identified. Finally, opposing intestinal gross anatomy, histology, and fecal output between the 2 mouse lines were found. Taken together, these findings suggest an impact of GH on gut microbial profile (abundance and maturity), metabolic pathways regulated by microbial community (acetate, butyrate, heme B, and folate biosynthesis) and intestinal phenotype (gross anatomy, histology, inflammation and fecal output).

The gut microbiome is a complex ecosystem, complementary and tightly interconnected with human host for the regulation of functions, such as the fermentation of indigestible dietary components, the synthesis of important elements, the removal of toxic compounds, the out competition of pathogens, the strengthening of the intestinal barrier, and the stimulation and regulation of the immune system (1). A healthy person typically hosts trillions of microbes, which differ between individuals, being the taxonomic composition of the gut microbiome influenced by factors including lifestyle and drugs (2). Recent studies have suggested a relationship between the gut microbiota and GH/IGF-1 axis. Gut microbiota exerts an anabolic effect on bone, likely mediated by the dynamic regulation of IGF-I levels (3). An altered microbiota composition has been detected in Ames dwarf mice, which have multiple hormonal deficiencies including GH, prolactin, and thyrotropin due to a mutation of the Prop1 gene (4).

This study shows, for the first time, a close relationship of GH status with gut microbiota and the metabolic pathways regulated by microbiota. Further studies are warranted to clarify the mechanisms underlying this relationship.

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4.10. IGF-I in cord blood is predictive of final height in monozygotic twins with intratwin birth weight differences

Kasner C, Schulte S, Schreiner F, Fimmers R, Stoffel-Wagner B, Bartmann P, Woelfle J, Gohlke B
Children's University Hospital Bonn, Dept. Pediatric Endocrinology and Diabetology, Adenauerallee 119, 53113, Bonn, Germany
bettina.gohlke@ukbonn.de

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Children born either small for gestational age (SGA) or preterm usually do not achieve a final height (FH) in the mid-parental target height (MPTH) range, despite showing catch-up growth in the first years of life (1). The influence of intrauterine metabolic and hormonal factors has been proposed and, in particular, IGF-I plays a key

role in both pre and post-natal growth (2), (3), (4). This paper stems from a longitudinal study in monozygotic twins who suffered from twin to twin transfusion syndrome (TTTS). These children shared the same genetic, prenatal environmental and family background, but differed in intrauterine nutrient availability. 28 twin pairs were selected and analyzed at 1 year, 4 years and pubertal age up to near FH (height velocity <2 cm/year). Anthropometry and cord blood IGF-I were assessed. Subjects were defined as discordant (10) or concordant (18) according to intra-twin birth weight difference more or less than 1 SD., respectively.

Significant intra-twin length/height differences remained for 26/28 (93%) of all twins and for all initially discordant twins. Mean difference in intra-twin height among concordant birth weight twin pairs was 0.18 SDS, corresponding to an absolute mean intra-twin difference at FH of only 1.31 cm. In the discordant birth weight group, all the initially smaller twins remained substantially shorter than their respective co-twin (mean difference 1.23 SDS). This corresponded to an absolute mean difference in intra-twin height at FH of 7.9 cm. A strong correlation between intra-twin difference in FH and differences in birth weight and birth length was observed. A significant correlation between cord blood concentrations of IGF-I and FH for both twin cohorts was observed. The greater the intra-twin difference in IGF-I cord blood concentrations, the larger the intra-twin difference in FH.

The results of this powerful long-term follow-up study suggest that IGF-I levels in cord blood are predictive for FH. Whether IGF-I concentrations are the drivers or just a mirror of the biological/environmental mechanisms regulating the differing growth outcomes in twins has to be established.

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4.11. NPR2 Variants are frequent among children with familial short stature and respond well to growth hormone therapy

Plachy L, Dusatkova P, Maratova K, Petruzelkova L, Zemkova D, Elblova L, Kucerova P, Toni L, Kolouskova S, Snajderova M, Sumnik Z, Lebl J, Pruhova S

Department of Pediatrics, 2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol, Prague, Czech Republic

stepanka.pruhova@fnmotol.cz

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This study aimed to assess the frequency of natriuretic peptide receptor type B gene (*NPR2*) variants in 87 children with familial short stature (FSS) and evaluate their response to GH therapy. By applying whole-exome or custom-targeted NGS panel sequencing, *NPR2* variants were found in 5 children (5.7%) belonging to 4 families. These variants were classified as pathogenic ($n=2$) or likely pathogenic ($n=3$) according to the American College of Medical Genetics and Genomics guidelines. Three children were born SGA and 3 were classified as GH deficient (GHD). Two children had disproportionate short stature with short limbs and one had a dysplastic middle phalanx of the fifth finger. All parents with short stature carried the same *NPR2* variant as the probands. GH therapy improved growth velocity after the first year of treatment (from 3.6 to 4.2 cm/year) and the gain in height ranged from 0.33 to 0.79 SD. after 1 year of GH therapy, 0.43 to 1.34 SD. at 2 years, and 1.2 to 1.8 SD. after 5 years.

C-type natriuretic peptide (CNP) is present in high concentrations in chondrocytes and regulates many types of bone cells (1). In animals, inactivating mutations of its gene cause severe dwarfism and impaired endochondral

ossification (1). By contrast, high levels of natriuretic peptides due to transgenic overexpression or reduced clearance cause skeletal overgrowth (1). CNP acts by binding to NPR2, whose loss of function also leads to dwarfism in animal models (1) and whose homozygous loss of function mutations are observed in patients with a rare autosomal recessive form of short-limb dwarfism, named acromesomelic dysplasia, type Maroteaux (1). Interestingly, patients with a single mutated *NPR2* allele are shorter than average and may display mild signs of bone dysplasia (2)(3). Genome-wide association studies have highlighted the CNP-NPR2 system in influencing adult height and previous studies have reported that *NPR2* mutations are present in up to 6% of children classified as having idiopathic short stature (3). The importance of this pathway in regulating bone growth and shape is demonstrated by a recent trial showing that subcutaneous administration of a biologic analogue of CNP increases growth velocity in patients with achondroplasia (4).

FSS refers to a child with a stature below the third centile but within the parental target range and with at least one short parent, and the exclusion of other causes of short stature. Mild forms of FSS are likely related to a polygenic inheritance whereas monogenic traits cause more severe forms. This study shows, for the first time, that *NPR2* variants could account for some cases of FSS. Although the response to GH therapy in these cases seems promising, no conclusive data about the efficacy of GH treatment can be drawn due to the small number of subjects and their surprising heterogeneity, including both SGA and GHD subjects. Furthermore, no functional study was performed to demonstrate the pathogenicity of the observed *NPR2* variants.

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New paradigms

4.12. IGSF1 Deficiency results in human and murine somatotrope neurosecretory hyperfunction

Joustra SD, Roelfsema F, van Trotsenburg ASP, Schneider HJ, Kosilek RP, Kroon HM, Logan JG, Butterfield NC, Zhou X, Toufaily C, Bak B, Turgeon MO, Brûlé E, Steyn FJ, Gurnell M, Koulouri O, Le Tissier P, Fontanaud P, Bassett JHD, Williams GR, Oostdijk W, Wit JM, Pereira AM, Biermasz NR, Bernard DJ, Schoenmakers N
Department of Medicine, Division of Endocrinology, Leiden University Medical Center, Leiden, Netherlands
naaa2@cam.ac.uk

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A cohort of 21 adult males (aged 19 to 89 years) harboring hemizygous pathogenic *IGSF1* gene mutations underwent anthropometry, endocrine testing, testis ultrasonography, and body composition assessment to define the pathophysiological role of *IGSF1* in influencing GH secretion. In addition, two lines of *Igsf1*-deficient male mice were used to assess auxological parameters, organ sizes, bone features, pituitary GH content, GH secretion and IGF-I levels.

More than half of the human *IGSF1*-deficient adults (52.4%) were classified as acromegalic and had increased head circumference SDS and finger soft-tissue thickness. They also had higher IGF-I levels and 7 patients showed GH hypersecretion on 24-hour GH secretion profiles. Some patients reported symptoms of GH excess, such as increasing shoe size, sleep apnea, acroparesthesia and oily skin. Consistently, *IGSF1*-deficient animals had elevated IGF-I serum levels, pituitary GH protein levels, body weight, organ and skeletal size, and lean body mass.

The Immunoglobulin superfamily member 1 (*IGSF1*) gene encodes for a plasma transmembrane glycoprotein abundantly expressed in Rathke's pouch and adult pituitary gland. Loss-of-function mutations or deletions of

IGSF1 cause an X-linked syndrome with variable phenotypes. Heterozygous female carriers show either no endocrine anomalies, central hypothyroidism (33%) or hypoprolactinemia (11%). Affected males are characterized by central congenital hypothyroidism and macro-orchidism (1). Further features include prolactin deficiency (69%), disharmonious pubertal development (normal timing of testicular growth but delayed rise of testosterone), and increased body mass index (BMI) (2). IGF-I levels are within the normal range but a partial and transient GHD is present in approximately 13% of children (2). Interestingly, on retesting in young adults, GH secretion is normal with a tendency to increasing circulating IGF-I levels during adult life. Acromegalic signs occur in some patients (1)(3). In animals, *Igsf1*-deficiency is associated with reduced pituitary and serum TSH concentrations, decreased serum T3, and increased body mass (1).

The mechanisms underlying *IGSF1* mutations and their different clinical features are still unknown (4). The paucity of reliable antibodies for IGSF1 protein has hampered histo-chemical studies in human pituitary (5). Consistent with previous data, the current study shows that in adult *IGSF1* deficiency is associated with symptoms typical of excessive GH secretion. These findings suggest that the effect of *IGSF1* on pituitary GH-secreting cells axis is complex and likely variable throughout life.

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4.13. IGF1-mediated human embryonic stem cell self-renewal recapitulates the embryonic niche

Wamaitha SE, Grybel KJ, Alanis-Lobato G, Gerri C, Ogushi S, AMcCarthy A, Mahadevaiah SK, Healy L, Lea RA, Molina-Arcas M, Devito LG, Elder K, Snell P, Christie L, Downward J, Turner JMA, Niakan KK
Human Embryo and Stem Cell Laboratory, The Francis Crick Institute, 1 Midland Road, London, UK
Kathy.niakan@crick.ac.uk

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The signaling pathways involved in the regulation of early human development are largely unknown and the IGF system has been proposed to play a major role (1). The knowledge of mechanisms and actors involved in early life development is crucial to develop successful strategies for maintaining pluripotent human embryonic stem cells (hESCs).

This sophisticated study performed a comprehensive analysis of signaling associated transcripts expressed in human preimplantation embryos. IGF-I ligand and the IGF-I and insulin receptors were found expressed in the human extraembryonic primitive endoderm (PE) and in pluripotent epiblast (EPI) cells, respectively. A minimal chemically-defined culture medium characterized by the supplementation of IGF-I together with Activin (AI medium) instead of fibroblast growth factor (FGF) was developed and evaluated. This medium was effective to derive hESCs from embryos, as well as induced pluripotent stem cells (iPSCs) reprogrammed from fibroblasts. AI-cultured hESCs exhibited a transcriptional expression profile similar to hESCs cultured under conventional conditions, retained the ability to differentiate into a variety of cell types, and were able to be genetically modified using CRISPR/Cas9 mutagenesis. IGF-I affected hESC self-renewal by acting on downstream PI3K signaling pathway and exerted a proliferative effect on the inner cell mass of the human embryo.

These results shed light on the signaling pathways involved in early stages of human embryo development and demonstrates the possibility of using an easily reproducible medium to culture hESCs. At the same time, this study raises many doubts about the ethics of experiments on human embryos. The Editors of this chapter dissociate morally from this approach based on manipulation of human life, though at embryo stage.

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4.14. Circulating IGF-1 independently predicts blood pressure in children with higher calcium-phosphorus product levels

Xargay-Torrent S, Dorado-Ceballos E, Benavides-Boixader A, Lizárraga-Mollinedo E, Mas-Parés B, Montesinos-Costa M, De Zegher F, Ibáñez L, Bassols J, López-Bermejo A
Pediatric Endocrinology Research Group, (Girona Biomedical Research Institute) IDIBGI, Salt, Spain
alopezbermejo@idibgi.org

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The aim of this prospective longitudinal study of 521 healthy children (8.8 $\pm$ 0.1) was to evaluate the correlation of circulating IGF-I levels with metabolic and cardiovascular parameters and the potential interaction with the Calcium-Phosphorus product (Ca*P). 158 subjects were re-evaluated after a follow-up of 5 years. Baseline IGF-I and IGF-I/IGFBP-3 molar ratio were associated with worse metabolic profiles (higher BMI, waist, systolic blood pressure (SBP), pulse pressure, insulin, HOMA-IR and triglycerides). Associations with both baseline and follow-up SBP remained independent in children in the highest Ca*P tertile even after adjusting for confounding variables.

IGF-I regulates metabolic pathways and exerts effects on the cardiovascular system. Data on the association between IGF-I levels and cardiometabolic disease risk are conflicting. In obese adults, IGF-I levels are inversely related to cardiometabolic disease risk, with low IGF-I levels associated with higher risk of ischemic heart disease (1). In the elderly, both low and high IGF-I levels have been associated with higher cardiovascular disease risk (2). In some studies obese adolescents, IGF-I is negatively associated with parameters of cardiometabolic disease risk (3). By contrast, we did not find any relationship between IGFs and metabolic syndrome components in obese children and adolescents (4).

Serum Ca*P has also been associated with higher cardiovascular disease risk, including vascular calcification and atherosclerosis. The current paper shows that IGF-I is an independent predictor of SBP and that this association strengthens with higher Ca*P. Nevertheless, as the presence of an association does not imply a causative role, further studies are required to clarify whether IGF-I, by influencing calcium metabolism, regulates cardiometabolic parameters.

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5. Bone, Growth Plate and Mineral Metabolism

Adalbert Raimann[a], Raja Padidela[b], Outi Mäkitie[c,d], Ola Nilsson[e,f]
[a]Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria; [b]Department of Pediatric Endocrinology, Royal Manchester Children's Hospital, Manchester University NHS Foundation Trust, Manchester, UK; [c]Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [d]Department of Molecular Medicine and Surgery, Karolinska Institutet, and Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden; [e]School of Medical Sciences, Örebro University and University Hospital, Örebro, Sweden; [f]Divison of Pediatric Endocrinology and Center for Molecular Medicine, Karolinska Institutet and University Hospital, Stockholm, Sweden

Preface

The skeletal research field continues to develop rapidly and produced several seminal findings during the last year, including advances in the treatment of rare skeletal disorders, and an ever deeper understanding of skeletal stem cell biology and hormone receptor signalling mechanisms. The targeting of the C-type natriuretic peptide (CNP) pathway as a way to antagonize the overactivity of the FGFR3 pathway in achondroplasia has been a subject of tremendous interest and excitement during the last couple of years and we here highlight the first phase 2 study with growth data up to 42 months of treatment with a CNP analogue (vosoritide) in children with achondroplasia. We also highlight skeletal outcomes after discontinuation of bisphosphonates in patients with osteogenesis imperfecta, as well as a clinical follow up study detailing the skeletal changes following hematopoietic stem cell transplantation in patients with osteopetrosis.

Additional highlights include a study from the Journal of Clinical Investigation unravelling the downstream mediators PTH/PTHrP receptor signalling in skeletal development and metabolism, a Nature article revealing that lipid availability is an important determinant of skeletal stem cell differentiation in fracture healing. In addition, the powerful technology of cell-tracing continues to reveal more details on the origin and fate of different skeletal cell populations. A report in the Journal of Bone and Mineral Research shows that Pthrp positive growth plate chondrocytes adjacent to the perichondrium act as mesenchymal stem cells providing metaphyseal osteoblast/osteocytes as well as reticular stromal cells to the bone marrow, confirming the stemness and versatility of growth plate chondrocyte.

In addition to these areas of progress, the chapter reports several exciting translational findings including a specific phosphate binding site in the calcium-sensing receptor that stabilizes the inactive conformation, thus increasing PTH expression during hyperphosphatemia, as well as promising results in a mouse model of fibrous dysplasia indicating that RANKL-inhibition can not only prevent the development of new fibrous dysplasia lesions, but also decrease the size and increase mineralization of existing lesions.

Novel Treatments for Rare Skeletal Disorders

5.1. C-Type natriuretic peptide analogue therapy in children with achondroplasia

Savarirayan R, Irving M, Bacino CA, Bostwick B, Charrow J, Cormier-Daire V, Le Quan Sang KH, Dickson P, Harmatz P, Phillips J, Owen N, Cherukuri A, Jayaram K, Jeha GS, Larimore K, Chan ML, Huntsman Labed A, Day J, Hoover-Fong J
Royal Children's Hospital, University of Melbourne, Parkville, VIC, Australia
N Engl J Med. 2019 Jul 4;381(1):25–35.
https://pubmed.ncbi.nlm.nih.gov/31269546/

In brief: Inhibition of endochondral ossification in Achondroplasia leads to disproportionate short stature. In this phase 2 study, daily subcutaneous injection of vosoritide, a biologic analogue of C-type natriuretic peptide

and a potent stimulator of endochondral ossification, results in sustained increase in the annualized growth velocity for up to 42 months, with minimal side effects.

Commentary: Achondroplasia is characterised by rhizomelic short stature with macrocephaly. Associated complications include hydrocephalus, obstructive sleep apnea and foramen magnum stenosis and cervicomedullary compression, leading to an increased risk of sudden death in infancy. Mortality is increased from birth to 4 years of age and 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, and in turn inhibits endochondral ossification. C-type natriuretic peptide, encoded by NPPC, and its receptor, natriuretic peptide receptor 2 (NPR2), are potent stimulators of endochondral ossification at the level of the growth plate. Continuous intravenous infusion of exogenous C-type natriuretic peptide restores the impaired bone growth observed in mice with achondroplasia and increases long-bone growth in wild-type monkeys. It acts, at least in part, by inhibiting the FGFR3-mediated MAPK signaling pathway. Vosoritide is a recombinant C-type natriuretic peptide analogue with longer half-life.

In this phase 2 study, 35 children (5–14 years of age) with achondroplasia were enrolled in four sequential cohorts to receive vosoritide at a once-daily subcutaneous dose of 2.5 μg per kg body weight (8 patients in cohort 1), 7.5 μg/kg (8 patients in cohort 2), 15.0 μg/kg (10 patients in cohort 3), or 30.0 μg/kg (9 patients in cohort 4). After 6 months, the dose in cohort 1 was increased to 7.5 μg/kg and then to 15.0 μg/kg, and in cohort 2, the dose was increased to 15.0 μg/kg; the patients in cohorts 3 and 4 continued on their initial doses.

During the study period, the most common treatment-related adverse events were mild, transient injection-site reactions. Therapy was discontinued in 6 patients (only in 1 because of an adverse event). The annualized growth velocity increased from baseline in all cohorts during each 12-month interval by 1.10–2.34 cm/year for up to 42 months. Among patients who received the 15.0 μg/kg dose, the mean annualized growth velocity was 5.51 cm/year, as calculated between 30 and 42 months. This change represents an annual increase of 1.46 cm (95% CI, −0.15 to 3.07) from baseline. Among patients who received the 30.0-μg-per-kilogram dose, the mean annualized growth velocity was 5.60 cm/year, as calculated between 18 and 30 months, representing an annual increase of 1.10 cm (95% CI, −0.27 to 2.48) from baseline. Whether earlier start and longer duration of this treatment would produce clinically meaningful improvements in growth and other features of the condition remains to be determined.

A phase 3, randomized, double-blind, placebo-controlled trial (NCT03197766) is currently evaluating the efficacy and safety of the 15.0 μg/kg dose of vosoritide in up to 110 children (age range, 5 to <18 years) with achondroplasia. An open-label phase 3 extension study (NCT03424018) will further evaluate the efficacy and safety of vosoritide until patients reach final adult height.

Advances in Clinical Practice

5.2. Osteogenesis Imperfecta: Skeletal outcomes after bisphosphonate discontinuation at final height

Robinson ME, Trejo P, Palomo T, Glorieux FH, Rauch F
Hospital for Children, Montreal, Canada
J Bone Miner Res. 2019 Dec;34(12):2198–2204.
https://pubmed.ncbi.nlm.nih.gov/31356699/

In brief: In Osteogenesis imperfecta (OI), intravenous (IV) cyclical bisphosphonates are often discontinued after cessation of growth in adolescents. This study showed that, four years after discontinuation of treatment, none of the patients sustained new vertebral compression fractures, and the proportion of individuals with long-bone fractures decreased compared with the two years before cessation of treatment.

Commentary: IV bisphosphonates are used worldwide in OI to increase bone mineral density (BMD) and decrease fracture rates. They are typically given throughout the growing years and are often discontinued when growth is completed.

The benefits of bisphosphonates in growing children are well established and cessation of bisphosphonate treatment in growing children with OI has been associated with poor skeletal outcomes. However, the skeletal outcomes after bisphosphonate discontinuation at final height have not been investigated in detail. This study assessed changes in BMD and fracture incidence during the first 4 years after IV bisphosphonate discontinuation in individuals with OI who had received treatment during skeletal growth and who had discontinued the treatment after achieving final height.

Thirty-one patients (22 females) with OI who had started IV bisphosphonates (either pamidronate or zoledronic acid) before 13 years of age, were treated for at least 2 years (range 4.7–15.7 years), and discontinued treatment after completion of growth, at age range 13.4–20.0 years (mean 16.4 years). At 4 years after treatment discontinuation, lumbar spine areal bone mineral density (BMD) had increased by 4% ($P < 0.05$). Peripheral quantitative computed tomography of the radius showed a decrease in trabecular volumetric BMD at the distal metaphysis of 19% but an increase in cortical volumetric BMD of 4% ($P < 0.05$ for both). None of the patients sustained a new vertebral compression fracture during follow-up. The proportion of patients with new long-bone fractures was higher in the 2 years before treatment discontinuation than in the last 2 years of follow-up (42% and 16%, respectively; $P < 0.05$).

In summary, stopping bisphosphonate therapy after completion of growth is not associated with a decline in bone mass at the lumbar spine or radius shaft, nor an increased number of vertebral compression or long-bone fractures. Decrease in bone mass at the distal radius is likely due to resorption of bisphosphonate-induced metaphyseal transverse lines. There were fewer long-bone fractures in the 4 years after bisphosphonate discontinuation compared with 2 years before discontinuation. Cessation of bisphosphonates after completion of growth is not associate with adverse outcomes.

There were concerns that discontinuing IV bisphosphonates after cessation of growth may adversely impact on the skeleton which is still mineralizing. This study is reassuring and suggests that it is safe to allow a bisphosphonate drug holiday of up to 4 years in most individuals with moderate to severe OI as they become skeletally mature. However, additional studies are required to better define the optimal dose range, dosing interval, duration of treatment and effects on bone mineralization and adverse events during childhood as well as in adults.

5.3. Skeletal changes following hematopoietic stem cell transplantation in osteopetrosis

Shapiro G, Fishleder J, Stepensky P, Simanovsky N, Goldman V, Lamdan R
Pediatric Orthopedics Unit, Assuta Ashdod Medical Center, Ashdod 7747629, Israel
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https://pubmed.ncbi.nlm.nih.gov/32329913/

In brief: Hematopoietic stem cell transplantation remains the only curative treatment in children with severe osteopetrosis. According to this study, increased serum calcium and phosphate serve as good markers of successful engraftment, which leads to significant but incomplete normalization of bone mineral distribution and bone morphology.

Commentary: Osteopetrosis is a genetically heterogeneous group of skeletal disorders that share a common feature: increased bone mass due to deficient bone resorption by osteoclasts. High bone mass may lead to severe complications involving the hematological, neurological and other organ systems. Genetic causes of this rare bone disease are relatively well known and mutations in several genes related to osteoclast development and resorptive capacity have been described. Despite these scientific advances, little progress has been made in the therapeutic approaches for severely affected children. Hematopoietic stem cell transplantation (HSCT) remains the only curative treatment but its success rate and long-term effects are inadequately explored. This study is the first large cohort study evaluating long-term skeletal effects of HSCT in children with osteopetrosis. Altogether 35 children treated between 2003 and 2016 were included in this retrospective study; all but two underwent HSCT. Disease-causing mutations involved five genes but mutations in TCIRG1 accounted for more than half of the cases. Outcome was evaluated by clinical, hematological and biochemical parameters and by careful evaluation of plain radiographs.

The authors show that hematopoietic recovery after HSCT coincides with a peak in bone remodelling, evidenced by significant changes in serum calcium and phosphate levels. These electrolyte measurements are widely available and used for baseline and monitoring studies. They conclude that serum calcium and phosphate could be used as auxiliary indicators of engraftment in addition to neutrophil counts, platelet counts and donor chimerism. While complete blood counts are readily available, they are difficult to interpret as they are confounded by blood product transfusions.

All radiographs taken before 3 months post-HSCT and radiographs taken later were assessed for morphological and cortex-to-medullary density changes following HSCT. After the HSCT, radiographs of the femur were much less sclerotic overall and the medullary cavity was visibly less radiodense than the cortex, suggesting significant remodeling and bone mineral redistribution. Not only skeletal mineral density but also long bone morphology was strikingly changed following HSCT. Before HSCT, Erlenmeyer flask deformities could be clearly seen, especially in the distal femoral metaphysis, whereas later radiographs showed a gradual widening of the diaphysis and a narrowing of the metaphysis, transforming the Erlenmeyer flask deformity into a more normal, but not completely normal, morphology. The authors conclude that neither mineral distribution nor bone morphology were completely rescued following HSCT, suggesting that even after HSCT the patients may be vulnerable to skeletal and other complications related to hyperostosis.

5.4. Effects of estrogen replacement on bone geometry and microarchitecture in adolescent and young adult oligoamenorrheic athletes: A randomized trial

Ackerman KE, Singhal V, Slattery M, Eddy KT, Bouxsein ML, Lee H, Klibanski A, Misra M
J Bone Miner Res. 2020;35(2):248–260.
www.ncbi.nlm.nih.gov/pubmed/?term=31603998

In brief: This randomized open-label trial in oligoamenorrheic athletes evaluated the effects of transdermal estrogen, oral estrogen or no replacement on bone metabolism, geometry, and microarchitecture. Transdermal estrogen treatment resulted in significantly greater increases in volumetric bone mineral density and other bone parameters compared to oral estrogen.

Commentary: Estrogen replacement is not the standard of care for athletes with functional hypothalamic amenorrhea. However, oligoamenorrheic athletes are estrogen deficient, have lower bone mineral density (BMD), impaired bone microarchitecture, and higher fracture rates compared to eumenorrheic athletes, and it has also been demonstrated that transdermal estrogen can mitigate some of the negative effects on bone metabolism. However, bone microarchitecture in oligoamenorrheic athletes has not been studied. In the current study, 75 oligoamenorrheic athletes (ages 14–25 years) were randomized to receive treatment with transdermal or oral estrogen, or no treatment. Bone geometry and bone microarchitecture were assessed using high-resolution peripheral quantitative CT at the distal tibia and distal radius at baseline and 1 year.

Transdermal estrogen treatment resulted in significantly greater increases in several bone parameters, including total and trabecular volumetric BMD (vBMD), cortical area and thickness, and trabecular number with transdermal estrogen compared to oral estrogen in the tibia. However, compared to no treatment, only cortical area was improved in the transdermal group and trabecular area was even decreased. Therefore, the overall findings of this study support the current guidelines that estrogen replacement is not the standard of care for athletes with functional hypothalamic amenorrhea and should only be considered in athletes who have failed to respond to non-pharmacological therapy for at least one year or who develop significant fractures with low bone density.

5.5. Six-year follow-up of a trial of antenatal vitamin D for asthma reduction

Litonjua AA, Carey VJ, Laranjo N, Stubbs BJ, Mirzakhani H, O'Connor GT, Sandel M, Beigelman A, Bacharier LB, Zeiger RS, Schatz M, Hollis BW, Weiss ST
Department of Pediatrics, Golisano Children's Hospital at Strong, University of Rochester Medical Center, Rochester, NY, USA
N Engl J Med. 2020;382(6):525–533.
https://pubmed.ncbi.nlm.nih.gov/32023372/

In brief: High dose vitamin D supplementation of 4400 IU compared with 400 IU during the prenatal period alone to mothers of offspring at risk for asthma did not influence the incidence of asthma and recurrent wheeze in children aged 6-years.

Commentary: Vitamin D deficiency has been implicated in the increasing prevalence of asthma and allergies in westernized societies. Because asthma and wheezing illnesses begin very early in life, studies of prenatal and early-life risk factors are crucial. These authors had previously reported a reduction in asthma and recurrent wheeze in 3-year-old children of pregnant women who had received a higher dose vitamin D supplementation of 4400 IU compared with 400 IU (average treatment time 25 weeks). However, the 6.1% reduction in incidence with higher dose supplementation was not statistically significant.

Here, the same authors followed the children to age 6 years to determine the course of asthma and recurrent wheeze. Throughout the study, investigators and participants remained unaware of the treatment assignments.

There was no effect of maternal vitamin D supplementation on asthma and recurrent wheeze in either an intention-to-treat analysis or an analysis with stratification according to the maternal 25-hydroxyvitamin D level during pregnancy. There was no effect of prenatal vitamin D supplementation on most of the prespecified secondary outcomes. This study found no effects of prenatal supplementation on spirometric indexes. Although there was a very small effect on airway resistance as measured by impulse oscillometry, this finding was of uncertain significance.

This study concluded that vitamin D supplementation during the prenatal period alone to mothers of offspring at risk for asthma did not influence the 6-year incidence of asthma and recurrent wheeze in the children. This study has number of limitations mainly in relation to lack of postnatal supplementation and enrolment of pregnant women in the trial was without regard to their initial vitamin D concentration. Nevertheless, it provides insight into the lack of benefit of antenatal exposure of high dose Vitamin D to prevent atopic disease in children.

5.6. Genetic variation in GC and CYP2R1 affects 25-hydroxyvitamin D concentration and skeletal parameters: A genome-wide association study in 24-month-old Finnish children

Kämpe A, Enlund-Cerullo M, Valkama S, Holmlund-Suila E, Rosendahl J, Hauta-Alus H, Pekkinen M, Andersson S, Mäkitie O
Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
PLoS Genet. 2019 Dec 16;15(12):e1008530.
https://pubmed.ncbi.nlm.nih.gov/31841498/

In brief: This genome-wide association study in a cohort of healthy infants shows that, already during the first two years of life, genetic variation in the genes encoding Vitamin D binding protein and Vitamin D 25-hydroxylase correlate with serum 25(OH)D levels and responses to vitamin D supplementation.

Commentary: Previous studies have shown that several genetic factors influence vitamin D status. However, no genome-wide association studies (GWAS) have evaluated the role of such genetic factors in infants and young children. This study by Kämpe *et al.* applied a GWAS approach in a cohort of 975 healthy Finnish infants, recruited to a randomized intervention study to evaluate the effects of 10 μg vs 30 μg supplemental vitamin D from age 2 weeks to 2 years (1). The original trial results showed that the higher vitamin D supplemental dose did not provide additional benefits for bone strength or susceptibility to infections (1). Here, the authors evaluated how genetic variation in these infants influences serum 25(OH)D concentrations, responses to vitamin D supplements, and skeletal outcomes. The GWAS identified two strong association signals involving the genes GC (Vitamin D binding protein) and CYP2R1 (Vitamin D 25-hydroxylase). Based on 25(OH)D measurements in umbilical cord and 24 months samples, the authors were able to assess genetic variation in relation to vitamin D supplementation responses. The GC locus was associated with the magnitude of 25(OH)D change during intervention. The original intervention study (1) found no differences in bone parameters at 24 months, as measured by peripheral quantitative computed tomography (pQCT), between the two vitamin D intervention groups. However, this study showed that haplotypes at the GC locus and the CYP2R1 locus showed strong associations with pQCT parameters, suggesting that low 25(OH)D does causally negatively impact on bone in 24-month-old children.

Both identified genes – GC and CYP2R1 – have previously been associated with serum 25(OH)D levels in several GWAS reports in older cohorts but this study is the first to confirm that genetic variation in these genes influences vitamin D metabolism and even responses to supplementation already in infancy. The cohort was genetically homogeneous (ethnic Finns), which may have enabled identification of these strong associations despite the relatively small cohort size. Further, the participants were also relatively homogeneous regarding their baseline vitamin D status as most were vitamin D sufficient already at onset of vitamin D supplementation. It remains to be evaluated whether these genetic findings can be replicated in larger and genetically and biochemically more heterogeneous cohorts of infants. The findings suggest that infants with certain risk haplotypes may benefit from higher vitamin D supplemental doses, and in contrast, others may be at increased risk of unnecessary high serum 25(OH)D levels with increased supplemental doses. Future follow-up of this cohort will hopefully elucidate whether GC and CYP2R1 genotypes predict longer-term health outcomes.

Reference

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Translational Highlights

5.7. RANKL Inhibition in fibrous dysplasia of bone: A preclinical study in a mouse model of the human disease

Palmisano B, Spica E, Remoli C, Labella R, Di Filippo A, Donsante S, Bini F, Raimondo D, Marinozzi F, Boyde A, Robey P, Corsi A, Riminucci M

Department of Molecular Medicine, Sapienza University, Rome, Italy

J Bone Miner Res. 2019;34(12):2171–2182.

https://asbmr.onlinelibrary.wiley.com/doi/abs/10.1002/jbmr.3828

In brief: The effect of RANKL inhibition on fibrous dysplasia development is explored here using a mouse model of a fibrous dysplasia-like disorder. Anti-mouse RANKL monoclonal antibody treatment prevented the development and growth of lesions. However, after discontinuation of anti-mouse RANKL antibody treatment, disease progression resumed and newly formed bone was remodelled into fibrous dysplasia.

Commentary: Fibrous dysplasia (FD) is a rare skeletal disease caused by mosaic gain-of-function mutations in Gsα causing fibro-osseous tissue and under-mineralized bone, rich in osteoclasts, to replace normal bone. Enhanced bone resorption driven by RANKL is a recurrent histological feature of FD and likely a major cause of pain and fragility of affected bones (1). Therefore, inhibition of RANKL appeared to be an attractive therapeutic approach. Consistently, in an early case report, the anti-RANKL monoclonal antibody Denosumab reduced bone turnover, bone pain and also was suggested to reduce growth of FD lesions. However, discontinuation of treatment was followed by life-threatening hypercalcemia, raising serious safety concerns (1,2).

In the current study, mice with a constitutively active Gsα (EF1α-Gs αR201C), which develop an FD-like phenotype, were treated with an anti-mouse RANKL monoclonal antibody (a denosumab analogue). The treatment not only prevented lesion formation but also promoted differentiation of FD cells to bone-forming osteoblasts that produced highly mineralized bone matrix. Thus, it raised the hope for an efficient treatment that can both limit the development of new FD lesions and reduce the size of current lesions. However, the report also shows that discontinuation of treatment is followed by rebound of bone resorption and FD, highlighting the safety concerns previously observed in humans with anti-RANKL monoclonal antibody therapy and that further work to elucidate prevention and management of relapse after treatment discontinuation is needed.

Nevertheless, this report represents an important proof-of-concept showing that RANKL inhibition, maybe in combination with a bisphosphonate or other anti-resorptive agent, have the potential to act as an effective therapeutic option to limit development and normalize the bone at FD lesions.

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5.8. Glucocorticoids decrease longitudinal bone growth in pediatric kidney transplant recipients by stimulating the FGF23/FGFR3 signaling pathway

Delucchi Á, Toro L, Alzamora R, Barrientos V, González M, Andaur R, León P, Villanueva F, Galindo M, Las Heras F, Montecino M, Moena D, Lazcano A, Pinto V, Salas P, Reyes ML, Mericq V, Michea L
Division of Nephrology, Department of Medicine, Hospital Clínico Universidad de Chile, Santiago, Chile
J Bone Miner Res. 2019;34(10):1851–1861.
https://pubmed.ncbi.nlm.nih.gov/31099911/

In brief: In a pediatric kidney transplant cohort, glucocorticoid treatment was independently associated with FGF23 levels. Using *in vivo* and *in vitro* rat model systems, blocking of Fgfr signalling rescued glucocorticoid-induced skeletal manifestations.

Commentary: The deleterious effects of chronic glucocorticoid (GC) treatment on the growing skeleton represent a major cause of short stature and secondary osteoporosis in children. Based on associations between GC intake and FGF23 levels in renal transplant patients, Delucchi *et al.* investigated a potential role of FGF23 in the pathogenesis of GC-induced skeletal symptoms. In a translational approach, the authors identified associations between GC-exposure, skeletal manifestations and FGF23 levels in a pediatric kidney transplant cohort. As expected, early GC-withdrawal was associated with improved height-z scores and BMD in renal transplant patients. Further, FGF23 levels positively correlated with GC-dosage independently of renal function, PTH and calcitriol levels. In a subsequent step, consistent with their hypothesis, they found a stimulating effect of GCs on skeletal FGF23 expression. Blocking of FGFR3, the major receptor for FGF23 action, rescued the phenotype including normalization of linear growth and histologic growth plate phenotype. In addition, local administration of a FGFR antagonist (PD173074) completely ameliorated the growth inhibiting effect of GCs in growing rats.

With the combination of clinical and animal data, these authors approached the immanent clinical challenge of GC-induced bone disease. The identification of FGF23 as a potential player in the deleterious effects of GC on the skeleton opens the perspective of pharmacologic interventions, including FGFR3 inhibition or FGF23-blocking antibodies, as modulators of the described mechanisms. Although the precise regulation of GC-induced FGF23 expression remains unclear, the work of Delucchi *et al.* raise a new alternative in the development of treatments to improve skeletal health in patients with chronic GC-treatment.

5.9. Sclerostin antibody treatment increases bone mass and normalizes circulating phosphate levels in growing Hyp mice

Carpenter KA, Ross RD
Department of Cell and Molecular Medicine, Rush University Medical Center, Chicago, IL, USA
J Bone Miner Res. 2020;35(3):596–60.
https://pubmed.ncbi.nlm.nih.gov/31743490/

In brief: Antibody treatment against the Wnt antagonizing sclerostin substantially improves FGF23 levels, serum phosphate and mechanical bone properties in a murine model of X-linked hypophosphatemic rickets, suggesting a role of sclerostin in phosphate metabolism.

Commentary: Associations between the expression of the Wnt-antagonist sclerostin and FGF23 have been suggested in recent human and murine studies, although a direct interaction was not proven so far.

Based on the hypothetical interactions between the two osteocyte-derived endocrine factors, Carpenter and Ross used the well-established murine Hyp model for XLH to investigate effects of sclerostin inhibiting antibodies (SclAb) on increased FGF23 expression and its sequelae during skeletal growth. In line with their initial assumption, Scl-Ab treatment increased circulating phosphate and decreased intact FGF23 levels in both WT

and Hyp mice with a more pronounced effect in the disease model. SclAb did not normalize the impaired growth or osteoid but increased bone mass and strength in Hyp and wildtype mice to a comparable extent. Thus, these data could prove a partial rescue of the Hyp phenotype by SclAb treatment. Although sclerostin clearly has to be added to the list of regulating factors in the still poorly understood pathogenesis of XLH, the distinct regulatory pathways remain unclear. Confounding effects such as increased 1,25-OH-Vitamin D levels in SclAb treatment further complicate the interpretation of the study's results.

Nevertheless, the pharmacologic inhibition of sclerostin represents a novel therapeutic perspective in the treatment of XLH and FGF23-related hypophosphatemia. While beneficial effects might be limited on phosphate levels and bone strength, combined approaches with established agents such as Burosumab might give hope to further optimize the pharmacological treatment of XLH.

Novel Receptor Signaling Mechanisms

5.10. Salt-inducible kinases dictate parathyroid hormone 1 receptor action in bone development and remodelling

Nishimori S, O'Meara MJ, Castro CD, Noda H, Cetinbas M, da Silva Martins J, Ayturk U, Brooks DJ, Bruce M, Nagata M, Ono W, Janton CJ, Bouxsein ML, Foretz M, Berdeaux R, Sadreyev RI, Gardella T, Jüppner H, Kronenberg HM, Wein MN
Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA
J Clin Invest 2019;129:5187–5203.
https://pubmed.ncbi.nlm.nih.gov/31430259/

In brief: This report establishes inhibition of salt-inducible kinases as a central mechanism by which the parathyroid hormone 1 receptor (PTH1R) exerts its effects in both growth plate chondrocytes and osteoblasts/osteocytes during skeletal development, growth, and remodelling.

Commentary: The parathyroid hormone 1 receptor (PTH1R) is a G protein-coupled receptor that is critical for mineral homeostasis, but also for skeletal development, growth and bone metabolism. Its importance in skeletal development was first indicated by the finding that Jansen type metaphyseal chondrodysplasia, characterized by severe short stature, short bowed limbs, and hypercalcemia and hypophosphatemia despite the lack of parathyroid abnormalities, is caused by activating mutations in PTH1R (Ref PubMed: 7701349) and later that biallelic inactivating mutations causes Blomstrands lethal chondrodysplasia (PubMed: 9649554), characterized by short limbs, hydrops fetalis, increased bone density, and advanced skeletal maturation. Mouse studies have detailed the cellular mechanism by which PTH1R signalling regulates growth plate chondrogenesis and bone metabolism, and recombinant PTH is now used clinically as a treatment for both osteoporosis and hypoparathyroidism. It is well established that PTH1R signalling leads to accumulation of cAMP. However, understanding of the downstream signaling of PTH1R remains incomplete.

The current article sheds new light on the downstream signalling mechanism of Pth1r. The authors demonstrated that salt-inducible kinase 3 (Sik3), assisted by Sik1, and -2, drives hypertrophy of growth plate chondrocytes and that a large part of the effect of Pth1r signalling is due to its inhibitory effects on Sik3 and other Sik proteins. Consequently, deletion of Sik3 rescued the lethal phenotype caused by chondrocyte hypertrophy and mineralization in homozygous Pthrp knockout mice. Combined deletion of Sik2 and Sik3 in osteoblasts and osteocytes caused high bone mass with accelerated bone turnover, mimicking the high-turnover phenotype in mice with constitutive Pth1r activation in these cells. Furthermore, the data indicated that class IIa histone deacetylases (e.g., HDAC4) are downstream mediators of Sik action in growth plate and osteoblasts/osteocytes.

Taken together, the presented data establish Sik inhibition as a central mechanism in Pth1r action on skeletal development and metabolism and suggest that cAMP-regulated Sik activity is a key mechanism mediating the action of G protein-coupled receptors.

5.11. Phosphate acts directly on the calcium-sensing receptor to stimulate parathyroid hormone secretion

Centeno PP, Herberger A, Mun H. *et al.*
Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK
Nat Commun 10, 4693 (2019).
https://pubmed.ncbi.nlm.nih.gov/31619668/

In brief: A specific anion binding site senses moderate changes of inorganic phosphate levels leading to rapid inhibition of CaSR responsiveness and PTH secretion in a murine and human *in vitro* model system.

Commentary: Phosphate sensing represents a crucial process in the regulation of phosphate homeostasis which remains only partially understood despite ongoing scientific efforts. On a systemic level, the association of inorganic phosphate levels (Pi) and PTH secretion is a well-known phenomenon, but the mechanism of parathyroid phosphate sensing was unclear. With the crystal structure characterisation of CaSR described in 2016, the G-protein coupled receptor revealed multiple phosphate binding sites mainly for stabilization of its inactive state.

Centeno *et al.* elegantly used *in vitro* and *ex vivo* models including freshly isolated human parathyroid cells to study phosphate-specific effects on CaSR signaling. The authors identified a rapid, dose-dependent attenuation of CaSR activity by moderate changes in Pi levels and identified a single residue in an anion-binding site as a crucial site for phosphate-dependent inhibition. The specific characteristics of this phosphate-sensing mechanism might be of particular relevance for patients with chronic kidney disorder by the ability of sensing small deviations in Pi concentrations, the receptor is shifts towards inactive configuration thus increasing PTH expression under hyperphosphatemic condition. Since phosphate levels at the upper end or above the normal range are a common feature of this patient cohort, the response to calcimimetics in the control of secondary hyperparathyroidism might therefore be enhanced by tighter control of serum phosphate levels.

These novel insights mark a new horizon in the understanding of systemic phosphate homeostasis. Further, the role of the described mechanism in the complex interplay between Ca and Phosphate levels, PTH, FGF23/Klotho and calcitriol levels might reveal new perspectives in the understanding of other disorders of mineral metabolism, such as XLH.

Advances in Skeletal Biology

5.12. Lipid availability determines fate of skeletal progenitor cells via SOX9

van Gastel N, Stegen S, Eelen G, Schoors S, Carlier A, Daniëls VW, Baryawno N, Przybylski D, Depypere M, Stiers PJ, Lambrechts D, Van Looveren R, Torrekens S, Sharda A, Agostinis P, Lambrechts D, Maes F, Swinnen JV, Geris L, Van Oosterwyck H, Thienpont B, Carmeliet P, Scadden DT, Carmeliet G
Laboratory of Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism and Ageing, KU Leuven, Leuven, Belgium
Nature 2020;579:111–117.
https://pubmed.ncbi.nlm.nih.gov/32103177/

In brief: In large fracture calluses, skeletal progenitors activate the chondrogenesis program, whereas in smaller calluses, direct osteogenesis is the preferred path. Here, the authors show that lipid availability determines whether skeletal stem cells repair a fracture through endochondral bone formation or direct ossification.

Commentary: Fracture repair reiterates normal skeletal development and is initiated by skeletal progenitor cells at the fracture site, which proliferate, condensate and differentiate into chondrocytes that form an avascular cartilage template which is then remodelled into bone. However, in the event of a small fracture or crack, the skeletal stem cells do not differentiate into chondrocytes, but instead directly to osteoblasts which

repair the fracture through direct bone formation. The determinants of skeletal stem cell differentiation into chondrocytes or osteoblasts at fractures sites have not been previously determined.

The authors first show *in vivo* that the lack of vasculature in large calluses is associated with chondrogenic differentiation, whereas small calluses have abundant vasculature and that blocking vasculature in-growth in small calluses leads to chondrogenic, rather than osteogenic differentiation. They next culture skeletal stem cells under different conditions and demonstrate that a large part of the chondrogenic effect of serum deprivation is reproduced by culturing skeletal stem cells in media deprived of lipids, and that skeletal progenitors cultured under lipid scarce conditions activate forkhead box O (FOXO) transcription factors, which bind to the SOX9 promoter and increase its expression. Besides initiating chondrogenesis, SOX9 acts as a regulator of cellular metabolism by suppressing oxidation of fatty acids, and thus adapts the cells to an avascular life.

These findings define lipids as a critical determinant of chondrogenic commitment, revealing a role for FOXO transcription factors during lipid starvation and identify SOX9 as a critical metabolic mediator. These data demonstrate the bone regulatory role of lipids and highlight the importance of the nutritional microenvironment in the specification of skeletal cell fate.

5.13. Perivascular osteoprogenitors are associated with transcortical channels of long bones

Root SH, Wee NKY, Novak S, Rosen CJ, Baron R, Matthews BG, Kalajzic I
Department of Reconstructive Sciences, UConn Health, Farmington, Connecticut, USA
Stem Cells, 2020;38(6):769–781.
https://pubmed.ncbi.nlm.nih.gov/32053258/

In brief: A novel population of osteoprogenitor cells in proximity to transcortical channels is found to persist during skeletal maturation. These migrate, expand and contribute to bone formation.

Commentary: Bone formation and regeneration requires multiple distinct populations of progenitor cells, contributing to a complex orchestration of cytokine production, cell migration and differentiation. Lineage tracing experiments revealed multiple cellular niches, including perivascular osteoprogenitors in the periosteum and bone marrow. Here, Root et al identified and characterized a perivascular stem cell niche which, in contrast to previously identified niches, is specific to cortical bone.

By combining inducible DMP1-dependent labelling with Col1-coupled GFP expression in a murine model system, the Authors localized a population of osteoprogenitors in proximity to transcortical channels residing independently of skeletal maturation. They proved in a cortical bone transplantation model that these perivascular progenitor cells contribute to bone formation and remodelling but have a rather marginal role in fracture repair. Given that this novel cell type is specific to cortical bone, the ability of migration, expansion and differentiation suggests a distinct role in intracortical remodelling. Since this still poorly understood process is of high clinical importance, the characterization of the involved cellular populations is particularly important to decipher the regulation of cortical bone mass and cortical porosity. However, the architecture of cortical vascular canals and osteons is highly species-specific and the lack of Harvesian canals in mice may hamper direct translation from murine data to human physiology. Future studies will be needed to investigate the existence and relevance of the identified cortical niche of osteoprogenitors, not at least as a potential target for the development of novel therapeutic approaches.

5.14. Growth plate borderline chondrocytes behave as transient mesenchymal precursor cells

Mizuhashi K, Nagata M, Matsushita Y, Ono W, Ono N
University of Michigan School of Dentistry, Ann Arbor, MI, USA
J Bone Miner Res 2019;34(8):1387–1392.

In brief: Cell tracing of the growth plate chondrocytes most adjacent to the perichondrium demonstrate that they transit into the metaphysis and contribute to the pool osteoblasts and reticular stromal cells of the bone marrow during growth in young mice.

Commentary: Borderline chondrocytes are the growth plate chondrocytes located as a ring around the growth plate and closest to the perichondrium. They form part of the ossification groove of Ranvier, which gives rise to the periosteal bone collar surrounding the growth plate. It was first described by Ranvier in 1889 (1) and its role, and the role of the borderline chondrocytes, have been discussed among developmental biologists ever since. Some have argued that they contribute to the appositional expansion of the growth plate, whereas others, including Ranvier argued that they move from the growth plate into the perichondrial groove, contributing to formation of the bone collar.

The current study authors used a pthrp-creER mouse line (allows for conditional labelling of Pthrp-expressing cells) to label and trace borderline chondrocyte. They showed that postnatal borderline chondrocytes transition into the metaphysis where they act as mesenchymal precursor cells, thus giving rise to osteoblasts forming metaphyseal bone and also to Cxcl12 positive reticular stromal "CAR" cells that are important to support haematopoietic stem cells in the bone marrow. Another recent report by Usami and co-authors (2) used an Axin2-creER which labelled postnatal borderline chondrocytes. After an extended chase, the labelled cells had populated the outer portion of the growth plate, including the outer part of the resting zone. Taken together, these studies indicate that the borderline chondrocyte niche is an important cell source, which contributes chondrocytes to the transverse appositional growth of the growth plate as well as new mesenchymal stem cells for the rapidly growing bone.

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5.15. A simple method based on confocal microscopy and thick sections recognizes seven subphases in growth plate chondrocytes

Fernández-Iglesias Á, Fuente R, Gil-Peña H, Alonso-Duran L, García-Bengoa M, Santos F *et al.*
Division of Pediatrics, Department of Medicine, Faculty of Medicine, University of Oviedo, Oviedo, Asturias, Spain
Sci Rep 2020;10:6935.
https://pubmed.ncbi.nlm.nih.gov/32332842/

In brief: A well-defined and optimized protocol using confocal microscopy allowed novel insight into growth plate chondrocyte differentiation by quantitative analysis of cellular characteristics of murine growth plate samples.

Commentary: The growth plate is a complex tissue with a unique morphology characterized by its anisotropic cellular organization. Limited sample availability, challenging isolation procedures and complex methodologies contributed to the scarce knowledge on growth plate chondrocyte differentiation, which is limited to single studies in recent years. Here, Fernández-Iglesia *et al.* developed a methodological approach for the standardized characterization of cell volume, cell shape and cytoplasm density during chondrocyte maturation by stringent optimization of protocols for confocal microscopy of growth plate tissue. The authors applied their novel protocol to identify seven clusters of growth plate chondrocytes in murine samples with specific cellular characteristics regarding cell volume, shape and cytoplasmic density. Interestingly, distinct clusters were observed in the proliferative zone, contradicting previous assumptions on homogenous cellular characteristics of proliferative chondrocytes. While this finding underlines the complexity of cellular subpopulations in growth plate cartilage, this work is of special value for providing detailed and comprehensive methodologies including a comparison of fixation techniques to preserve hypertrophic chondrocytes. Further, emphasis was given on reproducibility, feasibility and standardizations, such as by scoring systems for sample quality scoring. Thus, the availability of standardized growth plate imaging methodologies, such as the suggested protocol, might contribute to more detailed investigations of the cellular basis of linear growth, especially in disease models with focus on growth plate alterations and short stature.

6. Differences/Disorders of Sex Development and Transgender Medicine

Christa E. Flück, Grit Sommer, Anna Nordenström

Preface

In the last year the scientific community was again very active in research in both topics, differences/disorders of sex development (DSD) and transgender medicine.

In DSD, next generation sequencing approaches revealed novel variants in genes so far not suspected to participate in sex development (e.g. *MYRF, DHX37, PPP1R12A*). This expands the landscape of genes and proteins involved in DSD and provides further challenges for explaining how these factors work together in a concerted way. Research from recent and ongoing international collaborative networks such as DSD-LIFE (https://www.dsd-life.eu/), DSDnet (http://www.dsdnet.eu/) and endo-ERN (https://endo-ern.eu/) revealed important findings for the diagnostic workup and treatment of DSD individuals, including standardized assessment of the external genitalia using gender neutral terminology (the External Genital Score – EGS), state-of-the art hormonal analysis, and fertility preservation.

In transgender medicine, the topics of the year were the brain and psyche of transgender people, fertility, and late effects of current treatment options. Thereby the interest was especially on effects of gender-affirming therapies on psychological, cardiovascular and metabolic health.

Differences/Disorders of Sex Development: Reviews

6.1. Molecular characterization of XX maleness

Grinspon RP, Rey RA
Int J Mol Sci. 2019, Dec 3; 20.
doi: 10.3390/ijms20236089, PMID 31816857.
https://www.ncbi.nlm.nih.gov/pubmed/31816857

This excellent review approaches the diagnostic investigations in DSD from the perspective of an individual with 46,XX DSD. It provides valuable information for clinicians investigating an individual with 46,XX testicular DSD.

The knowledge of gene dosage effects and the opposing gene cascades driving differentiation of the gonadal ridge into testes or ovaries during early fetal development is elegantly explained. It provides insights into the molecular explanation of male development in an individual with XX sex chromosomes. The differentiation and development of the gonads with a detailed overview of the molecular genetics is described. Informative figures illustrate sex differentiation during embryonic and fetal life and the pathways that can lead to testicular and ovotesticular differentiation in 46,XX fetuses.

The review also discusses clinical aspects and the phenotypes of individuals with different variants of XX maleness. With its detailed description of the different genes involved and their respective specific phenotypes, it provides a road map for diagnostic investigations. Informative tables list the specific mutations or genetic defects, in the absence of SRY, and describes their clinical presentation with cross references to publications of clinical cases. This provides a useful resource for the clinical work-up of patients with these complex disorders.

6.2. A clinical algorithm to diagnose differences of sex development

Leon NY, Reyes AP, Harley VR

Lancet Diabetes Endocrinol. 2019, Jul; 7: 560–74.

doi: 10.1016/S2213-8587(18)30339-5, PMID 30803928.

https://www.ncbi.nlm.nih.gov/pubmed/30803928

This review provides a step-by-step approach for establishing the diagnosis in a newborn child with ambiguous genitalia. It compiles all recommended investigations in an excellent algorithm that comprises all established and emerging diagnostic tools, and puts them into the context of current knowledge and controversies.

It is crucial that clinicians follow a systematic decision-making path for the workup of patients with DSD that will currently lead to a precise diagnosis in about 50% of cases. An exact diagnosis is essential for the prediction of therapeutic and psychosocial consequences. It also informs communication between patients, parents, health care professionals from various disciplines as well as DSD researchers (1). DSD care has greatly improved in the past 15 years through the introduction of a clear, systematic nomenclature for DSD based on genetics, through collection of patient data in large registries (for research), and through the collaboration of DSD health professionals, researchers and DSD advocacy groups in international networks (1). Common diagnostic strategies for the newborn with ambiguous genitalia allow the DSD community to join efforts towards solving remaining research questions and to address ongoing controversies (2), e.g. about sex assignment and surgery, as mentioned in this review by Leon *et al*. It also enables quality control and will thereby improve the standards of DSD care, hopefully worldwide. The I-DSD registry (https://home.i-dsd.org/) is a helpful tool to systematically collect data. Its 2020 revision includes not only basic data of DSD patients (as comprised in the suggested diagnostic algorithm), but also longitudinal data that are will importantly inform lifelong holistic care of DSD patients beyond the newborn period (3, 4).

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Differences/Disorders of Sex Development: A DSDnet Paper

6.3. 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: P1–p15;

doi: 10.1530/eje-19-0831, PMID 32268295.

https://eje.bioscientifica.com/downloadpdf/journals/eje/182/6/EJE-19-0831.pdf

Diagnosis and monitoring of therapies for DSD patients require clinical, biochemical and genetic assessments. Biochemical analyses comprise the measurements of peptide and steroid hormones. This position statement, formulated by the COST action DSDnet (BM1303) and the Endo-ERN, provides laboratory guidelines for state-of-the art analysis and interpretation of peptide hormones (e.g. FSH, LH, AMH, inhibin B) in DSD. The authors recommend to measure these peptides in plasma and serum by immunoassays; laboratories should use sex- and age-related reference values and participate in external quality circles. Validation is crucial for harmonization and standardization of tests between different laboratories.

This paper is a 'must read and follow' for all health care professionals dealing with laboratory workup of DSD patients. It is written in a comprehensive yet easy-to-digest fashion and summarizes the preanalytical, analytical and postanalytical requirements for high quality laboratory testing of peptide hormones recommended for the workup of DSD. A table summarizes the expected peptide hormone values associated with the most often encountered DSD diagnoses, which is very helpful for interpretation of data during the diagnostic process.

It might be of interest to learn that experts participating in the DSDnet recently published two similar position statements, one on steroid hormone analysis in diagnosis and treatment of DSD (1) and the other on diagnostic genetic approach in DSD (2).

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Differences/Disorders of Sex Development: Basic Research

6.4. Dynamics of the transcriptional landscape during human fetal testis and ovary development

Lecluze E, Rolland AD, Filis P, Evrard B, Leverrier-Penna S, Maamar MB, Coiffec I, Lavoué V, Fowler PA, Mazaud-Guittot S, Jégou B, Chalmel F

Hum Reprod. 2020, May 15; 10.1093/humrep/deaa041.

doi: 10.1093/humrep/deaa041, PMID 32412604.

https://academic.oup.com/humrep/articleabstract/35/5/1099/5837511

This study provides a comprehensive reference of the protein-coding and non-coding genome from 24 testes and 24 ovaries of human fetuses. It used an RNA sequencing approach to investigate non-coding sequences, and to identify yet unknown candidate genes for sex development. The authors describe the transcriptome of gonadal tissues of 7 developmental stages (week 6–17 post conception) and compare expression patterns between testes and ovaries (sexually dimorphic transcripts) and between developmental stages without differences between sexes (non-sexually dimorphic transcripts). Principal component analyses revealed 14 expression clusters for the sexually dimorphic transcripts and 6 clusters for the non-sexually dimorphic transcripts. Sexually dimorphic expression at early sexual development uncovered promising new transcription factors, e.g. cAMP-responsive element modulator (CREM) and GLI family zinc finger 1. The interesting findings of this study included 680 novel antisense long RNAs and 318 novel unannotated transcripts, of which they confirmed 6% at the protein level.

In the last decade, new genomic techniques have uncovered numerous candidate genes in the complex process of human fetal sex development, but focused mainly on coding RNAs. It has become clear that non-coding RNAs have important regulatory functions. This study adds comprehensive information on non-coding transcripts to the current knowledge on regulation of sex development. It will be a valuable resource for

researchers and clinicians interested in the molecular processes of sex development, thereby contributing to address diagnostic challenges in persons with DSD.

6.5. Undifferentiated spermatogonia regulate Cyp26b1 expression through NOTCH signaling and drive germ cell differentiation

Parekh PA, Garcia TX, Waheeb R, Jain V, Gandhi P, Meistrich ML, Shetty G, Hofmann MC
FASEB J. 2019, Jul; 33: 8423–35.
doi: 10.1096/fj.201802361R, PMID 30991836.
https://www.ncbi.nlm.nih.gov/pubmed/30991836

Retinoic acid (RA) is essential for the regulation of many developmental events including germ cell differentiation. In the developing testis, tight spatiotemporal control of RA levels is maintained by the enzyme *CYP26B1*, which inactivates RA. *CYP26B1* expression is regulated by SOX9 and NR5A1/SF1 in Sertoli cells, but the mechanism that modulates its activity postnatally to allow germ cell differentiation from the niche of spermatogonial sperm cells was unknown. Here, Parekh *et al* performed *in vivo* and *in vitro* studies using mouse and cell models to reveal the underlying regulation. They found that NOTCH signaling through HEY1 and other transcription factor represses *CYP26B1*activity. They also show that Sertoli cells receiving NOTCH signaling by ligand JAG1 trigger germ cell differentiation by down-regulation of *CYP26B1*.

Studies investigating developmental regulation of germ cell differentiation enhance our understanding of basic biology. They reveal essential information to develop protocols for stem cell-based treatment opportunities for DSDs and for infertility in general.

6.6. CBX2-dependent transcriptional landscape: implications for human sex development and its defects

Sproll P, Eid W, Biason-Lauber A
Sci Rep. 2019, Nov 12; 9: 16552.
doi: 10.1038/s41598-019-53006-7, PMID 31719618.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6851130/pdf/41598_2019_Article_53006.pdf

A complex network of genes contributes to (human) sex development. Among these, *CBX2* is involved in the initial programming of the bipotential gonad to either the ovary or testis pathway. Chromosomal male mice lacking *M33*(the homolog of human *CBX2*) show complete sex reversal with additional developmental anomalies in skeleton, spleen, adrenals and kidneys, and are infertile (1). Similarly, Biason-Lauber reported a 46,XY girl with a double heterozygous variant in *CBX2.1* resulting in male-to-female sex reversal with ovary-like gonads (2).

To further explore the role of *CBX2* in human sex development, her group now performed genome wide protein-DNA interaction (Dam-ID) studies in combination with transcriptome studies in a Sertoli cell model after knockdown/-out and overexpression of *CBX2.1*. Using an unbiased bioinformatic approach to look for *CBX2.1* interacting genes and proteins, they identified novel and known targets of sex determination, differentiation and fertility. Overall, *CBX2.1* seems to be a stimulator of the male gonadal pathway and an inhibitor of the female pathway.

This work nicely illustrates how next generation sequencing approaches and bioinformatic tools can help in elucidating the role of novel gene variants identified in DSD individuals; in addition, hidden interacting partners may be uncovered. Using such approaches, the complex puzzle of sex development may be solved step-by-step and genotype-phenotype correlations may be elucidated.

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Differences/Disorders of Sex Development: Genetics

6.7. MYRF haploinsufficiency causes 46,XY and 46,XX disorders of sex development: Bioinformatics consideration

Hamanaka K, Takata A, Uchiyama Y, Miyatake S, Miyake N, Mitsuhashi S, Iwama K, Fujita A, Imagawa E, Alkanaq AN, Koshimizu E, Azuma Y, Nakashima M, Mizuguchi T, Saitsu H, Wada Y, Minami S, Katoh-Fukui Y, Masunaga Y, Fukami M, Hasegawa T, Ogata T, Matsumoto N

Hum Mol Genet. 2019, Jul 15; 28: 2319–29.

doi: 10.1093/hmg/ddz066, PMID 30985895.

https://academic.oup.com/hmg/article/28/14/2319/5424416

This study provides evidence that *MYRF* is important in the development of coelomic endothelial derived cells, and early gonadal development in both males and females. It combines detailed phenotypic assessment of patients and whole genome sequencing with basic biology using single cell gene expression analysis and comprehensive mapping of putative target genes and bioinformatics. The authors conclude that *MYRF* may be involved in not only in the development of the bipotential gonad but also in the early development of the Mullerian ducts.

MYRF (OMIM 608329; 11q12.2), encoding myelin regulatory factor, is a transcription factor that regulates proliferation and migration of coelomic endothelial derived cells (CEDC); it is essential for the development of multiple organs and plays a role in oligodendrocyte development.

This case–control exome sequencing study identified rare heterozygous truncating variants in *MYRF* by comparing rare damaging variants in 26 DSD cases with 2625 controls. Three patients with XY karyotype had micro-penis, hypospadias, small testis and cryptorchidism as signs of impaired Sertoli and Leydig cell function. Their laboratory measurements showed low testosterone and AMH and increased gonadotrophins. One patient had congenital diaphragmatic hernia, a defect in the development of pleuroperitoneal folds. In monozygotic twin with XX karyotype, the mutation led to hypoplasia of Müllerian derivatives and ovaries with increased gonadotrophins.

The expression patterns of *MYRF* in fetal gonads was studied using single-cell RNA sequencing of human male and female gonads. The findings revealed that male fetal gonadal cells express *WT1* and *AMH* markers of Sertoli cell lineage, and *NR2F2* was expressed which corresponds to the Leydig cell lineage. The female gonads expressed *UPK3B*, a marker of coelomic epithelial cells, *FOXL2*, a granulosa cell marker and *NR2F2*, a thecal cell lineage marker.

6.8. Pathogenic variants in the DEAH-box RNA helicase DHX37 are a frequent cause of 46,XY gonadal dysgenesis and 46,XY testicular regression syndrome

McElreavey K, Jorgensen A, Eozenou C, Merel T, Bignon-Topalovic J, Tan DS, Houzelstein D, Buonocore F, Warr N, Kay RGG, Peycelon M, Siffroi JP, Mazen I, Achermann JC, Shcherbak Y, Leger J, Sallai A, Carel JC, Martinerie L, Le Ru R, Conway GS, Mignot B, Van Maldergem L, Bertalan R, Globa E, Brauner R, Jauch R, Nef S, Greenfield A, Bashamboo A

Genet Med. 2020, Jan; 22: 150-9.

doi: 10.1038/s41436-019-0606-y, PMID 31337883.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6944638/pdf/41436_2019_Article_606.pdf

Massive parallel sequencing of 145 46,XY DSD patients revealed 13 individuals with heterozygous missense pathogenic variants in the RNA helicase *DHX37*, explaining 11% of cases of 46,XY gonadal dysgenesis, and 25% of 46,XY testicular regression syndrome (TRS). Likewise, the group of Domenice (1) found familial and sporadic DSD cases with *DHX37* pathogenic variants in 17/87 (19%) 46,XY DSD subjects. Half of the individuals with a TRS had a heterozygous *DHX37* variant.

The DSD category of 46,XY gonadal dysgenesis is clinically heterogeneous. Abnormal gonadal development leads to a broad phenotype manifesting with different degrees of undervirilization of the external genitalia. TRS belongs to this group of DSD and is characterized by the absence of gonadal tissue on one or both sides due to presumed involution *in utero* or soon after birth.

DHX37 is an RNA helicase protein, but its exact role especially in relation to sex development is unknown. RNA helicase proteins are involved in basic RNA-related cell processes, such as transcription, splicing, translation and degradation, as well as ribosome biogenesis. They are known to cause ribosomopathies that are yet poorly understood. Homozygous missense variants of *DHX37* cause severe microcephaly syndromes without the mention of genital anomalies (2), but those microcephaly-associated *DHX37* variants do not lie in the highly conserved helicase core region where all the heterozygous DSD-associated variants were found. In support of the current findings, chromosomal aberrations of 12q24 containing the *DHX37* gene cause multiple dysmorphic features including atypical genitalia (3).

It is interesting to observe how an unbiased next generation sequencing approach reveals novel genes causing DSD in biological processes never considered before. *DHX37* seems to be an important player in male gonadal differentiation and maintenance. It explains a considerable number of DSD cases classified as gonadal dysgenesis (and especially TRS) and may therefore deserve to be included in first line (routine) genetic analysis of this type of 46,XY DSD, together with *SRY, NR5A1/SF1* and *MAP3K1*.

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6.9. Loss-of-function variants in PPP1R12A: From isolated sex reversal to holoprosencephaly spectrum and urogenital malformations

Hughes JJ, Alkhunaizi E, Kruszka P, Pyle LC, Grange DK, Berger SI, Payne KK, Masser-Frye D, Hu T, Christie MR, Clegg NJ, Everson JL, Martinez AF, Walsh LE, Bedoukian E, Jones MC, Harris CJ, Riedhammer KM, Choukair D, Fechner PY, Rutter MM, Hufnagel SB, Roifman M, Kletter GB, Delot E, Vilain E, Lipinski RJ, Vezina CM, Muenke M, Chitayat D

Am J Hum Genet. 2020, Jan 2; 106: 121-8.

doi: 10.1016/j.ajhg.2019.12.004, PMID 31883643.

https://www.cell.com/ajhg/pdf/S0002-9297(19)30468-9.pdf

Protein phosphatase 1, regulatory subunit 12a (PPP1R12A) is an important developmental factor involved in cell migration, adhesion, and morphogenesis. It is involved in the formation of myosin phosphatase and is regulated by phosphorylation.

This paper identifies mutations in the *PPP1R12A*gene in 12 individuals with holoprosencephaly and DSD, using next generation sequencing in two projects. The clinical manifestations of affected cases showed a broad spectrum with no clear genotype-phenotype correlation. Five had neurological phenotypes with midbrain malformations, nine had urogenital malformations, and two had both. The genital manifestations included a broad spectrum, from partial gonadal dysgenesis with micropenis, hypospadias, and ambiguous genitalia with

Müllerian duct remnants, to complete gonadal dysgenesis in a 46,XY individual. In nine patients the karyotype was 46,XY and in three 46,XX.

Using next generation sequencing and in situ hybridization studies in mice and human embryonic tissues, this study adds an important piece of information to the knowledge on the early sequence of events resulting in normal genital development and the pathophysiology leading to a DSD, including complex syndromic forms.

Differences/Disorders of Sex Development: Clinical Studies

6.10. The external genitalia score (EGS): A European multicenter validation study

Van der Straaten S, Springer A, Zecic A, Hebenstreit D, Tonnhofer U, Gawlik A, Baumert M, Szeliga K, Debulpaep S, Desloovere A, Tack L, Smets K, Wasniewska M, Corica D, Calafiore M, Ljubicic ML, Busch AS, Juul A, Nordenström A, Sigurdsson J, Flück CE, Haamberg T, Graf S, Hannema SE, Wolffenbuttel KP, Hiort O, Ahmed SF, Cools M

J Clin Endocrinol Metab. 2020, Mar 1; 105.

doi: 10.1210/clinem/dgz142, PMID 31665438.

https://academic.oup.com/jcem/article-abstract/105/3/e222/5609091?redirectedFrom=fulltext

This cross-sectional study collected measures of genital development from 181 premature neonates, 378 term neonates, 308 babies up to 24 months, and in 111 babies with atypical genitalia from eight European countries within the European Cooperation in Science and Technology (COST) Action BM1303. The authors developed a clinical tool to describe external genitalia, validated this new External Genitalia Score (EGS) and provide reference values for babies up to two years of age. The EGS comprises five anatomic measurements: (1) refined categories for labioscrotal fusion; (2) position of the urethral meatus; position of the right (3) and left (4) gonads; and (5) size of the genital tubercle. Each five feature is scored, and then summed to give a final EGS ranging from 0 (female appearance) to 12 (male). Babies with EGS beyond the 10th and 90th percentiles (i.e. EGS > 0 and $\leq$ 10.5 for term-born infants) should receive assessment by a specialist DSD team.

The anogenital distance, the Prader Score, and the External Masculinization Score (EMS) are all well-established methods to describe the external genitalia, but they exhibit limitations. The Prader Score was originally designed to characterize the genital anomalies in adrenogenital syndromes, while the EMS is describes undervirilized male external genitalia. The new EGS adapted the EMS and enables a standardized measure that is importantly described in gender neutral words.

The EGS cannot replace detailed clinical evaluation, as it does not incorporate other atypical genital features or the internal sex organs. However, it is extremely useful for pediatricians and general practitioners, as it represents a simple and low-cost method to identify infants with atypical genitalia of both sexes who will require specialist care. It introduces a standardized assessment for the whole spectrum from female to male and thus facilitates research in DSD across multiple centers.

6.11. Disorder of sex development with germ cell tumors: Which is uncovered first?

Faure-Conter C, Orbach D, Fresneau B, Verite C, Bonneau J, Thebaud E, Poiree M, Thouvenin S, Pluchart C, Mure PY, Dijoud F, Morel Y

Pediatr Blood Cancer. 2020, Apr; 67: e28169.

doi: 10.1002/pbc.28169, PMID 32020769.

https://www.ncbi.nlm.nih.gov/pubmed/32020769

This thought-provoking paper looked at the malignancy risk in DSD from a different perspective. In this study, patients with malignant germ cell tumors (GCT) were identified in registries of pediatric GCT treatment from 1995 to 2005 and were then cross-referenced with DSD diagnoses. A total of 276 ovarian, 160 testicular, and 24 mediastinal germ cell tumor patients were identified. Among those, 16 individuals had a DSD diagnosis. In 11 of these 16 cases, the DSD diagnosis was unknown at the time of the tumor diagnosis. In addition, in 11 cases the tumor was located at the ovarian site and observed in females with non-ambiguous phenotype. Klinefelter

syndrome was diagnosed in 4/24 cases with mediastinal germ cell cancer. Only one person with a DSD (operated for hypospadias) had testicular germ cell cancer, which may be an underestimation since not all cases had karyotyping. The median age of individuals with GCT and DSD was 15 years, although one patient was only 6 years of age. The median age for the entire cohort was younger, 12.8 years.

The study illustrates that the clinical identification of patients with a DSD and a GCT tumor can be difficult, since hormone production from the tumor may be the first presentation and mimic pubertal development. It is well known that individuals with a DSD diagnosis carry an increased tumor risk. This study stresses that clinicians following individuals with DSD should be aware that pubertal development, including breast development, could be due to hormone production by a tumor. Conversely, oncologists should rule out a DSD diagnosis, especially in patients with a GCT in a mediastinal or ovarian location.

6.12. Clinical but not histological outcomes in males with 45,X/46,XY mosaicism vary depending on reason for diagnosis

Ljubicic ML, Jorgensen A, Acerini C, Andrade J, Balsamo A, Bertelloni S, Cools M, Cuccaro RT, Darendeliler F, Fluck CE, Grinspon RP, Maciel-Guerra A, Guran T, Hannema SE, Lucas-Herald AK, Hiort O, Holterhus PM, Lichiardopol C, Looijenga LHJ, Ortolano R, Riedl S, Ahmed SF, Juul A

J Clin Endocrinol Metab. 2019, Oct 1; 104: 4366–81.
doi: 10.1210/jc.2018-02752, PMID 31127831.
https://www.ncbi.nlm.nih.gov/pubmed/31127831

This retrospective observational study compared long-term health outcomes between 46,X/46,XY individuals diagnosed early in life due to genital anomalies ($n = 35$) and those diagnosed later due other reasons ($n = 28$). Data came from 16 clinical centers worldwide who participated in the international I-DSD registry and/or partners of the European Cooperation in Science and Technology (COST) network DSDnet. Compared to the non-genital diagnostic group, patients diagnosed earlier due to genital anomalies had poorer health outcomes, were less likely to show spontaneous puberty, received testosterone more often, were shorter and had more often genital surgery. Both groups had a similar prevalence of growth hormone treatment, cardiac or renal comorbidities, and cancer.

Histology of gonadal tissue was available in 44 patients across both groups: about one-half had bilateral testicular tissue; the other half had testicular tissue on one side and mostly a streak gonad on the other side. The prevalence of germ cell cancer was high, affecting 11% of patients. Of the 17 patients with available semen samples, three produced live spermatozoa and 14 had azoospermia. Almost one-half of patients had germ cells present histologically and up to one-quarter had focal spermatogenesis. These results highlight that clinicians should discuss fertility preservation with 46,X/46,XY mosaicism patients, as *in vitro* spermatogenesis may become an option in the future.

Another study from Brazil (1) described the clinical phenotype in patients with testicular dysgenesis, among which were 14 boys with 45,X/46,XY. They found differences in growth and fertility between 46,XY and 45,X/46,XY patients, but similar androgenization of external genitalia and similar gonadal characteristics.

It is important to use the term ‘mixed gonadal dysgenesis’, as referring to the sex chromosome karyotype may be misleading in the description of 45,X/46,XY patients.

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1. Clinical Findings and Follow-Up of 46,XY and 45,X/46,XY Testicular Dysgenesis. Andrade JGR, Fabbri-Scallet H, Dos Santos AP, Cools M, Werner R, Hiort O, de Mello MP, Guerra-Junior G, Maciel-Guerra AT. Sex Dev. 2019; 13: 171-7; DOI 10.1159/000504239, PMID 31816618. https://www.ncbi.nlm.nih.gov/pubmed/31816618

Differences/Disorders of Sex Development: Fertility

6.13. Establishing reproductive potential and advances in fertility preservation techniques for XY individuals with differences in sex development

Islam R, Lane S, Williams SA, Becker CM, Conway GS, Creighton SM
Clin Endocrinol (Oxf). 2019, Aug; 91: 237–44.
doi: 10.1111/cen.13994, PMID 31004515.
https://www.ncbi.nlm.nih.gov/pubmed/31004515

Fertility issues in individuals with a DSD has attracted increasing attention over the past decade and are summarized in this and other reviews. The various genetic defects that cause DSD and their underlying mechanism may impair fertility in a variety of ways. In addition, in many cases gonadectomy is performed due to high malignancy risks. With increasing knowledge on gonadal differentiation and development, the possibilities for fertility treatment may increase in the future.

Cryopreservation of sperm is standard care in most places. Today many centers also perform cryopreservation of testicular tissue or spermatogonial stem cells, mainly before cancer treatment. For prepubertal boys, this is a possibility to try to preserve future fertility.

Studies in humans and animals indicate that *in vitro* maturation of spermatogonial stem cells may be possible in the future. However, whether spermatogonial stem cells of testicular tissue from individuals with 46,XY DSD can be used for fertility treatment is unknown. Moreover, cryopreservation of prepubertal testicular tissue and spermatogonial stem cells is still experimental.

The efficacy of the techniques used in assisted reproduction, testicular sperm extraction (TESE), intracytoplasmic sperm injection (ICSI) *in vitro* fertilization (IVF) has improved (1), increasing the potential of fertility for individuals with 5-alfa-reductase deficiency and ovotesticular DSD. In addition, there is evidence that sperm may be present in the gonads of some individuals with other forms of DSD. In up to 40% of persons with a Klinefelter syndrome, TESE may lead to success because of focal sperm production. Similarly, among men with mixed gonadal dysgenesis (MGD) focal areas of spermatogenesis have been identified (2).

More studies are certainly required, but if the results continue to be encouraging, clinicians should inform families with DSD about their fertility preservation opportunities and provide access to these treatment options.

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Differences/Disorders of Sex Development: Psychology and Mental Health

6.14. Mental health of a large group of adults with disorders of sex development in six European countries

De Vries ALC, Roehle R, Marshall L, Frisen L, van de Grift TC, Kreukels BPC, Bouvattier C, Kohler B, Thyen U, Nordenstrom A, Rapp M, Cohen-Kettenis PT, dsd-LIFE Group
Psychosom Med. 2019, Sep; 81: 629–40.
doi: 10.1097/PSY.0000000000000718, PMID 31232913.
https://www.ncbi.nlm.nih.gov/pubmed/31232913

The cross-sectional European dsd-LIFE questionnaire study assessed self-reported health outcomes, including psychiatric diagnoses and symptoms, sexuality (1) and body image (2) in 1040 adolescents and adults with DSD

from six European countries. It compared mental health among DSD diagnostic groups and to the general population and identified determinants for poor mental health. Of all diagnostic groups, men with Klinefelter syndrome were the most vulnerable for poor mental health outcomes: They had the highest levels of anxiety and depression, exhibited most often autism and attention deficits, and had most often suicidal thoughts. Compared to the general population, participants with DSD conditions with androgen effects had more severe anxiety and depression, men with Klinefelter Syndrome and CAH women had more autism symptoms, and in all DSD diagnostic groups except Turner Syndrome, suicidal tendencies were higher. Reluctance to talk about their condition, lower self-esteem, poor body image and poor satisfaction with medical care were determinants of poorer mental health outcomes, and their impact differed between aspects of mental health and diagnostic groups. Participants reported impaired sex life (1), and female participants were more likely to report incomplete and dissatisfaction with their breast development than women from the normal population (2).

Psychological counseling in DSD care should focus on improving these predictive factors, individualized on the specific DSD condition. Specialized psychological care and a multidisciplinary approach for DSD persons plays a central role to help them improve acceptance of their body, build up self-esteem, and to achieve a good quality of life.

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Transgender Medicine: Reviews and Position Statements

6.15. Gender-affirming hormones and surgery in transgender children and adolescents

Mahfouda S, Moore JK, Siafarikas A, Hewitt T, Ganti U, Lin A, Zepf FD
Lancet Diabetes Endocrinol. 2019, Jun; 7: 484–98.
doi: 10.1016/S2213-8587(18)30305-X, PMID 30528161.
https://www.ncbi.nlm.nih.gov/pubmed/30528161

This review overviews the published literature on mental and physical health outcomes and treatment effects after gender-affirming hormone therapy and surgery in transgender adolescents and highlights important evidence gaps.

There is trend towards increasing incidence of transgender adolescents asking for gender transformation treatment, but many open questions remain. The authors state that gender-affirming hormone therapy is safe, and seems to improve gender dysphoria, mental health and quality of life. Testosterone treatment in transgender male adolescents affects on brain activity, increases hemoglobin concentrations and hematocrit, decreases high-density lipoprotein concentrations and promotes a leaner body composition. Estrogen treatment in transgender females increases high-density lipoprotein concentrations and body fat, and decreases lean body mass, waist-to-hip ratio and bone mineral density. Gender dysphoria and psychological functioning improve in transgender male adolescents who undergo mastectomy, and age at surgery has no influence on the risk of surgery complications. There are no quantitative studies on vaginoplasty in transgender female adolescents, probably because this feminizing surgery is rare in this young population.

The findings of this review are inconsistent with other studies published during the past years concluding that cardiovascular disease risk seems to increase with gender confirming hormone therapy, especially in female to male transition (see paper 6.20 by Klaver *et al*. below, and four other papers cited therein). Most of the few existing studies reviewed here face limitations, and larger, prospective studies are needed to provide more evidence on the effect of gender-affirming interventions during adolescence. The authors present a useful list of

open key issues for future research. One of these research priorities is to investigate whether an earlier start of cross-hormone treatment improves health outcomes.

6.16. European society for sexual medicine position statement "assessment and hormonal management in adolescent and adult trans people, with attention for sexual function and satisfaction"

T'Sjoen G, Arcelus J, De Vries ALC, Fisher AD, Nieder TO, Ozer M, Motmans J
J Sex Med. 2020, Apr; 17: 570–84.
doi: 10.1016/j.jsxm.2020.01.012, PMID 32111534.
https://www.ncbi.nlm.nih.gov/pubmed/32111534

This position statement provides an update for health-care professionals on recommended basic principles for care of transgender people, including male, female and nonbinary. It uses a developmental approach to describe the best care for prepubescent, pubescent and adolescent gender diverse people and emphasizes the importance of expertise of health-care providers, especially also in gender-affirming psychological aspects.

The recommendations are from a task force of the European Society for Sexual Medicine based on current available literature (including latest guidelines) and experience. They add important aspects to current guidelines in the prospect of an upcoming change in the diagnostic classification system that most European services will adapt. In this new ICD-11 classification, the diagnosis "gender incongruence" (GIC) has been moved out of the mental health chapter to depathologize transgender people. A total of 32 statements summarize and provide evidence for: general aspects, the assessment of gender diverse children, adolescents and adults, hormone therapies and aspects of sexual functioning and satisfaction in relation to surgical treatment.

For many topics, evidence is limited, and more studies are necessary, especially regarding long-term follow-up. Important statements related to healthcare of gender incongruent children and adolescents include the following: Overall 20–36% of transgender people identify beyond a binary gender. In prepubescent children, in contrast to adolescents and adults, gender incongruence may remit before puberty, but all age groups have much higher frequencies of emotional and behavioral problems than the general population, highlighting the need for mental health support. In children, support from the social network is essential, and this includes parents, school representatives and peers. Hormonal therapies should be clinically and biochemically monitored in an individual developmental context. It is key to inform transgender persons comprehensively about effects, side effects and possible adverse short-term and long-term outcomes for any type of offered hormonal or surgical treatment.

Transgender Medicine: Brain and Psychology

6.17. Similarity in transgender and cisgender children's gender development

Gülgöz S, Glazier JJ, Enright EA, Alonso DJ, Durwood LJ, Fast AA, Lowe R, Ji C, Heer J, Martin CL, Olson KR
Proc Natl Acad Sci U S A. 2019, Dec 3; 116: 24480-5.
doi: 10.1073/pnas.1909367116, PMID 31740598.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6900519/ pdf/pnas.201909367.pdf

In a large sample of 317 US American transgender children aged 3–12 years, the authors compared gender outcomes of transgender children with cisgender peers. They also assessed whether the time the children lived in their current gender, i.e. the time after gender transition, influences their gender development. The study confirms previous findings from smaller studies that transgender children's gender identity and preferences align with their current gender to a similar extent as in cisgender peers.

The most interesting and novel finding of the paper is that the time since gender transition did not influence gender identity or gender preferences, except that stereotyped clothing was a bit less pronounced for children who transitioned longer ago. These results indicate that gender assignment at birth and gender socialization does not define gender identity in transgender or cisgender children. It would be interesting to follow-up these children into adulthood and assess whether gender identity may change over time.

6.18. Cross sex hormone treatment is linked with a reversal of cerebral patterns associated with gender dysphoria to the baseline of cisgender controls

Kilpatrick LA, Holmberg M, Manzouri A, Savic I
Eur J Neurosci. 2019, Oct; 50: 3269-81.
doi: 10.1111/ejn.14420, PMID 30991464.
https://onlinelibrary.wiley.com/doi/full/10.1111/ejn.14420

This longitudinal study investigated the effects of cross-sex hormone treatment on brain morphology in 40 transgender men and 24 transgender women with gender dysphoria. The MRI changes were related to body perception as assessed by questionnaire. Interestingly, after correction for treatment-related changes in grey and white matter volumes, a different picture emerged. Cortical thickness in mesial prefrontal and parietal cortex decreased during treatment in both transgender men and women. The thinning of left parietal cortex correlated with increased perception of body congruence with treatment. The authors conclude that gender dysphoria may be associated with specific brain differences related to body perception, and that the pattern reverses to the pattern in individuals without gender dysphoria with treatment in both sexes. Similarly, other brain MRI studies provide evidence that transgender individuals have structural brain alterations with a different pattern than the sex differences seen in cisgender individuals (1).

Structural and functional brain organization in gender dysphoria and the development of gender is unclear. The brain differences and changes are complex and difficult to interpret. Both a neurodevelopmental cortical hypothesis based on different sex-related structural brain phenotypes and genetic polymorphisms of sex hormone receptors, and a functional-based hypothesis related to regions involved in the own body perception are plausible (2).

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2. Brain network interactions in transgender individuals with gender incongruence. Uribe C, Junque C, Gomez-Gil E, Abos A, Mueller SC, Guillamon A. *Neuroimage*. 2020, May 1; 211: 116613; DOI 10.1016/j.neuroimage.2020.116613, PMID 32057995. https://www.ncbi.nlm.nih.gov/pubmed/32057995.

6.19. Does sex hormone treatment reverse the sex-dependent stress regulation? A longitudinal study on hypothalamus–pituitary–adrenal (HPA) axis activity in transgender individuals

Fuss J, Claro L, Ising M, Biedermann SV, Wiedemann K, Stalla GK, Briken P, Auer MK
Psychoneuroendocrinology. 2019, Jun; 104: 228–37.
doi: 10.1016/j.psyneuen.2019.02.023, PMID 30897530.
https://www.sciencedirect.com/science/article/pii/ S0306453018312307?via%3Dihub

The effect of sex hormones on long-term regulation of the hypothalamic-pituitary-adrenal (HPA) axis was studied in 25 transgender individuals (10 trans-female) at baseline and after 3 months of gender-affirming hormonal therapy. After estrogen and antiandrogen treatment, both ACTH and cortisol increased in response to CRH in transfemale persons, but decreased in transmale subjects after testosterone treatment.

Previous studies observed sex differences in HPA axis activity, but complex and mixed results in various clinical and animal studies failed to explain the direct influence of sex hormones on the reactivity of the HPA axis, mostly due to methodical limitations. Here, Fuss *et al*. provide evidence in transgender people that sex hormones alone (albeit out of their cis-context and corresponding genetic background) affect the stress response in different directions. The study also confirms previous results showing that testosterone attenuates adrenal ACTH sensitivity and that copeptin measurements (as a surrogate of vasopressin, the second stimulant of ACTH secretion) are able to predict the HPA axis response to a dexamethasone/CRH test.

Overall, HPA axis response studies are often difficult to interpret as they may be influenced by many confounding factors. Most relevant to transgender might be changes observed in HPA axis activity with concomitant mental disorders such as depression and anxiety disorders, which are frequent in these individuals. Thus, long-term gender-affirming hormonal treatments affecting HPA axis reactivity may directly modulate mental health.

Transgender Medicine: Treatment and Cardiovascular Effects

6.20. Hormonal treatment and cardiovascular risk profile in transgender adolescents

Klaver M, de Mutsert R, van der Loos M, Wiepjes CM, Twisk JWR, den Heijer M, Rotteveel J, Klink DT
Pediatrics. 2020, Mar; 145.
doi: 10.1542/peds.2019-0741, PMID 32102929
https://pediatrics.aappublications.org/content/pediatrics/145/3/e20190741.full.pdf

Cardiovascular health of transgender people was a hot topic in the transgender literature last year, triggered by previous findings that these individuals could have elevated risks for cardiovascular diseases because of socioeconomic risk factors and unfavorable health behaviors.

This study investigated the combined effect of puberty suppression and hormonal treatment in 192 Dutch transgender adolescents on cardiovascular disease risk factors, by following them from start of gonadotropin-releasing hormone (GnRH) agonist treatment (mean age 15 years) to age 22 years. Changes over time in blood pressure, insulin resistance and lipid profile were similar to cisgender peers, but young transgender persons were more often obese. Novel findings from the US complemented these results, suggesting that body fat and lean tissue of both 19 transgender male and 13 transgender female adolescents under sex-affirming hormone treatment lies in between those of cisgender females and cisgender males (1).

In transgender adults, cardiovascular disease risk seemed to increase mainly in transgender men undergoing testosterone treatment: A prospective European multicenter study found unfavorable lipid profiles in 188 transmen after one year of hormone treatment (2), but not in transwomen. Endothelial function was impaired in 11 testosterone-treated US American transgender men, however transwomen were not studied (3). Finally, a cross-sectional study with a large random sample obtained from the US Centers for Disease Control and prevention (CDC) found that transgender women and men had a 2 and 4-fold increased odds for myocardial infarction compared to cisgender peers, after adjusting for age, race, chronic diseases and health behaviors (4).

Good weight management to lower obesity in transgender adolescents seems important to improve cardiovascular health later in life, in particular in transgender men, who seem to be especially vulnerable to cardiovascular disease.

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Transgender Medicine: Fertility

6.21. Attitudes toward fertility preservation among transgender youth and their parents

Persky RW, Gruschow SM, Sinaii N, Carlson C, Ginsberg JP, Dowshen NL
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https://www.ncbi.nlm.nih.gov/pubmed/32359942

Fertility preservation among transgender youth undergoing gender-affirming hormone treatment is a topic of increasing interest. This study describes the attitudes of youth and parents towards fertility and fertility preservation. Surveys in youths and their parents investigated the desire for biological children in the future and the willingness to delay gender-affirming hormone treatment in order to have fertility preserving therapy first. This is a cross-sectional study of 64 youths and 46 parents in Pennsylvania, USA. The mean age was 16.8 years (12–24 years), and 68% were assigned female at birth. Only 20% of youths and 13% of parents thought it was important to have biological children/grandchildren, and only 27–30% were willing to undergo procedures to preserve fertility. The factors influencing this decision were: discomfort with the body (in 69% of youth), delaying hormonal treatment (50%), and financial aspects (47%). Similarly, in another study of 79 youth, having children was the least important priority (1).

This is an important and difficult topic. Only a minority considered it important to have biological children in the future. On the other hand, priorities in life change over time for many people, and having children often becomes more important beyond adolescence. In addition, there was a large difference in attitudes to fertility preservation between transgender females and males: 60% of adolescent transgender females had sperm preservation, while none of the transgender males underwent oocyte preservation (2). For irreversible treatments, especially for treatments that affect fertility, it is important that the individual is old enough to understand the consequences of such a decision. It remains a question whether this is always the case. Further studies should investigate attitudes towards fertility issues later in life and possibilities for fertility treatment after initiation of gender-affirming hormone treatment (3).

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7. Puberty

Sara Moline[a], Delphine Franssen[b], Anne-Simone Parent[a,b]

[a]Department of Pediatrics, University Hospital Liège, Belgium; [b]Neuroendocrinology Unit, GIGA-Neurosciences, University of Liège, Belgium

Preface

In the puberty field this year, several high impact basic studies highlighted the role of neurotrophic factors and microRNA in GnRH neuron migration and activation around puberty. Fascinating discoveries were made regarding the transcriptome of developing GnRH neurons and testicular cells.

This chapter also summarizes new data regarding the genetic architecture of the control of puberty using GWAS studies in boys as well as much awaited data regarding estrogen reference values in children and sertoli function in boys with constitutional delay of growth and puberty treated with aromatase inhibitors.

Clinical Guidance

7.1. Anti-Müllerian hormone and letrozole levels in boys with constitutional delay of growth and puberty treated with letrozole or testosterone

Kohva E, Varimo T, Huopio H, Tenhola S, Voutilainen R, Toppari J, Miettinen PJ, Vaaralahti K, Viinamäki J, Backman JT, Hero M, Raivio T

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https://academic.oup.com/humrep/article/35/2/257/5709207. https://pubmed.ncbi.nlm.nih.gov/31958337/

This randomized controlled trial compares treatment with peroral letrozole or intramuscular low-dose testosterone in boys with constitutional delay of growth and puberty. Gonadotrophin and Sertoli cell marker levels show that letrozole may be beneficial for Sertoli cell proliferation and future spermatogenesis.

Until now, usual approach for constitutional delay of puberty involves watchful waiting or low-dose androgen treatment (1,2). Recently, peroral letrozole, an aromataze inhibitor, was explored as a new treatment for constitutional delayed puberty (3). It appears more efficacious in inducing hypothalamic-pituitary-gonadal (HPG) axis activation and testis growth than testosterone (3). However, men with a history of delayed puberty exhibit decreased sperm count (4) and so far, the concomitant changes in inhibin B and AMH levels (markers of seminiferous epithelium function, essential to future fertility) in boys receiving letrozole or testosterone had not been described.

The aim of this study was to compare the action of oral letrozole versus testosterone injection on AMH, Inhibin B and gonadotrophin serum levels and clinical outcomes in 28 boys. The authors show that AMH levels similarly decrease over time in both treatment groups, suggesting that letrozole does not cause unduly rapid maturation of Sertoli cells and does not have untoward effects on future sperm producing capacity. However, testosterone has been shown to suppress gonadotrophins and inhibin B, whereas letrozole increases these levels (4). Here, treatment-induced changes in circulating gonadotrophin levels were not associated with changes in serum AMH, but they correlated with changes in inhibin B. This supports the idea that inhibin B depends on gonadotrophin-dependent and Sertoli cell mass-related components (5). The authors also measured circulating levels of letrozole in treated boys (Letrozole 2.5 mg/day). Circulating letrozole levels are highly variable and explained by the dose per weight. There was no significant association between Letrozole levels and any markers of HPG axis activity.

This article paves the way for further studies to investigate the minimal efficient letrozole dose per Kg weight and strengthens the hypothesis that Letrozole-induced activation of HPG-axis will protect Sertoli cell proliferation and future spermatogenesis.

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7.2. Sex-specific estrogen levels and reference intervals from infancy to late adulthood determined by LC–MS/MS

Frederiksen H, Johannsen TH, Andersen SE, Albrethsen J, Landersoe SK, Petersen JH, Andersen AN, Vestergaard ET, Schorring ME, Linneberg A, Main KM, Andersson AM, Juul A

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https://academic.oup.com/jcem/article/105/3/754/5624050

The authors used state-of-the-art liquid chromatography tandem mass spectrometry to establish sex-specific reference ranges for estrone (E1) and estradiol (E2) throughout life and evaluate sex-differences.

Evaluating circulating estrogens is necessary in several clinical conditions in girls and women throughout life, as well as in specific situations in boys and adult men. This requires highly specific and sensitive standardized assays and liquid chromatography tandem mass spectrometry (LC–MS/MS) is now considered as the reference method (1). However, most reference values still rely on immunoasssays which lack sensitivity for very low ranges such as found in infants, prepubertal children or adolescent boys.

In this study, serum samples from 1838 healthy subjects (772 boys and men/1066 girls and women; selected from 5 different cohorts) were analyzed for estrogen concentrations. E2 was detectable in 96% and 63% of all female and male serum samples, respectively. E1 was detectable in 89% in both sexes. From infancy until puberty onset, E2 was detectable in 68% and 22% of the female and male samples, respectively, while E1 was detectable in 88% (female) and 74% (male). E3 was detectable in only very few samples. In infant girls, both E1 and E2 concentrations were significantly higher around 3 months of age than concentrations at other prepubertal ages, confirming the rise associated with mini-puberty (2,3). The authors found clear sex differences, as E2 levels in infant boys were mostly undetectable. E1 and E2 concentrations increased with age and pubertal stage both in boys and girls. The highest E2 concentration in prepubertal girls aged <7 years was 20 pmol/l, while older prepubertal girls (>7 years) had slightly higher E2 concentrations. Prepubertal girls had significantly higher levels of E2 than prepubertal boys. Almost all girls with breast stage B2 or more had levels >10 pmol/l. In conclusion, this article provides much needed reference ranges for estrogen values throughout life.

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7.3. Pubertal timing and adult fracture risk in men: A population-based cohort study

Vandenput L, Kindblom JM, Bygdell M, Nethander M, Ohlsson C
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This large scale population-based cohort study identifies a slightly higher risk for adult fracture in men who had later puberty.

The pubertal period plays a key role in bone mineral acquisition. Several papers have reported that pubertal delay, for both men and women, is associated with lower bone mineral density (1–5). While several studies in women have showed that later age at menarche is associated with higher risk of osteoporosis fracture (6–7), there is no evidence in men of a causal relationship between pubertal timing, defined by age at peak height velocity (PHV), and fracture risk in adulthood. Yet, given the aging of the population and the importance of osteoporosis, fragility fractures are a public health concern. For these reasons, it is necessary to better predict which individuals are at high risk of future fractures.

In a very large dataset of 31 971 Swedish men born between 1945 and 1961, the authors demonstrate for the first time that men with age at PHV in the oldest tertile (>14.5 years old) had a 15% higher relative risk of any fracture or non-vertebral fracture than men with age at PHV in the lowest tertile ($\leq$13.6 years). Adjustments for birthweight, childhood BMI at 8 years of age, adult educational level or young adult height did not attenuate these associations.

These findings suggests that pubertal timing might be taken into account to identify adult men at elevated risk of fracture, by combining bone density imaging and clinical risk factors. However, it should be noted that the increase in risk reported in this study was only moderate.

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7.4. Using kisspeptin to predict pubertal outcomes for youth with pubertal delay

Chan YM, Lippincott MF, Sales Barroso P, Alleyn C, Brodsky J, Granados H, Roberts SA, Sandler C, Srivatsa A, Seminara SB
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This longitudinal study (n = 16) suggests that responses to kisspeptin administration could predict later pubertal outcomes for patients with delayed puberty.

The management of delayed puberty still represents a clinical challenge given the difficulty to differentiate between patients with constitutional delay, who require only watchful waiting, and those with idiopathic hypogonadotropic hypogonadism (IHH) who will not attain adult reproductive function. Measurement of baseline and GnRH stimulated serum LH and FSH levels, and inhibin B have been suggested to differentiate

those clinical entities (1–3) but none of these tests has sufficient sensitivity and/or specificity to accurately predict pubertal outcomes.

The premise of this physiological study was based on previous findings in adults showing that a bolus of kisspeptin increases LH release in reproductively-intact but not IHH individuals (4–6). Here, responsiveness to kisspeptin was measured in 16 patients with delayed or stalled puberty. All of those patients ($n = 8$) who showed a rise in LH >0.8 mUI/ml after kisspeptin stimulation progressed spontaneously through puberty during the follow-up period, while all patients with an LH response < 0.4 mUI/ml ($n = 8$) failed to enter puberty. Furthermore, the kisspeptin stimulation test outperformed inhibin B, LH after GnRH stimulation, or basal or overnight LH and genetic testing to predict pubertal outcome.

These findings suggest that the kisspeptin stimulation test, coupled with others tests, may provide a more informative management for patients with delayed puberty and optimize later-life health outcomes.

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7.5. Can we rely on adolescents to self-assess puberty stage?

Campisi SC, Marchand J, Siddiqui FJ, Islam M, Bhutta ZA, Palmert MR

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This meta-analysis of 22 studies, comprising in total 21 801 individuals, evaluates the accuracy of self-assessment of pubertal stage by the patient him/herself, given the many challenges in obtaining clinician-assessed Tanner Staging.

Clinicians, researchers and health advocates know how challenging it can be to accurately evaluate pubertal stage in some adolescent patients. Nevertheless, monitoring of pubertal development is an important part of disease management, and even health monitoring given the fact that age at puberty is associated with many later-life health outcomes (1–3).

Previous studies have highlighted the wide interest in self-report measures of pubertal status (4–6), but this is the first systematic review, using formal meta-analysis methods and encompassing a large number of participants (including male patients). The findings provide reliable information on self-assessment of pubertal stage:

- Self-assessment is reliable to identify Tanner stages 1 and 5, or to categorize pubertal development into the three categories: pre-puberty; in or early puberty; and completing or late puberty. However, it is less accurate to identify Tanner stages 2, 3 and 4, for which expert clinician assessment remains paramount. Girls seem to better assess pubertal stages than boys.
- The use of some self-assessment tools, such as images and test descriptions, increases the agreement with clinician assessments.
- Overweight and obesity do not seem to weaken the reliability of self-assessment.

The authors described limitations in the published evidence, such as variable levels of examiner's confidence in pubertal examination, included studies were mostly from high-income countries with Caucasian youth, and no patients were included with incongruence or with delayed or precocious puberty. However, this study highlights the importance of being aware of these nuances when interpreting self-assessed pubertal development, for example in remote clinics during the COVID-19 pandemic.

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7.6. Age at menarche associated with subsequent educational attainment and risk-taking behaviours: The Pelotas 1982 Birth Cohort

Calthorpe LM, Gigante DP, Horta BL, Ong KK
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This cohort study identifies an association between pubertal timing in females and educational attainment as well as risk-taking behaviours in a Brazilian population during economic transition.

Earlier puberty timing is a risk factor for diverse adverse health, psychological and behavioural outcomes (1–5). However, the effect of puberty timing on educational outcomes is still a matter of debate (6–7).

The authors surveyed 2083 women from the Brazilian Pelotas 1982 cohort at age 23 years. Later on, 1912 of them were followed to age 30 years (8). The authors evaluated the link between timing of puberty, defined as early age at menarche (AAM), average AAM and late AAM, and years of full time education; secondary outcomes were sexual behaviours, substance use and socio-economic outcomes. Surprisingly, late AAM was associated with lower educational attainment, whereas the early AAM effect was not statistically significant when compared to average AAM. These results contradict numerous other studies which have reported an association between early AAM and lower educational attainment (9–10). As much of the existing evidence relies on data obtained in high-income countries, these findings highlight that the link between early AAM and education may involve different psychosocial mechanisms. Brazil represents a unique context to study the effects of puberty timing on education outcomes and behaviour, as it only recently underwent an economic transition.

Additionally, the study confirmed that early AAM is associated with younger age at first sexual intercourse and younger age at first alcohol consumption.

This study and its divergent findings highlight the importance to pursue investigations to better clarify the determinants of puberty and its implications on health, psychological parameters and education.

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Basic Science

7.7. Environmentally relevant perinatal exposures to bisphenol A disrupt postnatal Kiss1/NKB neuronal maturation and puberty onset in female mice

Ruiz-Pino F, Miceli D, Franssen D, Vazquez MJ, Farinetti A, Castellano JM, Panzica G, Tena-Sempere M

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This study identifies the effects of exposure to environmentally relevant doses of bisphenol A (BPA) on the development of hypothalamic kiss1/NKB neurons in female mice.

Bisphenol A (BPA) is a compound with estrogenic activity, which is widely used in the production of polycarbonate plastics and epoxy resins (1). It is the most studied endocrine-disrupting chemical and is potentially involved in a wide array of health problems including reproductive disorders (2–3). Puberty is an important marker of reproductive health at the population level, and may be correlated with downstream outcomes, such as irregular estrous cycles and reduced fertility. However, studies focusing on timing of pubertal onset report divergent results, ranging from no effect of BPA, to a delay, or early onset of puberty.

Using a mouse model, the authors showed that perinatal exposure to BPA significantly advances age at vaginal opening, a measure of puberty onset in female mice, consistent with some previous studies (4–5). The novelty of this study is the use of very low-doses of BPA given orally to the mothers which reinforces the clinical relevance of the data. Mice exposed perinatally to BPA exhibited a persistent, but divergent, impairment of Kiss1 neuronal maturation, with more kisspeptin cells in the rostral (RP3V) hypothalamus but consistently fewer kisspeptin neurons in the arcuate nucleus (ARC). Mice exposed to BPA had persistently lower Kiss1 expression during (pre)pubertal maturation, which was associated with lower Tac2 (encoding NKB) levels.

This comprehensive analysis illustrates the exquisite sensitivity of the Kiss1/NKB system to endocrine disruptors. Detecting brain region-specific BPA effects is a novel contribution and provides a potential explanation for abnormal programming of puberty. It draws attention to the necessity of precautionary measures concerning exposure to BPA, even at low doses that are currently considered as safe.

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7.8. Characterization of the human GnRH neuron developmental transcriptome using a GNRH1-TdTomato reporter line in human pluripotent stem cells

Lund C, Yellapragada V, Vuoristo S, Balboa D, Trova S, Allet C, Eskici N, Pulli K, Giacobini P, Tuuri T, Raivio T
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https://dmm.biologists.org/content/13/3/dmm040105.long

These authors developed an in vitro model to study human GnRH neuron transcriptome during developmental differentiation.

Gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus govern the hypothalamus-pituitary-gonadal (HPG) axis and regulate reproductive functions. Alteration of GnRH neuron development or signaling leads to congenital hypogonadotropic hypogonadism (CHH) or pubertal delay. However, the causal mechanisms are incompletely understood. A better understanding of the developmental differentiation of GnRH neurons is required to address this issue. During human fetal development, GnRH neurons differentiate from the nasal neuroepithelial compartment, which is derived from the olfactory placodes, and begin their migration to the hypothalamus (1). Neuronal progenitor subtypes that give rise to GnRH neurons have not been completely characterized yet.

Here, the authors generated a stable GnRH-TdTomato reporter cell line in human pluripotent stem cells (hPSCs) using CRISPR-Cas9 technology to model GnRH neuron development. CRISPR- Cas9 genome editing has recently become one of the key tools to study differentiation of specialized cell types, including GnRH neurons (2). To characterize the mRNA transcriptome, RNA-sequencing was performed on progenitors and differentiated GnRH neurons. This analysis revealed 6461 differentially-expressed genes. The findings highlight potential genes of interest regarding GnRH neuron development and disease, such as the transcription factor *ISL1*, a LIM/homeodomain family transcription factor with a known role as a specifying transcription factor in early spinal motor neurons (3). *ISL1* was highly upregulated in TdTomato-expressing GnRH neurons and its expression was confirmed in human fetal tissues. In addition, they detected 15 differentially-expressed genes that are already implicated in CHH, such as *TAC3*, *TAC3R*, and *ANOS1*, and some genes with known relevance to human puberty timing, such as *LEP* and *LEPR*. The results of this study include a large number of differentially-expressed genes not previously described in GnRH neurons. This study provides new insights into the early stages of human GnRH neuron development, and greatly increases the list of potential regulators.

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7.9. The dynamic transcriptional cell atlas of testis development during human puberty

Guo J, Nie X, Giebler M, Mlcochova H, Wang Y, Grow EJ, Donor Connect, Kim R, Tharmalingam M, Matilionyte G, Lindskog C, Carrell DT, Mitchell RT, Goriely A, Hotaling JM, Cairns BR
Cell Stem Cell vol. 26,2 (2020): 262–276.e4.
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https://www.sciencedirect.com/science/article/pii/S1934590919305235?via%3Dihub

This paper describes a transcriptional analysis of human spermatogonial stem cells during puberty and the involvement of testosterone in Sertoli cell maturation.

The testis is one of the few organs that defines most of its cell types, physiology, and function after birth. During puberty, major changes occur, such as proliferation and maturation of niche cells, spermatogonial differentiation,

and modulation of hormonal signaling to finally allow spermatogenesis (1). Our understanding of the molecular and genetic mechanisms involved in these dramatic changes are limited to rodent studies, while fundamental differences exist between humans and rodents regarding the onset of spermatogenesis (2).

Here, the authors describe a transcriptional cell atlas of the developing human testis during puberty, generated using Single-cell RNA sequencing. This technique allows the simultaneous examination of thousands of individual cells, representing the entire organ, without the need for prior sorting or enrichment procedures (3). They profiled 10 000 single-cell transcriptomes from whole-testes of four juvenile boys (7, 11, 13, and 14 years old) and compared these data to previously published data in one infant (1 year old) and one adult (25 years old). In addition, in order to explore the role of testosterone in the adult testis, they examined the expression profiles of testes from two adult transfemales who had undergone long-term suppression of testosterone. They determined distinctive phases of germ cell differentiation during puberty and observed that cell maturation could be reversed with testosterone suppression. Importantly, two modes of computational analysis allowed the identification of a common progenitor for Leydig and myoid cells prior to puberty. They also reported major differences between mice and humans regarding the markers for somatic cell development and their timing of appearance.

In summary, this study provides new insights into the development of human spermatogonial stem cells and their niche during puberty. A website has been designed to access the data: https://humantestisatlas.shinyapps.io/humantestisatlas1/.

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7.10. Microbial reconstitution reverses early female puberty induced by maternal high- fat diet during lactation

Wang M, Zhang Y, Miller D, Rehman NO, Cheng X, Yeo JY, Joe B, Hill JW
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https://academic.oup.com/endo/article/161/2/bqz041/5698115

This mouse study demonstrates the influence of maternal nutrition during lactation on offspring gut microbiota and its consequences on metabolism and puberty timing.

Childhood obesity is a risk factor for the development of precocious puberty in girls, and the metabolic state of children also depends on maternal nutrition (1). Increasing evidence shows that maternal high-fat diet (MHFD) induces gut microbial dysbiosis in offspring (2), and breastfeeding also has a critical role on gut microbiota maturation (3). In addition, MHFD has been shown to induce precocious puberty in animal offspring (4), but little is known about the role of the gut microbiota (GM) in pubertal development.

This mouse study investigated whether MHFD during lactation alters GM in offspring and influences the risk of obesity and early pubertal development. The authors observed that MHFD-exposed pups developed juvenile obesity, early puberty, irregular estrous cycles and signs of disrupted glucose metabolism. Offspring metabolic abnormalities induced by MHFD were linked to reduced GM diversity and richness, with an increase of *Streptococcaceae* and *Peptostreptococcaceae* families, which are strongly correlated with adiposity (5). It was recently shown that co-housing obese mice with lean mice restored a lean phenotype (6), as mice are coprophagic, so co-housed family members share gut microbiota through the fecal–oral route. The authors found that co-housing offspring of MHFD with those of normally-fed mothers increased GM richness, reversed early puberty, and improved insulin sensitivity.

This study provides novel potential therapeutic targets and a specific window to treat metabolic and reproductive diseases. Acting on microbial reconstitution during lactation may prevent early puberty associated with insulin resistance.

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7.11. Neuron-derived neurotrophic factor is mutated in congenital hypogonadotropic hypogonadism

Messina A, Pulli K, Santini S, Acierno J, Känsäkoski J, Cassatella D, Xu C, Casoni F, Malone SA, Ternier G, Conte D, Sidis Y, Tommiska J, Vaaralahti K, Dwyer A, Gothilf Y, Merlo GR, Santoni F, Niederländer NJ, Giacobini P, Raivio T, Pitteloud N
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By performing next-generation sequencing in 240 unrelated probands with congenital hypogonadotropic hypogonadism and follow-up in multiple animal models, this study identifies Neuron-Derived Neurotrophic Factor as a gene involved in GnRH neuron migration and implicated in Kallmann syndrome.

Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder caused by absent secretion or action of gonadotropin-releasing hormone (GnRH) and is characterized by infertility and the absence of puberty (1). CHH associated with anosmia defines Kallmann syndrome (KS). CHH is a complex genetic disorder for which more than 40 causal genes have been identified, each accounting for fewer than 10% of cases (2). Fifty percent of subjects with CHH do not carry mutations in the known CHH-associated genes. Previous next-generation sequencing studies for CHH included small population size and limited ability to detect statistically significant associations with variants at most known CHH-causing genes.

Here, the authors studied 240 unrelated CHH probands (140 KS and 100 normosmic CHH). They used an analysis strategy to combine collapsed gene-based burden testing with targeting of genes in the FN3 superfamily. Several proteins involved in GnRH neuron migration, and previously implicated in CHH, contain FN3 domains. Using this strategy, they identified an enrichment for protein-truncating variants in Neuron-Derived Neurotrophic Factor (NDNF), which encodes a secreted glycosylated disulfide-bond protein containing FN3 domains. Overall, ~3% of individuals with KS harbour NDNF mutations. In addition, authors showed that NDNF is expressed in the nasal region after formation of the olfactory placode in mice and humans. Knockdown of the ortholog of NDNF in zebrafish resulted in altered GnRH migration, and mice lacking NDNF showed delayed GnRH neuron migration and altered olfactory axonal projections to the olfactory bulb.

Together, these results provide strong evidence for a role of NDNF in GnRH neuron migration. Further investigations are needed to identify the molecular signalling pathway used by NDNF in GnRH neuron ontogeny, and to dissect the contribution of genetic and environmental factors potentially modifying the phenotype of subjects carrying NDNF loss-of-function mutations.

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7.12. Hypothalamic miR-30 regulates puberty onset via repression of the puberty-suppressing factor, Mkrn3

Heras V, Sangiao-Alvarellos S, Manfredi-Lozano M, Sanchez-Tapia MJ, Ruiz- Pino F, Roa J, Lara-Chica M, Morrugares-Carmona R, Jouy N, Abreu AP, Prevot V, Belsham D, Vazquez MJ, Calzado MA, Pinilla L, Gaytan F, Latronico AC, Kaiser UB, Castellano JM, Tena-Sempere M

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https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000532

This study unravels the role of the miR-30/Mkrn3 pathway in the hypothalamic regulation of puberty

Puberty onset is triggered by a hypothalamic network of interconnected genes regulated by a complex transcriptional network (1). Among transcriptional factors, *Mkrn3*, a maternally imprinted gene encoding the makorin RING-finger protein-3, has been recently identified as an essential inhibitory component of the gene network governing puberty. Mutations in *Mknr3* were found to cause precocious puberty in boys and girls (2) and its expression decreases throughout sexual maturation (3). However, the molecular mechanisms of *Mkrn3* regulation of puberty are still unknown. Recently, microRNAs (miRNAs), short noncoding RNAs, have emerged as relevant regulatory elements of puberty (4).

Using miRNA-target prediction bioinformatic tools, these authors identified that the 3'UTR of *Mkrn3* includes a binding site for the miR-30 family. Analyzing mRNA expression in the hypothalamus, they observed that miR-30b expression increases during peri-puberty while the level of *Mkrn3* decreases. The physiological role of the miR-30/Mkrn3 pathway in the control of puberty was attested by blocking miR-30 binding to the 3'UTR of *Mkrn3*. This blockage attenuated the decrease in hypothalamic *Mkrn3* during peri-puberty and significantly delayed puberty onset. Thus, the miR-30/Mkrn3 pathway is added to the newly identified sets of 'repressors of repressors' involved in the multilayered control of puberty, such as the Lin28/let-7 system (5), and the miR-200/Zeb1 and miR-155/Cebpb tandems (4), which control GnRH expression.

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7.13. Genomic analysis of male puberty timing highlights shared genetic basis with hair colour and lifespan

Hollis B, Day FR, Busch AS, Thompson DJ, Soares ALG, Timmers PRHJ, Kwong A, Easton DF, Joshi PK, Timpson NJ; PRACTICAL Consortium; 23andMe Research Team, Ong KK, Perry JRB

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This multi-trait genome-wide association study (GWAS) for male puberty timing identifies 76 independent signals for puberty timing and highlights relationships with natural hair colour and lifespan.

The timing of puberty varies widely among individuals and better understanding the genetic basis of such variations and the reasons why earlier puberty is associated with later life diseases is an important challenge. Most of genetic research regarding puberty is based on studies in women. However, despite the overlapping genetic architecture between males and females, a series of genetic signals differ between sexes *(1,2)*.

The authors combined data from two very large GWAS studies (23andMe and UK Biobank), including a total of 205 354 men, and identified new male-specific loci associated with age at voice breaking and thus involved in pubertal timing. Among the new candidates, a non-synonymous variant in *ALX4* was identified. *ALX4* encodes an homeobox gene involved in fibroblast growth factor signalling whose mutations lead to a male-specific hypogonadism *(3)*. Another missense variant was identified in *SRD5A2*, which encodes for Steroid 5-alpha-reductase, the disruption of which leads to disorders of sexual differentiation. They also discovered three loci for pubertal timing located proximal to genes known to regulate pigmentation, *HERC2*, *IRF4* and *C16orf55*, raising suspicion of a phenotypic relationship between inter-individual variation in natural hair colour and puberty timing. Phenotypic and genetic analyses confirmed that susceptibility to darker hair colour also confers earlier puberty timing in a relative sex-specific manner (the association being stronger in men). This suggests the existence of common regulators of melanocortins and gonadotropins or a direct impact of melanocyte signalling on puberty.

Another finding of this study is the genetic association between later puberty timing in males and longer lifespan, in accordance with previous phenotypic studies (4). Underlying mechanisms are still unclear, hypothesis range from a direct causal effect of earlier male puberty timing on earlier mortality to a widespread horizontal pleiotropy where genetic variants influence lifespan through mechanisms separate from puberty timing.

This study demonstrates the utility of a multi-trait large-scale GWAS to better understand the regulation and likely consequences of puberty timing.

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7.14. Patterns of aging biomarkers, mortality, and damaging mutations illuminate the beginning of aging and causes of early-life mortality

Kinzina ED, Podolskiy DI, Dmitriev SE, Gladyshev VN

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This study identifies that aging starts very early in life and is best defined by molecular damage rather than mortality.

Clinically, aging is classically defined by the age-related increases in frailty and mortality (1, 2). However, human mortality rates start high at birth then decline until puberty when it reaches its minimum (3). In this article, all-cause mortality rates were obtained from the Human Mortality Database for the years 1999–2015 and combined across 19 countries with very low child mortality and low adult mortality rates (4). Cause-specific mortality data from 1999 to 2013 were obtained from the US Center for Disease Control and Prevention (CDC) National Center for Health Statistics. The authors showed that the relationship between age and mortality is a U-shaped curve, with its lowest point before puberty at age 9 years. To the contrary, age-associated mutation accumulation in cancer tissues and DNA methylation changes showed a linear increase throughout the whole lifetime, suggesting that decreasing mortality in early life is a separate effect caused by non-aging related mechanisms.

In summary, the authors show that mortality rate reaches a nadir and then starts to increase before puberty. Separately, deleterious molecular changes begin very early in life and are the defining characteristics of aging.

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8. Adrenals

Svetlana Lajic[1], Leif Karlsson[1*], George Paltoglou[2*], Evangelia Charmandari[2,3]
[1]Department of Womeńs and Childreńs Health, Pediatric Endocrinology Unit; Karolinska University Hospital/Karolinska Institutet, 171 76 Stockholm, Sweeden; [2]Division of Endocrinology, Metabolism and Diabetes, First Department of Pediatrics, National and Kapodistrian University of Athens Medical School, 'Aghia Sophia' Children's Hospital, Athens, 11527, Greece; [3]Division of Endocrinology and Metabolism, Center of Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, 11527, Greece.
*Equal author contribution
(Author for Correspondence: Evangelia Charmandari, MD, MSc, PhD, MRCP(UK), CCST(UK) Professor of Pediatric and Adolescent Endocrinology, First Department of Pediatrics, National and Kapodistrian University of Athens Medical School, 'Aghia Sophia' Children's Hospital, Thivon and Papadiamantopoulou Street, Athens, 11527, Greece
Tel/Fax: +30-213-2013 384 Email: evangelia.charmandari@googlemail.com)

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, 2019 and May 31, 2020. Our search yielded more than 5000 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 2019, unless progress in the field has been incremental. Emerging themes for this year's chapter include: i) Neural circuits that link the cerebral cortex to the adrenal medulla; ii) Cognitive dysfunction in mice lacking proper glucocorticoid receptor dimerization; iii) Long-term outcome of Primary Bilateral Macronodular Adrenocortical Hyperplasia after unilateral adrenalectomy; iv) Efficacy and safety of levoketoconazole in the treatment of endogenous Cushing's Syndrome; v) Cognitive function of children and adolescents with Congenital Adrenal Hyperplasia; vi) Carriers of a classical CYP21A2 mutation have reduced mortality; and vii) Brain differences in the prefrontal cortex, amygdala, and hippocampus in youth with Congenital Adrenal Hyperplasia.

Mechanism of the Year: Neural circuits that link the cerebral cortex to the adrenal medulla

8.1. The mind-body problem: Circuits that link the cerebral cortex to the adrenal medulla

Dum RP, Levinthal DJ, Strick PL
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PMID: 31871146
https://pubmed.ncbi.nlm.nih.gov/31871146/

The 'mind–body connection' is essential for normal organ function and is viewed as the basis for psychosomatic disorders. There is abundant evidence that shows how the mind or mental processes influence our health and well-being, as well as the negative impact of emotional stress on the gastrointestinal, cardiovascular, metabolic and immune systems. The connection between the central nervous system and internal organs is mediated by sympathetic and parasympathetic subdivisions of the autonomic nervous system. Although ample information is available about the neural connections that link autonomic output from centers in the brainstem and spinal cord to specific organs (**1**), the neural circuits that link higher brain function and central sites (e.g., the cerebral cortex) to autonomic output and organ function have not been clearly defined (**2**). Specific questions that have

not been answered include the following: How does the mind (conceptually associated with the cerebral cortex) influence autonomic and endocrine systems that control internal organs? Also, which regions of the cerebral cortex represent the origin of descending commands to direct organ function?

To address these questions, Dum *et al*. used transneuronal transport of rabies virus (RV) in monkeys and rats to identify regions of cerebral cortex that have multisynaptic connections with a major sympathetic effector, the adrenal medulla. In rats, the authors also examined multisynaptic connections with the kidney. RV is transported transneuronally in the retrograde direction in a time-dependent fashion. By varying of the survival time, the extent of transport can be limited to 1st-order (sympathetic preganglionic 1 neurons), 2nd-order (spinal interneurons, brainstem, hypothalamus), 3rd-order (cerebral cortex) or 4th-order neurons (neurons at multiple sites, including cortical layers II–IV and VI) (**3**). The power of transneuronal tracing with RV is that it reveals the entire extent of the cortical influence over this system. In this way, it identifies the potential origins of the elusive 'central commands' from the cerebral cortex.

The authors found that in monkeys, the cortical influence over the adrenal medulla originates from 3 distinct networks that are involved in movement, cognition and affect. Each of these networks has a human equivalent. One clear implication of this organization is that the sympathetic responses that occur during activities such as exercise, the performance of demanding cognitive tasks, and the experience of emotions are generated by neural activity from the same cortical areas that are responsible for these behaviors. The largest influence originates from a motor network that includes all 7 motor areas in the frontal lobe. These motor areas are involved in all aspects of skeleto-motor control, from response selection to motor preparation and movement execution. The motor areas provide a link between body movement and the modulation of stress. The cognitive and affective networks are located in regions of the cingulate cortex. They provide a link between how we think and feel and the function of the adrenal medulla. Together, the 3 networks can mediate the effects of stress and depression on organ function and provide a concrete neural substrate for some psychosomatic illnesses. In rats, cortical influences over the adrenal medulla and the kidney originate mainly from 2 motor areas and adjacent somatosensory cortex. The cognitive and affective networks present in monkeys are largely absent in rats. Thus, nonhuman primate research is essential to understand the neural substrate that links cognition and affect to the function of internal organs. These observations provide a network perspective on the neuroanatomical organization of the cortical influences over the sympathetic nervous system.

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New Mechanisms

8.2. Cognitive dysfunction in mice lacking proper glucocorticoid receptor dimerization

Van Looveren K, Van Boxelaere M, Callaerts-Vegh Z, Libert C

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https://pubmed.ncbi.nlm.nih.gov/31869387/

Glucocorticoids (cortisol in humans, corticosterone in most rodents) are steroid hormones secreted by the adrenal cortex into the systemic circulation in an ultradian, circadian, and stress-related fashion under the control of the hypothalamic-pituitary-adrenal (HPA) axis. These cholesterol-derived molecules participate in the physiologic function of almost all organs and play an important role in the maintenance of resting and stress-related homeostasis (**1, 2**). At the cellular level, the actions of glucocorticoids are mediated by the glucocorticoid receptor (GR), which is encoded by the NR3C1 gene and belongs to the steroid/thyroid/retinoic

acid nuclear receptor superfamily of transcription factors (**3, 4**). Consistent with the pleiotropic effects of glucocorticoids, the hGR is ubiquitously expressed in all human tissues and cells and is necessary for life after birth (**5**). GR functions as a ligand-activated transcription factor that influences the transcription rate of numerous genes through well-described genomic and less well-defined nongenomic actions (**3-5**). GR regulates gene expression by either transcriptional activation (transactivation) or transcriptional repression (transrepression). Prior to binding to glucocorticoids, the hGR resides mostly in the cytoplasm of cells as part of a large multiprotein complex. Upon ligand-induced activation, the GR undergoes conformational changes that result in dissociation from this multiprotein complex and translocation into the nucleus, where it binds to glucocorticoid-response elements (GREs) in the promoter region of target genes. The ligand-activated GR can also modulate gene expression independently of DNA-binding, by interacting with other transcription factors, such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), p53 and signal transducers and activators of transcription (STATs) (**3, 4**).

In this study, the authors created $GR^{dim/dim}$ mutant mice with a point mutation (A458T) in the DNA binding domain (DBD) of the GR, in which GR dimerization is impaired and DNA binding is less robust, however, the monomeric GR functions remain intact. Subsequently, the authors evaluated the effect of poor GR dimerization on hippocampus-dependent cognition, as well as on exploration and emotional behavior under baseline and chronically increased stress hormone levels. Thus, they analyzed the behavioral phenotype of female $GR^{dim/dim}$ mice under baseline and under chronically elevated corticosterone levels compared with their wild-type littermates. Specifically, they compared behavior spanning general activity, emotional behavior and cognitive performance under these two conditions. In addition, they analysed expression levels of selected genes that have been shown to be differentially regulated by i) corticosterone and stress, (ii) in major depressive disorders in the hippocampus, and (iii) play an important role in cognitive processes especially in learning impairments reported in preclinical models and clinical population of depression. They found that $GR^{dim/dim}$ mice did not behave differently from $GR^{wt/wt}$ littermates under baseline conditions. However, after chronic elevation of stress hormone levels, $GR^{dim/dim}$ mice displayed impaired hippocampus-dependent memory compared to $GR^{wt/wt}$ mice, which correlated with differential expression of hippocampal Bdnf/TrkB and Fkbp5. These data suggest that the phenotypic problem in maintaining a good memory after chronic stress in $GR^{dim/dim}$ mice is not reflected in the lack of repression of these genes in the hippocampus, and it may be that other genes, not tested here or as yet not recognized, are regulated by GR dimers in this specific control.

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Important for Clinical Practice

8.3. Frequency and incidence of Carney complex manifestations: A prospective multicenter study with a three-year follow-up

Espiard S, Vantyghem MC, Assié G, Cardot-Bauters C, Raverot G, Brucker-Davis F, Archambeaud-Mouveroux F, Lefebvre H, Nunes ML, Tabarin A, Lienhardt A, Chabre O, Houang M, Bottineau M, Stroër S, Groussin L, Guignat L, Cabanes L, Feydy A, Bonnet F, North MO, Dupin N, Grabar S, Duboc D, Bertherat J

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Carney complex (CNC) is a rare multiple endocrine and nonendocrine neoplasia syndrome, described in 1985 by J. Aidan Carney (**1**). The diagnostic criteria include dermatologic manifestations (spotty skin pigmentation with typical periorificial distribution [known as lentigines], cutaneous myxomas), cardiac myxoma, primary pigmented nodular adrenal disease (PPNAD) causing adrenal Cushing, acromegaly due to growth hormone (GH)-producing pituitary adenoma, breast myxomatosis and breast ductal adenoma, large cell calcified Sertoli cell tumors (LCCSCT), thyroid carcinoma or multiple nodules, psammomatous melanotic schwannoma (PMS) and osteochondromyxoma (**2**). The diagnosis is based on the presence of 2 or more manifestations. In addition, other endocrine manifestations suggestive of CNC include hyperprolactinemia, elevated IGF-I concentrations or inadequate GH suppression following an oral glucose tolerance test, or paradoxical GH responses to thyrotropin releasing hormone (TRH) stimulation in the absence of clinical acromegaly, blue nevi, or a single thyroid nodule. Non-endocrine manifestations possibly associated with CNC have been described, such as cardiomyopathy, colonic polyps, bronchogenic cysts, hepatocellular adenoma and carcinoma, colonic or gastric carcinomas, retroperitoneal fibrous histiocytomas and pancreatic tumors (**2, 3**). Approximately 30% of cases are sporadic and the remaining 70% are dominantly inherited (**2**). The causal gene for CNC was identified in 2000 as the tumor suppressor gene *PRKAR1A,* located at 17q22-24 and encoding for the regulatory subunit type 1 alpha of the protein kinase A (**4, 5**). Affected patients harbor a germline heterozygous *PRKAR1A* alteration, more often a mutation rather than an intragenic deletion, with large deletions being rarely observed.

In the present multicenter national prospective study, the authors evaluated for the first time the frequency, incidence, and evolution of the different manifestations of the condition in patients with genetically confirmed CNC during a 3-year follow-up period. The cohort included 70 patients (50 female/20 male, mean age 35.4 $\pm$ 16.7 years, 81% carrying *PRKAR1A* mutation). The initial investigations allowed identification of several manifestations. At the end of the 3-year follow-up, the newly diagnosed manifestations of the disease were subclinical acromegaly in 6 patients, bilateral testicular calcifications in 1 patient, and cardiac myxomas in 2 patients. Recurrences of cardiac myxomas were diagnosed in 4 patients during the 3-year follow-up study period. Asymptomatic abnormalities of the corticotroph and somatotroph axis that did not meet the criteria of PPNAD and acromegaly were observed in 11.4% and 30% of the patients, respectively. Patients carrying the *PRKAR1A* c.709- 7del6 mutation had a mild phenotype.

This study underlines the importance of a systematic follow-up of patients with CNC, including biannual assessment for cardiac myxomas. By contrast, regular screening for the other manifestations after a first extensive workup could be spread out, leading to a lighter and more acceptable follow-up schedule for patients.

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8.4. Long-term outcome of primary bilateral macronodular adrenocortical hyperplasia after unilateral adrenalectomy

Osswald A, Quinkler M, Di Dalmazi G, Deutschbein T, Rubinstein G, Ritzel K, Zopp S, Bertherat J, Beuschlein F, Reincke M
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Endogenous Cushing syndrome (CS) is a severe condition characterized by excessive glucocorticoid production (**1**). In 20% of cases, cortisol is secreted autonomously by the adrenal cortex (**2**). Adrenal CS is mostly caused by unilateral cortisol-producing adrenal adenomas (CPAs) and less frequently by cortisol-secreting carcinomas. Very rare causes of adrenal CS include primary bilateral macronodular adrenocortical hyperplasia

(PBMAH), bilateral CPAs, and primary pigmented micronodular adrenal disease (**3, 4**). PBMAH presents on imaging with characteristic multiple bilateral macronodules (>10 mm) with hyperplasia and/or internodular atrophy (**5**). In most cases, PBMAH is a sporadic disorder, although familial cases have been described (**6**). The treatment of choice to control hypercortisolism in patients with PBMAH is bilateral adrenalectomy, which inevitably results in lifelong glucocorticoid dependence and risk of adrenal crisis (**6**). To avoid the induction of adrenal insufficiency, resection of only one adrenal gland has been advocated. Several recent studies have reported clinical and biochemical improvement after unilateral adrenalectomy (**7, 8**). However, the number of documented patients and associated follow-up time is limited.

Here, Andrea Osswald and colleagues report the largest series of 25 patients with PBMAH after unilateral adrenalectomy (unilat-ADX-PBMAH) with analysis of long-term results regarding biochemical and clinical outcomes, as well as morbidity and mortality. They compare outcomes their to 9 patients with PBMAH treated with bilateral adrenalectomy (bilat-ADX-PBMAH) and also 39 patients with CPA treated with unilateral adrenalectomy (unilat-ADX-CPA). The baseline clinical and biochemical parameters were comparable in patients with unilat-ADX-PBMAH, bilat-ADX-PBMAH, and unilat-ADX-CPA. Immediately after surgery, 84% of the patients with unilat-ADX-PBMAH experienced initial remission of Cushing syndrome, however at last follow-up (median, 50 months), only 32% of these patients remained biochemically controlled, which was lower than patients in the other two groups ($P < 0.001$). Adrenalectomy of the contralateral side had to be performed in 12% of patients with initial unilat-ADX-PBMAH. Three of 20 patients with unilat-ADX-PBMAH (15%) died during follow-up; no deaths occurred in the other two groups ($P < 0.008$). Deaths occurred exclusively in patients who were not biochemically controlled after unilateral ADX and were presumed to be of CS-related causes.

These findings suggest that unilateral adrenalectomy of patients with PBMAH leads to initial clinical remission and a lower incidence of adrenal crisis, but subsequently leads to less sufficient biochemical control of hypercortisolism, potentially leading to higher mortality.

This is the first study to provide data on the long-term outcome and mortality of patients with PBMAH after adrenalectomy in comparison with a reference patient group. The decision to accept mild persistent hypercortisolism against lifelong glucocorticoid dependence is justifiable as long as it does not lead to enhanced mortality. In view of the presented data, unilateral adrenalectomy should be reserved for patients with PBMAH with asymmetric hyperplasia or mild cortisol secretion. Although these findings do not allow to define a safe 24-hour urinary free cortisol (UFC) cut-off, persistently elevated levels of more than two times the normal upper limit should lead to additional measures to control hypercortisolism, such as adrenostatic treatment or (subtotal) contralateral adrenal surgery.

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8.5. Exposure to glucocorticoids in the first part of fetal life is associated with insulin secretory defect in adult humans

Riveline JP, Baz B, Nguewa JL, Vidal-Trecan T, Ibrahim F, Boudou P, Vicaut E, Brac de la Perrière A, Fetita S, Bréant B, Blondeau B, Tardy-Guidollet V, Morel Y, Gautier JF

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Prenatal treatment with glucocorticoids (GC) is used in several clinical indications. However, the long-term outcome on offspring metabolic, somatic and cognitive health has raised significant concern. In rodents, high glucocorticoid levels inhibit development of beta cells during fetal life and lead to insulin deficiency in adulthood. To investigate whether similar phenomena occur in humans, Jean-Pierre Riveline and colleagues compared the beta-cell function of adults exposed to glucocorticoids during the first part of fetal life with that of nonexposed subjects. The authors assessed beta cell function using the oral glucose tolerance test (OGTT) and the graded intravenous glucose and arginine tests in adults ($n = 16$) treated with dexamethasone (DEX) during the first trimester of fetal life. They were compared to untreated healthy participants ($n = 16$) with normal glucose tolerance, who were matched for age, sex, and body mass index. Subjects exposed to glucocorticoids were born to mothers who had been treated with DEX 1 – 1.5 mg/day from the sixth gestational week (GW) to prevent genital virilization in children at risk of 21-hydroxylase deficiency (**1**), but stopped DEX before the 18th GW following negative genotyping of the fetus. Insulin sensitivity was determined by the hyperinsulinemic-euglycemic-clamp.

Although there was no evidence of altered insulin sensitivity, DEX exposed participants showed significantly lower insulin secretion both on OGTT and arginine tests, indicating lower beta cell function. These findings confirm that GC exposure during the first part of fetal life is associated with a decrease in insulin secretion in response to IV glucose and arginine at adult age. The present human model of GC exposure is unique, since exposure was limited to the first part of gestation, a period corresponding to the differentiation of beta cells with low risk of confounding factors, such as gestational diabetes and hypertension. DEX, a fluorinated synthetic GC used in this model, evades inactivation by placental 11-beta-hydroxysteroid dehydrogenase type 2, which oxidizes cortisol to the inactive form cortisone (**2**). Therefore, when given to pregnant women, DEX enters directly into fetal circulation in its active form. This study provides the first evidence that prenatal treatment with DEX during the first part of fetal life is associated with decreased beta-cell function at adult age and may lead to glucose intolerance later in life. This new evidence adds to other existing concerns regarding the long-term safety of prenatal DEX treatment in CAH.

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8.6. Cognitive function of children and adolescents with congenital adrenal hyperplasia: Importance of early diagnosis

Messina V, Karlsson L, Hirvikoski T, Nordenström A, Lajic S

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Classical congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency requires life-long glucocorticoid (GC) replacement therapy. Previous studies on general cognitive ability in patients with CAH have been conflicting, and the majority focused on intelligence in adult patients. Some studies have reported normal intelligence in both women and men with CAH (**1**), whereas others have noted impaired intelligence (**2, 3**) and memory deficit (**4**) in children and adults with CAH. A recent investigation demonstrated that patients with CAH have impaired spatial perception and diminished quantitative abilities, most probably due to altered

executive functioning (**5-7**). On the contrary, others have reported superior performance on spatial tests in women with CAH (**8**). In addition to the effects of postnatal GC, an excess of prenatal androgens may be associated with permanent changes in brain structures, organization, or function (**9**). Prenatal treatment with dexamethasone (DEX) may also affect cognitive functions, given that poorer cognitive abilities were shown in DEX-treated women with CAH compared with women with CAH who received no prenatal DEX therapy (**7**).

This study evaluated the cognitive outcome in children with CAH and the impact of early diagnosis with neonatal screening. The effect of postnatal and prenatal GC treatment on cognitive function was evaluated. Forty-three children with CAH (mean age: 11.5 years; 23 girls, 20 boys; 11 prenatally treated with DEX) and 52 matched control children (mean age 10.7 years) were studied prospectively. All but one patient were identified by neonatal screening and were treated with hydrocortisone (HC). Twelve patients had a null genotype and 30 patients a non-null genotype. Only 2 patients with the salt-wasting phenotype exhibited sodium concentrations <131 mmol/l at diagnosis. In general, all patients had good metabolic control at the time of cognitive function assessment. Their cognitive abilities were assessed with standardized neuropsychological tests (Wechsler scales, Span Board Test, Stroop Interference Test, NEPSY list learning).

The results showed that children with CAH, who were diagnosed early via neonatal screening and treated with HC (mean dose 12.3 mg/m^2) to achieve a good metabolic control, had normal general intellectual ability including good executive functions, learning and memory. They performed at the same level as their matched controls from the general population. In addition, children with CAH who were treated with DEX prenatally performed equally well on most measures compared to children not treated with DEX, except that girls treated during the entire gestational period with DEX had lower scores on WISC-III Vocabulary (a proxy for general intellectual ability). However, due to low sample size of DEX treated cases (only 6 girls) the results should be interpreted with caution and need replication in larger studies. These findings confirm that children and adolescents with CAH, who were diagnosed early via neonatal screening and treated with HC, have normal psychometric intelligence and executive functions.

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8.7. Alternative pathway androgen biosynthesis and human fetal female virilization

Reisch N, Taylor AE, Nogueira EF, Asby DJ, Dhir V, Berry A, Krone N, Auchus RJ, Shackleton CHL, Hanley NA, Arlt W
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Androgen biosynthesis in the human fetus proceeds through the adrenal sex steroid precursor dehydroepiandrosterone (DHEA), which is converted to testosterone in the gonads, followed by further

activation to 5α-dihydrotestosterone (DHT) in genital skin, thereby facilitating male external genital differentiation (**1**). Congenital adrenal hyperplasia (CAH) due to P450 oxidoreductase deficiency (PORD) is an inborn disorder that results in disrupted DHEA biosynthesis, leading to undervirilization of affected boys. However, some affected girls present with severe genital virilization at birth, despite low circulating androgen concentrations (**2-4**). Here, Reisch *et al*. hypothesized that this might be explained by a prenatally active, alternative biosynthesis pathway to 5α-DHT. They investigated the presence of an alternative androgen-producing pathway in human fetuses.

Human fetal organ explant cultures of adrenal, gonad, and genital skin tissue collected at 6 to 10 weeks post conception from 30 fetuses were incubated with steroid precursors, and conversion products were measured using LC–MS/MS. In addition, urinary steroid profiles from three neonates with PORD were analysed and compared with profiles of 9 healthy controls during the first 90 days after birth. They found that explant incubations with female tissues convert some 17OHP to androstenedione via the classical pathway in the adrenal, ovary and genital skin, but testosterone was produced in only 2 of 9 female genital skin incubations, and not at all in adrenals and gonads. By contrast, all female tissues converted 17OHP to DHT via the proposed alternative androgen producing pathway and its intermediates. In male tissues, 17OHP was converted along the classical pathway to testosterone and in genital skin to DHT. In male adrenals, testis and genital skin, 17OHP was stepwise converted also via the alternative pathway to DHT. Confirmation with expression of the necessary steroidogenic enzymes was consistent with the above data. In addition, the authors found androgen receptor (AR) expression in male and female genital skin using immunohistochemistry, and demonstrated that both 5α-DHT and adrenal explant culture supernatant induce nuclear translocation of the androgen receptor in female genital skin primary cultures. In 3 neonates with a 46, XY karyotype and a PORD mutation known to cause normal male external genitalia, but female virilization in case of a girl, the authors identified urinary metabolites consistent with the adrenal gland being the major site of the alternative androgen producing pathway.

In conclusion, these data provide *in vitro*, *ex vivo*, and *in vivo* evidence for the existence and activity of an alternative pathway for the synthesis of the most potent androgen, DHT, during early human development. They demonstrate that, through co-operation of an adrenogenital steroidogenic unit, the alternative androgen pathway yields active androgen synthesis in the female fetus, with excess activity driving female virilisation in CAH due to PORD. Given that the alternative pathway substrate 17OHP also accumulates in 21-hydroxylase deficiency, it is conceivable that alternative pathway androgens contribute to prenatal virilization in this common form of CAH.

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8.8. Efficacy and safety of levoketoconazole in the treatment of endogenous Cushing's syndrome (SONICS): A phase 3, multicentre, open-label, single-arm trial

Fleseriu M, Pivonello R, Elenkova A, Salvatori R, Auchus RJ, Feelders RA, Geer EB, Greenman Y, Witek P, Cohen F, Biller BMK
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Endogenous Cushing's syndrome is a rare, serious endocrine condition characterized by chronic overproduction of cortisol (**1**). It is most often caused by a pituitary adenoma (i.e. Cushing's disease), while other causes include ectopic ACTH secretion or primary adrenal neoplasia (**2**). Patients with Cushing's syndrome have increased mortality, mainly as a result of cardiovascular complications. Surgical removal of the underlying lesion is first-line therapy, sometimes preceded by preoperative medical treatment (**1–3**). Medical treatments aim to suppress the excessive ACTH or cortisol production or to decrease cortisol activity (**1**). Few medications have been evaluated in well-designed prospective studies. Ketoconazole, an azole antifungal drug that inhibits steroidogenesis, is approved for the treatment of endogenous Cushing's syndrome in Europe and is used off-label in the USA. However, although ketoconazole can reduce urinary free cortisol concentrations in patients with endogenous Cushing's syndrome, its use is often limited by side-effects, such as hepatotoxicity and QT interval prolongation, as well as the potential for drug interactions. Levoketoconazole, the 2S,4R enantiomer of ketoconazole, decreases cortisol synthesis via potent inhibition of several enzymes in the steroidogenic pathway and might have lower risk of hepatotoxicity and an improved side-effect profile relative to ketoconazole (**4**).

This phase 3 multicentre, single-arm, non-randomised, open-label study (SONICS, Registration no: NCT01838551) investigated the efficacy, safety and tolerability of twice-daily oral levoketoconazole in patients with endogenous Cushing's syndrome. Ninety four patients with Cushing's syndrome [80 (85%) with pituitary Cushing's disease] were studied prospectively and received at least one dose of levoketoconazole. Patients were treated with oral levoketoconazole in a 2–21 week incremental dose-titration phase starting at 150 mg twice daily (150 mg increments until mean 24-hour UFC normalization to a maximum of 600 mg twice daily) and a 6-month maintenance phase. Levoketoconazole normalized 24-h UFC concentrations after 6 months of maintenance therapy (without a dose increase after establishing a therapeutic dose) in 31% of patients. In addition, it was associated with improvements in cardiovascular disease risk factors, as well as normalization of cortisol concentrations in some patients. Levoketoconazole was generally well tolerated, with no unexpected safety signals identified.

Therefore, levoketoconazole might represent a useful therapeutic option for the medical treatment of Cushing's syndrome. Although Cushing's syndrome is rare in children and adolescents, levoketoconazole might represent a useful therapeutic option when surgery is delayed, contraindicated or unsuccessful, given the reported acceptable safety and tolerability profile.

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8.9. Primary adrenal insufficiency: New genetic causes and their long-term consequences

Buonocore F, Achermann JC

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Primary adrenal insufficiency (PAI) is a relatively rare but potentially life?threatening condition that requires urgent diagnosis and treatment (**1**). Although the most common causes are congenital adrenal hyperplasia (CAH) in childhood and autoimmune adrenal insufficiency in adolescence and adulthood, there is an ever-expanding list of rare genetic causes (**2**). These genetic causes frequently have variable inheritance patterns, while some milder or non-classical forms of these conditions may present for the first time in adolescence or adulthood (**2**). In some situations, patients may have been labelled as having 'Addison's' disease and more detailed genetic investigations to find a specific cause have not been undertaken.

In this narrative review, the authors present the recent insights into the genetics and molecular mechanisms of rare forms of PAI and show how reaching a specific diagnosis benefit for management and long-term care. Specifically, they discuss the role of the nuclear receptors DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human adrenal and reproductive dysfunction (**3, 4**); multisystem growth restriction syndromes due to gain-of-function in the growth repressors CDKN1C (IMAGE syndrome) and SAMD9 (MIRAGE syndrome), or loss of POLE1 (**5, 6, 7**); non-classical forms of STAR and P450scc/CYP11A1 insufficiency that present with a delayed-onset predominantly adrenal phenotype and represent a surprisingly prevalent cause of undiagnosed PAI or resembling familial glucocorticoid deficiency (FGD) (**8, 9**); and a new sphingolipidosis causing PAI due to defects in sphingosine-1-phosphate lyase-1 (SGPL1) (**10, 11**).

Reaching a genetic diagnosis of PAI in childhood can have important implications for counselling and management, while clinical monitoring for the emergence of potential associated features and devising of treatment strategies is of paramount importance in this diverse group of patients. Detecting affected family members before the onset of disease is also important. When presented with a child or young person with newly diagnosed adrenal insufficiency, several aspects of the history, clinical features or focused tests may give a clue to the underlying cause. The suggested approach is for single gene testing in conditions such as 21-hydroxylase deficiency or X-linked adrenoleukodystrophy, where there are diagnostic biochemical markers. Focused panels are also available that include many of the genetic causes of PAI. Ultimately, in the future, whole exome or whole genome sequiencing with targeted analysis of relevant genes will likely be the best approach, as all known genes can be reviewed initially and, if the cause is not found, data can subsequently be reanalysed as new genetic causes are identified or the relevance becomes established of intronic changes that may affect splicing. In addition, knowledge of geographical hotspots is potentially important to implement targeted genetic testing quickly and cost-effectively, especially in resource-limited settings. The authors finally offer insights in gene discovery approaches using genome wide analysis that have the potential to give better understanding of human adrenal development and function.

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New Hope

8.10. Combined gestational age- and birth weight-adjusted cutoffs for newborn screening of congenital adrenal hyperplasia

Pode-Shakked N, Blau A, Pode-Shakked B, Tiosano D, Weintrob N, Eyal O, Zung A, Levy-Khademi F, Tenenbaum-Rakover Y, Zangen D, Gillis D, Pinhas-Hamiel O, Loewenthal N, de Vries L, Landau Z, Rachmiel M, Abu-Libdeh A, Eliakim A, Strich D, Koren I, German A, Sack J, Almashanu S

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PMID: 30865229
https://pubmed.ncbi.nlm.nih.gov/30865229/

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency was among the first genetic disorders included in newborn screening (NBS) programs worldwide, based on 17-hydroxyprogesterone (17OHP) concentrations determined in dried blood spots (**1**). However, the success of NBS for CAH is limited by high false positive rates, especially in preterm and low-birth weight infants (**1, 2**). The aim of this study was to establish cut-off values adjusted for both gestational age (GA) and birth-weight (BW) to reduce false positive rates. The authors conducted a cross-sectional, population-based study that summarized 10 years of experience of the Israeli NBS program for CAH. Multi-tiered 17-OHP cut-off values were stratified according to both BW and GA.

Between the years of 2008 and 2017 a total of 1 387 132 newborns were included in the NBS program of Israel and 88 newborns were diagnosed with CAH; 84/88 by NBS. Blood sampling occurred at 36 to 72 hours after birth. 17OHP concentrations (nmol/l) were determined by time-resolved fluoro-immunoassay using the 112 AutoDELFIA neonatal 17OHP kit (PerkinElmer). The combined parameters-adjusted approach significantly reduced the recall false positive rate to 0.03% (425 false positive cases; recall sample found within normal NBS limits) and increased the positive predictive value to 16.5%. The sensitivity among those referred for immediate attention increased significantly (94%). There were four false negative cases (sensitivity, 95.4%), all ultimately diagnosed as simple-virilizing CAH. The sensitivity and specificity were 95.4% and 99.9%, respectively, and the percentage of true-positive cases from all newborns referred for evaluation following a positive NBS result was 96%. These results are similar to those by Gidlöf *et al* (**3**) in Sweden, where only GA was used to determine cut-off values. Therefore, the use of cut-off values of 17OHP concentrations based on a combination of GA and BW for newborn screening of classical CAH is safe and more efficient than either factor alone. This strategy lowers false positive rates and reduces the costs and emotional strain that accompany unnecessary recalls and referrals of false positive cases.

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8.11. Carriers of a classic CYP21A2 mutation have reduced mortality: A population-based national cohort study

Nordenström A, Svensson J, Lajic S, Frisén L, Nordenskjöld A, Norrby C, Almqvist C, Falhammar H
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https://pubmed.ncbi.nlm.nih.gov/31393570/

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency has an incidence of 1 in 10 000 to 20 000 in most populations. It is one of the most common monogenic autosomal recessive disorders (**1**). It has been suggested that the condition is common because it may confer a survival advantage to carriers, 1 in 15 000 in most populations (**2**). The hypothesized mechanism is that the increased and more prompt cortisol response to stress (due to an upregulated HPA-axis) seen among *CYP21A2* mutation carriers enables a more rapid return to homeostasis. Previously, it was shown that *CYP21A2* mutation carriers (parents of children with CAH) are more likely to have a psychiatric diagnosis both before and after the birth of the child with CAH, indicating less vulnerability to stress (**3**).

Here, Nordenström *et al*. investigated the mortality and the cause of death in obligate *CYP21A2* mutation carriers (i.e. parents of children with CAH). In particular, infection as the cause of death was investigated, since infectious disease from an evolutionary perspective has been a threat to mankind, and a more effective stress response or resistance to infections could confer a survival advantage explaining the high percentage of *CYP21A2* mutation carriers in most populations.

A total of 1143 obligate carriers of a *CYP21A2* mutation were compared with population controls matched for sex and age (100 controls per carrier). Mortality and cause of death were investigated via the Swedish Cause of Death Registry. All-cause mortality was lower in *CYP21A2* mutation carriers than in the general population (Hazard Ratio, HR 0.79, $P=0.002$), with a slightly stronger protective effect in carriers of more severe mutations (HR 0.65, $P=0.003$) than carriers of simple-virilizing CAH mutations (HR 0.71, $P=0.02$). Parents of children with non-classical CAH or CAH of unknown severity did not differ from population controls, indicating that the severity of the mutation (and hence the cortisol response) may be important. In all groups, women had a lower HR than men, as seen in the general population. Risk of death from infection was lower in carriers (HR 0.65, $P<0.01$), in particular death from pneumonia (HR 0.22, $P=0.03$), but not from sepsis, erysipelas, or hepatitis. In addition, no difference in deaths from cancers or cardiovascular disease was detected.

In summary, the more efficient cortisol response to somatic and psychological stress in carriers of a *CYP21A2* mutation may represent a survival advantage, in particular from death caused by infections. This protection may contribute to the high incidence of *CYP21A2* mutation carriers seen worldwide.

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8.12. HSD3B1 genotype identifies glucocorticoid responsiveness in severe asthma

Zein J, Gaston B, Bazeley P, DeBoer MD, Igo RP Jr, Bleecker ER, Meyers D, Comhair S, Marozkina NV, Cotton C, Patel M, Alyamani M, Xu W, Busse WW, Calhoun WJ, Ortega V, Hawkins GA, Castro M, Chung KF, Fahy JV, Fitzpatrick AM, Israel E, Jarjour NN, Levy B, Mauger DT, Moore WC, Noel P, Peters SP, Teague WG, Wenzel SE, Erzurum SC, Sharifi N
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PMID: 31932420
https://pubmed.ncbi.nlm.nih.gov/31932420/

Since their discovery ~70 years ago, glucocorticoids (GC) have been widely used to elicit a systemic anti-inflammatory response, and currently play a major role in the treatment of asthma and other inflammatory diseases (**1**). However, unresponsiveness to GC in some individuals is a major limitation in the treatment of asthma, and the mechanisms underlying this clinical entity are not fully elucidated (**1**). Indeed, severe asthma is generally defined as asthma that remains symptomatic despite high-dose inhaled GC and/or systemic GC therapy. GCs inhibit production of adrenal androgens, which may have potential benefits in asthma. The enzyme 3β-hydroxysteroid dehydrogenase-1 (3β-HSD1) catalyzes the peripheral conversion of adrenal dehydroepiandrosterone (DHEA) to more potent androgens. At a missense polymorphism in its gene *HSD3B1,* the 1245A restrictive allele limits DHEA metabolism to potent androgens, whereas the 1245C permissive allele increases conversion. The 1245A restrictive genotype is associated with GC resistance, and this effect appears to be driven by GC suppression of 3β-HSD1 substrate (2). In population studies, animal models and cell culture experiments, androgens are associated with several benefits in asthma, however, the role of GC-induced androgen suppression in the pathophysiology of severe, GC-resistant asthma in humans is not established (**3, 4**).

In this retrospective cohort study ($n=318$), Zein *et al*. tested the hypothesis that the restrictive *HSD3B1* allele that limits DHEA-S conversion to potent androgens impairs pulmonary function specifically when GC treatment suppresses adrenal DHEA-S production, limiting substrate availability for 3β-HSD1 and possibly providing a mechanistic explanation for GC-resistant severe asthma. In the Severe Asthma Research Program (SARP) III cohort, they tested the association between DHEA-S and percentage predicted forced expiratory volume in 1 s (FEV1PP). DHEA-S levels were positively associated with FEV1PP, and these levels were suppressed on those with GC treatment. Among homozygotes for the *HSD3B1*(1245A) restrictive genotype, GC patients had lower FEV1PP than noGC patients (54.3% vs. 75.1%; $P<0.001$). Among homozygotes for the *HSD3B1*(1245C) permissive genotype, there was no difference in FEV1PP between GC vs. noGC patients (73.4% vs. 78.9%; $P=0.39$). The adrenal restrictive HSD3B1(1245C) genotype was associated with GC resistance and this effect appeared to be driven by GC suppression of 3β-HSD1 substrate.

These data suggest the possibility that *HSD3B1* genotype is predictive of which patients might benefit from systemic GC therapy and, for those who are resistant, who might benefit from androgen replacement in severe asthma. Furthermore, this study provides evidence that implicate an androgen synthesis variant in GC-resistance in asthma. In addition, it demonstrates an adverse consequence of adrenal androgen suppression with GC therapy, showing a positive relationship between circulating adrenal DHEA-S levels and lung function. These data, although limited by the inclusion only of Caucasian patients, provide a potential mechanism for the gender and pubertal maturation differences observed in asthma or other inflammatory diseases (**5, 6**).

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8.13. Adverse childhood experiences, DNA methylation age acceleration, and cortisol in UK children: a prospective population-based cohort study

Rosalind Tang, Laura D Howe, Matthew Suderman, Caroline L Relton, Andrew A Crawford, Lotte C Houtepen

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A large body of evidence has documented the long-term consequences of adverse childhood experiences (ACEs) on social and health outcomes in later life (**1**). However, the underlying mechanisms remain unclear. Epigenetic mechanisms may explain the lasting effects of early life adversity (**2**). 'Epigenetic clocks' are sets of DNA methylation (DNAm) markers (CpG sites) that accurately predict chronological aging (**3**) and higher DNAm-predicted age relative to chronological age [DNAm age acceleration (AA)] is associated with higher risks for cardiovascular disease, cancer, and all-cause mortality (**4**). DNAm AA has also been associated with childhood exposure to adversity, including parental depression, violence, sexual abuse, low socioeconomic status, and cumulative exposure to sexual abuse, physical abuse, or neglect (**5**). Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is a potential mediator between childhood adversity, epigenetics, and poor health in later life. DNAm AA has also been associated with elevated diurnal and baseline salivary cortisol in adolescents.

Here, the authors investigated the associations of individual types of ACEs, as well as cumulative ACE exposure, with DNAm AA and plasma cortisol concentrations in the prospective population-based UK Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort ($n = 974$). Cumulative ACE exposure, emotional abuse, and physical abuse between age 0 and 14 years were each associated with older DNAm AA at age 17 years in girls but not in boys. These findings indicate a sex dimorphic epigenetic response to early life adversities, where girls appear to be more vulnerable than boys.

The present study adds to a growing body of evidence that suggest that effects from early life adversities may affect the individual later in life and that epigenetic mechanisms may partly explain how these effects may persist throughout the life-span. Understanding the mechanisms by which epigenetic mechanisms mediate these effects is important in order to determine how to ameliorate or prevent long-term negative effects.

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8.14. Brain differences in the prefrontal cortex, amygdala, and hippocampus in youth with congenital adrenal hyperplasia

Herting MM, Azad A, Kim R, Tyszka JM, Geffner ME, Kim MS

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Classical congenital adrenal hyperplasia (CAH) is characterized by impaired glucocorticoid, and often also mineralocorticoid, secretion and increased adrenal androgen production (**1**). Given the widespread expression of androgen and glucocorticoid receptors throughout the brain (**2, 3**), there has been a growing interest in

understanding how hormonal imbalances related to CAH may impact distinct subregions of the developing brain (**4**).

Here, the authors characterized brain gray matter morphology in the prefrontal cortex and subregions of the amygdala and hippocampus using structural magnetic resonance imaging in young patients with CAH ($n=27$, mean age 12.6 years) compared with unaffected controls ($n=35$, 13 years). Patients with CAH had smaller intracranial volumes and larger cerebrospinal fluid volumes compared with controls. Patients with CAH showed smaller volumes of the bilateral superior frontal cortex, bilateral caudal middle frontal cortex, and left rostral middle frontal cortex, as well as smaller left hippocampus and several subregions of the amygdala. The results cannot be inferred to be directly associated with differences in cognition or behaviour, however, they highlight areas for possible future research.

While the clinical care of patients with CAH has improved over the years, long-term negative effects from their life-long treatment with glucocorticoids remain a concern. Identifying long-term sequelae and their contributing factors is important to optimize treatment. This study demonstrates that young CAH patients have reduced intracranial volume as well as regional volumes of the prefrontal cortex, amygdala and hippocampus. It significantly adds to the evidence for long-term effects on brain morphology in patients with CAH, and raises the question whether differences in clinical care might possibly contribute to these differences.

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8.15. Altered gray matter structure and white matter microstructure in patients with congenital adrenal hyperplasia: Relevance for working memory performance

Van't Westeinde A, Karlsson L, Thomsen Sandberg M, Nordenström A, Padilla N, Lajic S

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Congenital adrenal hyperplasia (CAH), most commonly caused by 21-hydroxylase deficiency, is an autosomal recessively inherited life-threatening impairment in cortisol and, in the severe salt wasting form, aldosterone synthesis. The implementation of neonatal screening programs for CAH and the continuous improvement in its clinical care has led to CAH being now a lifelong chronic disease. However, over time patients with CAH are at risk of metabolic and cognitive sequelae.

Here, the authors performed an observational study using structural magnetic resonance imaging in adolescents and young adults with CAH in order to investigate possible alterations in brain morphology and their associations with genotype/phenotype, cognition and behaviour, as well as DNA methylation. Patients with CAH ($n=37$, mean age 22 years) were compared with sex- and age-matched population controls ($n=43$). They also included a small group of patients prenatally treated with dexamethasone ($n=8$). The major findings were that CAH patients have smaller whole brain volume, as well as altered structures of the prefrontal, parietal, and superior occipital cortex, areas that are hubs of the visuospatial working memory and default mode networks. Interestingly, these structural alterations correlated with visuospatial working memory performance, and CAH patients performed worse on this task. Finally, prenatal dexamethasone treatment was associated with smaller surface area and volume in parietal regions.

This study provides evidence that patients with CAH show alterations in brain structure, specifically in regions important for working memory performance, and these alterations might underlie the observed cognitive

deficits. Early diagnosis and optimal lifelong treatment with glucocorticoids are important factors that may ameliorate cognitive deficits and alterations in brain structure.

New Paradigms

8.16. GDF15 is elevated in conditions of glucocorticoid deficiency and is modulated by glucocorticoid replacement

Melvin A, Chantzichristos D, Kyle CJ, Mackenzie SD, Walker BR, Johannsson G, Stimson RH, O'Rahilly S

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GDF15 is a stress-induced hormone that acts in the hindbrain to activate neural circuits involved in aversive responses and reducing food intake and body weight in animal models (1). In humans, GDF15 is widely expressed, with highest concentrations seen in placental trophoblasts, followed by kidney, bladder, prostate, gastrointestinal, pancreatic, lung, liver, and adipose tissue (2). GDF15 expression is increased when the cellular integrated stress response (ISR) pathway is activated, suggesting that GDF15 represents an endocrine arm of the cellular ISR (3). Interestingly, GDF15 has been reported to have a diurnal rhythm with a peak at ~0000 h and nadir at ∼1200 h, but it is unknown whether this is entrained by the normal circadian rhythm of cortisol secretion (4).

This randomized cross-over, single-blind trial of subjects with Addison's disease tested whether circulating GDF15 concentrations are regulated by glucocorticoids and may contribute to the nausea, vomiting or anorexia symptoms frequently observed in adrenal insufficiency. GDF15 concentrations were elevated in untreated patients with primary adrenal insufficiency compared with matched healthy controls, and glucocorticoid replacement reduced these differences in a dose-dependent manner.

This study shows that GDF15 concentrations are negatively regulated by glucocorticoids. Further studies are needed on the causal relationships between glucocorticoids and GDF15 concentrations on symptoms of nausea, vomiting, anorexia, and weight loss in adrenal insufficiency, and also the typical increased appetite and weight gain in states of glucocorticoid excess.

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Food for Thought

8.17. Plasma renin measurements are unrelated to mineralocorticoid replacement dose in patients with primary adrenal insufficiency

Pofi R, Prete A, Thornton-Jones V, Bryce J, Ali SR, Faisal Ahmed S, Balsamo A, Baronio F, Cannuccia A, Guven A, Guran T, Darendeliler F, Higham C, Bonfig W, de Vries L, Bachega TASS, Miranda MC, Mendonca BB, Iotova V, Korbonits M, Krone NP, Krone R, Lenzi A, Arlt W, Ross RJ, Isidori AM, Tomlinson JW

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Mineralocorticoid (MC) replacement therapy along with glucocorticoid (GC) replacement is crucial to avoid life-threating adrenal crises in Addisońs disease (AD) and in classical CAH (1–3). MC is administered with the aim of achieving plasma renin concentration (PRC) within the upper limit of the reference range. Taking into account the complex regulation of PRC, this study explored the relationship between MC dose regimens and clinical and biochemical variables in clinical practice to determine whether those variables can be used to guide titration of MC dosing.

This retrospective observational study used data from the International CAH Registry (I-CAH) collected from 1982 to 2018, and data from local adrenal patient databases. After exclusions, data were available on 735 visits in 243 patients (204 SW-CAH, 39 AD). Seven variables were included in the multivariate models: serum sodium, serum potassium, mean arterial blood pressure (MAP), PRC, MC dose, age and body mass index (BMI). PRC levels were categorized as low, normal, or high according to the local reference ranges.

In univariate analyses, a positive relationship was observed between MC dose and PRC in adults and children. In children, but not in adults, a positive relationship was also observed between MC dose and BP. However, in multiple regression models, sodium was the only factor that predicted PRC in adults. In summary, these findings suggest that MC dose titration should be based not only on PRC normalization, but also on clinical data, such as BP and electrolyte concentration.

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8.18. Interaction between hypothalamic–pituitary–adrenal axis genetic variation and maternal behavior in the prediction of amygdala connectivity in children

Pozzi E, Bousman CA, Simmons JG, Vijayakumar N, Schwartz O, Seal M, Yap MBH, Allen NB, Whittle SL

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A large body of evidence suggests that the early environment can influence the health and wellbeing in children later in life. Variability in susceptibility to environmental stimuli might be mediated by interactions between genetic variations and the environment. The hypothalamic-pituitary adrenal (HPA) axis is the key biological stress response system, and dysregulation of this system is associated with depressive symptoms. Genes that are involved in HPA axis function and regulation are candidates to explain individual differences in sensitivity.

Here, Pozzi *et al*. investigated the associations between HPA-related genetic variation, maternal parenting behaviors and amygdala activity and connectivity during implicit emotion processing in a community sample of 80 children (46 girls, mean age: 10.0 years). Maternal behaviour was observed during mother-child interactions. Children underwent functional magnetic resonance imaging while performing an implicit emotion-processing task, and mothers and children completed measures of child internalizing symptoms. An HPA genetic risk score was calculated by combining 10 genotypes at 4 genes (*FKBP5, CRHR1, NR3C2* and *NRC31*). The results showed that higher HPA genetic risk score was associated with greater amygdala-precuneus connectivity, which in turn was associated with greater child self-reported levels of depressive symptoms. The interaction between HPA genetic risk score and maternal negative behavior was associated with greater connectivity between the amygdala and the superior frontal gyrus, parietal operculum cortex, post-central gyrus and anterior cingulate cortex. Finally, the HPA genetic risk score neither alone nor in interaction with maternal parenting behavior was associated with amygdala reactivity.

Therefore, the authors demonstrated that genetic variation of the HPA axis directly, and in interaction with maternal negative parenting behavior, was associated with amygdala connectivity with regions involved in self-

referential processing and emotion regulation, and may confer risk for depressive symptoms via an effect on these circuits. These findings further elucidate the complexity between gene and environment interaction in connection with early life environment and the HPA axis, and may help identify risk factors for the development of depression.

Reviews

8.19. Fixing the broken clock in adrenal disorders: Focus on glucocorticoids and chronotherapy

Minnetti M, Hasenmajer V, Pofi R, Venneri MA, Alexandraki KI, Isidori AM

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Human physiology and behavior are adapted to daily environmental cycles by means of endogenous circadian clocks. Biological tasks, including cell proliferation, differentiation, energy storage and immune regulation, are preferentially confined to specific periods of the 24-h cycle. This circadian rhythm derives from the integration of many signals that shape the expression of clock-related genes in a 24-h cycle (**1**). Of interest, the fluctuations of cortisol and the expression of its receptor are crucial in modulating endogenous and exogenous signals and preparing the transition to activities confined to periods of light or darkness, acting (along with the autonomous nervous system) as a bridge between the suprachiasmatic master clock and almost all peripheral clocks (**2**).

In this narrative review, the authors describe and summarize the hierarchical control of the hypothalamic–pituitary–adrenal (HPA) axis circadian rhythm, the importance of peripheral mediators (nutrient intake, metabolism, inflammation and the autonomous nervous system) and discuss if the glucocorticoid effector system is the key component of this system as well as the interplay with other circadian systems (**3**). Relevant paradigms are taken from adrenal disorders that are considered models of HPA circadian rhythm disruption, particularly conditions of corticosteroid excess (hypercortisolism and adrenocortical tumors) and of corticosteroid insufficiency. These conditions, while seemingly opposite in their clinical presentation, share two common pathophysiological pathways that are described as sensitive to clock regulation: impaired immune function and increased atherosclerotic risk (**4, 5, 6, 7**). Of interest to Pediatric Endocrinology is the discussion on glucocorticoid-correcting medications, both replacement and reduction therapies, and the benefits of mimicking the normal daily peaks and troughs of cortisol in order to avoid exposure to glucocorticoids late in the evening and at night and to act in phase with the endogenous clock (**8, 9**).

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8.20 11-Oxygenated androgens in health and disease

Turcu AF, Rege J, Auchus RJ, Rainey WE
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The adrenal gland is the primary source of 11-oxygenated 19-carbon steroids, also termed 11-oxyandrogens, which have several roles in human physiology and disease. These include 11β-hydroxyandrostenedione (11OHA4), 11-Ketotestosterone, (downstream metabolite of 11OHA4, mostly produced in peripheral tissues), and its 5α-reduced product, 11-ketodihydrotestosterone (**1**). The 11-oxyandrogens are bioactive androgens, with potencies equivalent to those of testosterone and dihydrotestosterone, while concentrations of 11-oxyandrogens are elevated in several disorders of androgen excess (**2**).

This narrative review summarizes and discusses the physiology and pathology of adrenal C19 steroids, emphasizing the previously under-recognized roles of 11-oxyandrogens both as part of pathophysiological mechanisms as well as potential biomarkers, of disease control in conditions of androgen excess, such as Congenital Adrenal Hyperplasia (CAH). Of particular interest to Pediatric Endocrinologists is the potential of use as biomarkers of disease control in CAH because, in contrast to more extensively studied traditional androgens, circulating 11-oxyandrogens concentrations are highly adrenal specific and do not demonstrate variation due to age or steroid treatment (**3, 4**). Finally, the 11-oxyandrogens appear to be major contributors to the androgen excess of premature adrenarche (**5**).

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8.21. P450 oxidoreductase deficiency: A systematic review and meta-analysis of genotypes, phenotypes, and their relationships

Dean B, Chrisp GL, Quartararo M, Maguire AM, Hameed S, King BR, Munns CF, Torpy DJ, Falhammar H, Rushworth RL
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P450 oxidoreductase deficiency (PORD) is a rare autosomal recessive variant of congenital adrenal hyperplasia (CAH) arising from homozygous or compound heterozygous mutations to the gene encoding the enzyme P450 oxidoreductase (*POR*) (1). Patients with PORD have a range of skeletal malformations, glucocorticoid deficiency, and disorders of sexual development (2). The extent of skeletal malformations can be assessed using a scoring system (2).

This systematic review and meta-analysis summarises the skeletal malformations in a PORD cohort, as well as maternal virilization in pregnancy, adrenal insufficiency, hormone concentrations, blood pressure, and DSD,

with particular reference to genotype-phenotype relationships. Although skeletal malformations were identified in 84% of patients with PORD, no specific skeletal anomaly, or group of anomalies, was found to be characteristic of PORD and malformations can be either widespread or localized to particular parts of the skeleton. In addition, as previously reported, PORD patients were found to have a characteristic hormonal profile, where serum concentrations were typically elevated for progesterone, pregnenolone, 17OHP, corticosterone, and DOC, but were variable for DHEA, baseline cortisol, aldosterone, and androstenedione (3). Patients were typically normotensive at the time of investigation, but 20% of patients were mildly hypertensive, most likely secondary to elevated DOC, as previously suggested (2).

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8.22. Cushing syndrome: Old and new genes

Tatsi C, Flippo C, Stratakis CA

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Cushing's syndrome (CS) is the constellation of signs and symptoms resulting from excessive exposure to cortisol (1). While exogenous CS is relatively common, endogenous CS accounts for only 2.3 to 3.2 new cases per million per year; 10% of these present in children (2–4). Endogenous CS is caused by either ACTH-dependent sources (pituitary or ectopic) or ACTH-independent (adrenal) hypercortisolemia.

This review summarizes current knowledge regarding the genetics of CS, reviewing well-established genetic causes (e.g. mutations in *MEN1, GNAS* or *TP53*), as well as novel genes implicated in its pathogenesis. Moreover, the authors categorize known CS genes by disease types (Cushing's disease, ACTH-independent CS or ectopic CS) together with their accompanying phenotypic features. They also provide recommendations on genetic screening in CS cases and discuss the appropriate techniques during clinical management. They highlight the need for careful review of the patient's personal and family history for clues to a specific genetic syndrome, as well as the importance of understanding the diagnostic value of genetic testing and its value to guide treatment options. In summary, this article provides an overview of the various genetic mechanisms underlying hypercortisolemia in CS and provides helpful guidance on the use of genetic testing.

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9. Oncology and Chronic Disease

Stefania Pedicelli, Sara Ciccone, Carla Bizzarri
Unit of Endocrinology, Bambino Gesu Children's Hospital, IRCCS, Rome, Italy

Preface

Survival rates of childhood cancer have been steadily improving over the past 3 decades, with 5-year overall survival now approaching 85% in Western countries. These changes have shifted parental and patients' concerns. While survival was previously the primary concern, the late effects of cancer treatment are now becoming major healthcare issues. Epidemiological studies in childhood cancer survivors (CCS) have shown a significant decrease in male fertility when compared to their healthy siblings. Low sperm count or azoospermia are the most common characteristics of infertility in these patients. Studies of sperm from adult cancer patients showed that cancer itself and its treatment are able to induce sperm aneuploidy, chromatin damage, and epigenetic changes that persist for years post chemotherapy. In childhood cancer, it is still unclear whether the sperm produced years after the completion of cancer treatment may still contain chromatin and DNA damage.

The increasing awareness of severe late health effects includes subsequent malignant neoplasms. These neoplasms occur after an initial cancer treatment or HSCT, and represent histologically distinct primary malignancies. They account for nearly one-half of CCS non-relapse mortality. Previous treatment with radiotherapy is a major risk factor for second neoplasias. Thyroid cancer accounts for 10–20% of subsequent malignant neoplasia in CCS.

Finally, the impairment of cardiometabolic fitness and the increased risk of cardiovascular disease (CVD) are major emerging concerns in CCS. Several cohort studies from the USA and Europe have shown that young adult CCS have an up to 8-fold higher risk of death from CVD, compared to age- and sex-matched individuals. Endothelial dysfunction and chronic low-grade inflammation are supposed to be the first steps in the development of early atherosclerosis leading to CVD. New insights in CVD pathogenesis have allowed the detection of sensitive and noninvasive biomarkers of a pre-atherosclerotic status in young adult CCS.

Fertility-Related Issues

9.1. Anti-Müllerian hormone and Inhibin B after stem cell transplant in childhood: A comparison of myeloablative, reduced intensity and treosulfan-based chemotherapy regimens

Leiper A, Houwing M, Davies EG, Rao K, Burns S, Morris E, Laven J, van der Kooi AL, van den Heuvel Eibrink M, Nussey S
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a.leiper@nhs.net

Anti-Müllerian hormone (AMH), secreted by granulosa cells of growing pre- and early antral ovarian follicles, reflects the reserve of primordial ovarian follicles at any time from birth to menopause. Serum AMH levels are low during childhood, increase to a plateau in the mid-twenties and progressively decline to the menopause. lnhibin B is produced by Sertoli cells, serum levels surge during puberty and then plateau. Inhibin B measurement is considered a suitable surrogate marker of spermatogenesis, even if cannot replace semen analysis. Low AMH and lnhibin B levels reflect the entity of gonadal damage related to chemotherapy and represent promising tools to predict infertility in childhood cancer survivors (CCS).

In this study, serum levels of AMH and inhibin B were analysed to assess fertility potential in survivors of childhood hematopoietic stem cell transplantation (HSCT) after 3 different chemotherapy-based conditioning treatments. Group A received a treosulfan-based low-toxicity regimen; Group B had reduced-intensity regimen based on fludarabine/melphalan (Flu-Mel) and Group C received a busulphan/cyclophosphamide (Bu-Cy) myelo-ablative regimen.

In females, AMH levels expressed as mean standard deviation scores (SDS) were below zero in all groups, but were significantly higher after treosulfan (group A) and Flu-Mel (group B) than after Bu-Cy (group C). Gonadotropin levels were normal in group A, and elevated in group B and C.

In males, gonadotrophins were normal for age in all groups, but FSH levels were significantly higher in Group B, than in group C. Mean Inhibin B levels in boys were significantly higher in Group A than in Group B and C, with the Flu-Mel group showing the greatest impairment.

The treosulfan-based regimen is confirmed to be less gonadotoxic than myeloablative Bu-Cy in both sexes. Flu-Mel appears to be less gonadotoxic than Bu-Cy and is comparable to treosulfan in girls, while it is associated with the most severe damage in boys. Late recovery of spermatogenesis in the Bu-Cy myeloablative group may explain this sex difference. This study also confirms that AMH and inhibin B are more reliable than gonadotrophins in the early prediction of gonadal failure in patients treated with conditioning regimens.

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9.2. Pregnancy, time to pregnancy and obstetric outcomes among female childhood cancer survivors: Results of the DCOG LATER-VEVO study

Van Dijk M, van Leeuwen FE, Overbeek A, Lambalk CB, van den Heuvel-Eibrink MM, van Dorp W, Tissing WJ, Kremer LC, Loonen JJ, Versluys B, Bresters D, Ronckers CM, van der Pal HJ, Beerendonk CCM, Kaspers GJL, van Dulmen-den Broeder E, van den Berg MH

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marloes.vandijk@amsterdamumc.nl

Chemo- and radiotherapy administered during childhood may compromise female reproductive function leading to premature depletion of the ovarian follicle pool. Childhood cancer survivors (CCS) women who pursue pregnancy may experience a lengthening of the time required to become pregnant (time to pregnancy: TTP), similar to that seen in aged mothers. This retrospective study is part of the DCOG LATER-VEVO study, a nationwide multicenter cohort study evaluating long-term fertility among Dutch female CCS. Data were collected by questionnaires. The study included CCS sisters and a random sample of women from the general population as controls.

Among the subgroup of CCS women who ever pursued pregnancy, the chance of becoming pregnant was significantly lower than in normal women. Overall TTP was 1.1 times longer for CCS women compared to controls and it was significantly longer in survivors of tumours of central nervous system (CNS) and kidney. Frequency of adverse obstetric outcomes (miscarriage, still birth, or induced abortion) was not different in CCS and controls, but CCS had an increased risks of preterm delivery and caesarean section. Lower abdominal/pelvic radiotherapy was strongly associated with adverse obstetric outcomes.

This is one of the first studies analysing TTP and adverse obstetric outcomes in CCS using a large, nationwide register. Strengths of the study include the evaluation of the chance of becoming pregnant among women who actually pursued pregnancy, the inclusion of a large control group, and the availability of detailed information about previous cancer treatment. On the other hand, data were collected by a questionnaire and only 63% of the invited CCS and 49% of the controls completed this, clearly limiting the value of the results. Moreover, the proportion of CCS women treated with chemotherapy-only was significantly higher among participants compared to non-participants. This may indicate that the more aggressively treated CCS were not included. Consequently, the reported risks of adverse obstetric outcomes may be an underestimation of the actual risk.

TTP analyses are valid only for CCS who were able to conceive and ignore women who pursued but failed to achieve pregnancy. Finally, the analyses were not corrected for individual factors negatively affecting pregnancy outcome, such as BMI, smoking behavior, and menstrual cycle characteristics.

9.3. The influence of different intensity of treatment on hormonal markers of gonadal function in acute lymphoblastic leukemia survivors

Krawczuk-Rybak M, Płonowski M, Leszczyńska E, Latoch E, Sawicka-Żukowska M, Muszyńska-Rosłan K, Skalska-Sadowska J, Wachowiak J, Sga-Pondel D, Kazanowska B, Chybicka A, Stachowicz-Stencel T, Stefanowicz J, Malinowska I, Matysiak M, Kotan A, Wysocki M, Pobudejska-Pienążek A, Szczepański T, Przybyszewski B, Badowska W, Szymańska-Miller D, Kowalczyk JR, Kamieńska E, Urasiński T, Wawrzeńczyk A, Żelazowska-Rutkowska B, Cylwik B
Hematol Oncol. 2019 Dec;37(5):609–616.
mar26@mp.pl

Acute lymphoblastic leukaemia (ALL) is the most common childhood neoplasia with an actual survival rate $>80\%$. ALL treatment has changed considerably during last 30 years; radiotherapy with 24 Gy to the central nervous system (CNS) has been eliminated or reduced to lower doses (12 or 18 Gy) and chemotherapy regimens are now tailored to the specific disease risk. For these reasons, ALL survivors are usually considered to have a low risk of gonadotoxicity. In BFM (Berlin-Frankfurt-Munster) protocols, used in most European countries, patients are stratified into 3 risk groups: standard risk (SR), intermediate risk (IR), and high risk (HR), according to age at diagnosis, initial leucocyte count, immunophenotype of leukaemic blasts, their genetic abnormalities, and response to treatment. The total doses of chemotherapy and the use of cranial irradiation differ among the risk groups and so the impact of late toxicity can be different.

In the present study, sex hormone levels were analysed in 69 young ALL survivors stratified into SR, IR, and HR risk groups, and in 80 healthy controls of similar age. Compared to controls, HR group males showed higher levels of FSH and LH, lower inhibin B, and normal testosterone. Males in SR + IR groups showed hormonal values similar to controls. In females of all risk groups showed similar levels of FSH, LH, E2, and inhibin B to controls, however, mean AMH levels were slightly lower in SR + IR group females and significantly reduced in the HR group.

Semen analysis is the gold standard to assess male fertility, but it is often not feasible in young patients. FSH measurement is frequently used in clinical practice as a surrogate marker of spermatogenesis. Similarly, FSH measurement is used in females, as an index of ovarian reserve, despite its cyclic variability. Recently, inhibin B and AMH have been proposed as more reliable tools to assess gonadal function in CCS. Inhibin B, a glycoprotein hormone produced by Sertoli cells, plays an important role in the regulation of the hypothalamic-pituitary-gonadal axis and is an indirect marker of spermatogenesis. AMH is produced by granulosa cells and correlates with the number of mature follicles.

Due to the implementation and tailoring of therapy and supportive care, the burden of late effects observed in ALL survivors has changed, but some survivors still present significant sequelae. Testicular function can be affected by ALL itself, chemotherapy, administration of higher doses of gonadotoxic cytostatics, such as alkylating agents, radiation to the pelvic/gonadal area, cranial irradiation or total body irradiation. All of these factors can lead to poor sperm quality and/or oligospermia. In females, anticancer therapy can induce oocyte depletion. The present data indicate that even upgraded ALL treatment, usually considered less gonadotoxic, can cause gonadal dysfunction. ALL patients stratified into the HR group show signs of germ cell damage without overt hypogonadism. All of these patients should be informed about the possible late impact of ALL treatment on gonadal function and the need for lifelong follow up.

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9.4. Sperm DNA integrity in adult survivors of paediatric leukemia and lymphoma: A pilot study on the impact of age and type of treatment

Beaud H, Albert O, Robaire B, Rousseau MC, Chan PTK, Delbes G
PLoS One. 2019;14(12):e0226262.
geraldine.delbes@iaf.inrs.ca

Male childhood cancer survivors (CCS) show reduced fertility, mostly due to low sperm count. The links between DNA damage caused by cancer and its treatment, pubertal stage at diagnosis and future infertility are still unclear. This Canadian pilot study analysed reproductive parameters and sperm characteristics in adult survivors of childhood leukaemia and lymphoma, divided into 2 groups according to whether cancer diagnosis occurred before or after puberty. They were compared to age-matched men with no history of cancer. No differences were observed in mean serum testosterone and FSH levels, but 4 CCS had FSH levels >8 U/ml. No differences in average sperm count or motility were observed across groups, but 5 of the 13 CCS were affected by azoo/oligozoospermia, regardless of the time at cancer diagnosis. Analysis of sperm chromatin and DNA integrity revealed no differences among the groups. The authors observed a significant negative correlation between sperm count and the cumulative dose of alkylating agent, determined by the cyclophosphamide equivalent dose, and a strong positive correlation between the cumulative dose of anthracyclines and the DNA fragmentation index. A limitation of the study is the small sample size, which makes it difficult to generalize the results. It is noteworthy that both groups of CCS had a similar prevalence of azoo/oligozoospermia, supporting the hypothesis that the prepubertal testis is not protected from damage in children who undergo cancer treatment.

9.5. Leydig cell function in male survivors of childhood cancer: A report from the St Jude lifetime cohort study

Chemaitilly W, Liu Q, van Iersel L
J Clin Oncol. 2019;37(32):3018–3031.
wassim.chemaitilly@stjude.org

This retrospective study, with cross-sectional health outcomes analysis, evaluated Leydig cell function in 1516 childhood cancer survivors at a median duration of 22.0 years after cancer diagnosis. These patients are part of the St Jude Lifetime Cohort. An age- and sex-matched community control group was recruited among friends and relatives of patients. Leydig cell failure (LCF) was defined as serum total testosterone <250 ng/dl and LH >9.85 IU/l, while Leydig cell dysfunction (LCD) was defined as testosterone ≥ 250 ng/dl and LH >9.85 IU/l. Follow-up data were available on 683 participants (45.2%). The point prevalence of LCF and LCD at the most recent evaluation was 6.9% and 14.7%, respectively; 42/104 patients with LCF (40.4%) used hormone replacement therapy. Eight controls, (4.8%) were receiving testosterone, but the indications for treatment were not available. No control had LCF, while 4 individuals had LCD. Independent risk factors for LCF included: age ≥ 26 years at assessment, testicular radiotherapy, and alkylating agents at cyclophosphamide equivalent doses ≥ 4000 mg/m^2. LCF was also associated with: abdominal obesity, diabetes mellitus, erectile dysfunction (ED), muscle weakness, and all-cause mortality. Hormone-treated LCF was associated with increased waist circumference, ED, muscle weakness, and depression, but not with all-cause mortality. LCD was associated with unilateral orchiectomy and the same risk factors as LCF. No significant associations of LCF and LCD with adverse physical or psychosocial outcomes were found.

The study reveals that despite the availability of hormonal replacement therapy, only a minority (40.4%) of patients with LCD receive such treatment. On the other hand, many of the associations between LCF and long-term adverse physical and psychosexual health outcomes persisted even among individuals who reported to be treated with hormonal replacement therapy. The selection of control subjects may be questioned, as some of them were treated with testosterone for unclear reasons. Finally, the cohort of survivors was not homogeneous, but included patients with different cancer diagnoses and prevalence estimates have to be interpreted with caution, given the substantial number of nonparticipants.

9.6. Assessment of ovarian function in adolescents and young adults after childhood cancer treatment-How accurate are young adult/parent proxy-reported outcomes?

George SA, Williamson Lewis R, McKenzie L, Cherven B, Patterson BC, Effinger KE, Mertens AC, Meacham LR
Pediatr Blood Cancer. 2019;66(12):e27981.
sgeorg4@emory.edu

This cross-sectional study tested the concordance between young adult (or parent proxy)-reported premature ovarian insufficiency (POI) and biochemical evidence of POI, defined as FSH $\geq$40 mIU/ml, in 182 childhood cancer survivors (CCS) who had received gonadotoxic therapy. Reported POI was defined as the survivor taking hormone replacement therapy (HRT) for ovarian failure or having been told they were affected by ovarian failure. 85% of CCS reported a spontaneous menarche; 55.5% reported regular menses without HRT use, and 14.8% reported requiring HRT to have menses. Overall, 71.4% of CCS had been screened for biochemical POI; these CCS were more likely to be older, to use hormonal therapy and to have been seen in the survivor clinic and/or by an endocrinologist at least once prior to the survey. Among them, 17.7% reported POI whereas 18.5% had FSH $\geq$40 mIU/ml (moderate agreement). The highest agreement between patient-reported and biochemical POI was with young adult survivors with >5 survivor clinic and/or endocrinologist visits. The large cohort of adolescent and young adult female CCS investigated for menstrual patterns and POI represents a significant strength of this study. However, it is surprising that only 71% of the study population underwent FSH levels assessment and the definition of biochemical POI was limited to at least one elevated FSH level and did not include a history of menstrual irregularities.

Continuous education is crucial for young CCS and their parents; in particular, they should be appropriately informed about their ovarian function prior to transition to adult care, so that they can take care of their reproductive health, particularly in medical settings outside of survivor clinics.

9.7. Testicular Function of childhood cancer survivors: Who is worse?

Duca Y, Di Cataldo A, Russo G, Cannata E, Burgio G, Compagnone M, Alamo A, Condorelli RA, La Vignera S, Calogero AE
J Clin Med. 2019;8(12):2204.
sandrolavignera@unict.it

This cross-sectional study evaluated morpho-volumetric development of the testis, endocrine function and sperm parameters in 102 young adult childhood cancer survivors (CCS). About 1/3 of patients showed low testicular volume ($<$24 ml); the Hodgkin disease group showed significantly lower total testicular volumes, compared to the rest of the group. CCS who underwent hematopoietic stem cell transplantation (SCT) showed significantly lower total testicular volume, followed by those who received chemotherapy plus radiotherapy. Eighty-seven CCS underwent hormonal evaluation; 3/87 showed low LH levels, 5/87 high LH levels, 8/87 high FSH values. Twenty-eight out of 84 patients (33.3%) had suboptimal testosterone values ($<$3.5 ng/ml), while 5/84 showed frankly reduced levels ($<$2.3 ng/ml). AMH levels were measured in 17 CCS and were normal in all of them. Thirty-four patients accepted to undergo sperm analysis; sperm count was reduced in 7/34 patients (20.6%), while azoospermia was found in 6/34 patients (17.7%). All patients with azoospermia had a primary testicular failure (FSH levels >8 UI/l and low testicular volume) and had received radiotherapy or SCT. History of Hodgkin's disease, SCT, and non-cranial irradiation associated with chemotherapy were risk factors for reduced sperm concentration. Patients treated during puberty showed lower total testicular volume than patients treated before puberty, probably due to a more aggressive treatment in older patients. Sperm concentration was more closely related to testicular volume than to FSH levels, while AMH values were unrelated to testicular function. In this study, only one third of patients accepted to undergo sperm analysis. The same reluctance has been described by other authors. This common attitude among CCS has prompted the search for surrogate markers of spermatogenesis to be used in infertility screening. Inhibin B has proved to be a better marker for spermatogenesis than FSH. Surprisingly, inhibin B was not analysed in this study, while as expected, AMH was not useful in the evaluation of testicular function. Similarly, the cumulative dose of alkylating agents, already identified as a detrimental factor affecting gonadal function in these patients, was not evaluated.

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New Fertility Preservation Strategies: Lights and Shadows

9.8. Does co-transplantation of mesenchymal and spermatogonial stem cells improve reproductive efficiency and safety in mice?

Kadam P, Ntemou E, Onofre J, Van Saen D, Goossens E
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ellen.goossens@vub.be

Infertility due to spermatogonial stem cell (SSC) loss is one of the major late adverse effects related to both chemotherapy and radiotherapy, and several researchers are now focusing on the development of new techniques to preserve germ cells.

Semen sample storage is not feasible in prepubertal patients, so cryostorage of testicular tissue is considered an alternative option. Transplantation of testicular tissue or SSCs is a promising strategy to restore fertility in these patients, even if caution is needed for the risk of contamination by malignant cells in boys affected by systemic or metastatic disease. In primates, autologous transplantation of pre-pubertal testicular tissue yielded complete spermatogenesis and the live birth of a healthy monkey. New techniques transplant not only SSCs, but also the associated mesenchymal stem cells (MSCs). These cells do not trans-differentiate into germ cells, but produce paracrine factors that play a role in restoring the damaged SSC niche, thus improving the efficiency of transplantation.

This experimental study tested the safety and reproductive efficiency of TGFβ1-induced MSCs and SSCs transplant (MSi-SSCT) in a mouse model of long-term infertility, and compared fertility parameters with a control group of non-treated fertile mice. TGFβ1 was used to improve engraftment efficiency, through the reduction of inflammatory factors that cause migration of MSCs away from the testis. The overall tubular fertility index (TFI), representing the percentage of tubules containing spermatogenesis, was higher after MSi-SSCT than after SSCT alone, but the expression levels of DNA methyltransferase (DNMT) 1 and 3A in donor-derived gem cells were low, suggesting a derangement of DNA methylation pattern. The complex process of epigenetic reprogramming through DNA methylation is a crucial step during spermatogenesis. Any abnormality is likely to cause infertility, and the risk of inheriting an abnormal epigenome causing congenital defects in the offspring cannot be neglected. In this study, even if the expression levels of DNMT 1 and 3A in donor-derived gem cells were low, normal levels were restored in the offspring of transplanted mice, and no differences of DNA methylation patterns were found between healthy fertile control mice and the offspring of transplanted mice.

The results of this study are encouraging, but more extensive epigenetic analyses are needed in order to define the reproductive safety of this strategy.

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9.9. Advanced glycation end products and chronic inflammation in adult survivors of childhood leukemia treated with hematopoietic stem cell transplantation

Felicetti F, Cento AS, Fornengo P, Cassader M, Mastrocola R, D'Ascenzo F, Settanni F, Benso A, Arvat E, Collino M, Fagioli F, Aragno M, Brignardello E

Pediatr Blood Cancer. 2020 Mar;67(3):e28106.

ebrignardello@cittadellasalute.to.it

Premature aging has been proposed as a paradigm to explain the early cardiovascular disease (CVD) that affects young-adult childhood cancer survivors (CCS). Protein glycation is recognized as one of the main factors contributing to aging. Advanced glycation end products (AGEs) are a heterogeneous group of compounds that contribute to the development of chronic low-grade inflammation through various mechanisms. Extracellular AGEs accumulation causes an inflammatory response that alters the structure and function of essential proteins as collagen, hemoglobin, myocardial matrix protein, fibrinogen, and low-density lipoproteins. The alteration of protein folding processes impairs mitochondrial function, leading to reduced production adenosine triphosphate (ATP), increased synthesis of reactive oxygen species (ROS), and reduced antioxidant activity. Finally, the interaction of AGEs with their cell-bound receptor (RAGE) increases gene expression and release of proinflammatory cytokines, as well as ROS generation. The stimulation of endothelial proliferative and fibrotic processes induces vascular inflammation and atherosclerosis, leading to myocardial dysfunction and other vascular disruption–related diseases. Interleukin (IL)-1β is considered as the first step of the proinflammatory cytokine chain. It induces the synthesis of multiple inflammatory mediators, as IL-17 that acts on vessel and cardiac cells, leading to inflammation, coagulation, and thrombosis. High-sensitivity C-reactive protein (hs-CRP) has been defined as the downstream biomarker of chronic low-grade inflammation.

Among patients affected by childhood acute lymphoblastic leukaemia (ALL), those who received hematopoietic stem cell transplantation (HSCT) after conditioning regimens involving myeloablative total-body irradiation (TBI) show the highest risk of CVD. This cohort study evaluates AGEs plasma levels in 18 adult survivors of ALL treated with TBI + HSCT; 30 age-matched healthy controls were recruited for comparison. TBI-exposed ALL survivors showed a seven-fold increase of AGEs levels, in comparison with healthy controls. Circulating levels of L-1β, IL-17 and hrCRP, as well as the glutathione/reduced glutathione ratio (expression of antioxidant reserve) in ALL survivors were remarkably higher than in controls. ALL survivors also showed significantly higher levels of triglycerides and apolipoprotein B.

The small sample size represents the major limitation of this study, even if the study group was highly homogeneous. Survivors affected by other conditions, such as diabetes mellitus, chronic graft versus host disease, liver or renal dysfunction, which could potentially influence AGEs accumulation and a chronic inflammatory status, were excluded. If confirmed by larger studies, these findings could lead to development of AGEs-targeted therapeutic strategies in order to prevent late effects related to premature aging. The high circulating levels of AGEs and inflammatory cytokines could contribute to explain not only the increased risk of CVD, but also the pathogenesis of other severe complications including second malignant neoplasms.

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9.10. Childhood leukemia survivors and metabolic response to exercise: A pilot controlled study

Pegon C, Rochette E, Rouel N, Pereira B, Doré E, Isfan F, Grèze V, Merlin E, Kanold J, Duché P

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e_rochette@chu-clermontferrand.fr

This 5-month prospective case-control study was designed to evaluate the impairment of metabolic response to exercise in 20 childhood acute leukaemia survivors (CALSs), by the analysis of substrate oxidation during submaximal exercise, in comparison with 20 matched healthy controls.

The different signs of metabolic syndrome are frequently observed in CALSs, due not only to previous treatments, but also to lifestyle changes (reduced physical activity, increased fatigue, reduced energy expenditure). Metabolic fitness can be estimated by indirect calorimetry, measuring the ability to oxidize lipids and carbohydrates during incremental exercise; this reflects the physiological status of the muscles.

This study shows that CALSs have reduced carbohydrate and fat oxidation rates, compared to healthy controls. They also show lower relative and absolute VO2 peak, which is an indicator of impaired cardiorespiratory fitness associated with higher fatigability during physical exercise. The authors speculated that these findings were mainly related to the effect of anthracyclines on cardiac function (despite a normal echocardiography at rest). Damage of the pulmonary, musculoskeletal and neuronal adaptative mechanisms that regulate the physiological response to exercise, as adverse effects of different chemotherapeutic agents, may be associated. Specifically, the impaired muscle function may be related to the catabolic effects of treatments, such as vincristine and corticosteroids, which cause limitation of the ability of muscle fibers to consume oxygen. This already damaged oxidative system could be further affected by a low level of physical activity and sedentary lifestyle habits, causing impairment of metabolic fitness.

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9.11. Retinal vessel analysis as a novel screening tool to identify childhood acute lymphoblastic leukemia survivors at risk of cardiovascular disease

Azanan MS, Chandrasekaran S, Rosli ES, Chua LL, Oh L, Chin TF, Yap TY, Rajagopal R, Rajasuriar R, MacGillivray T, Trucco E, Ramli N, Kamalden TA, Ariffin H

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taftkamalden@um.edu.my

Childhood cancer survivors (CCS) have a markedly increased incidence of early cardiovascular disease (CVD). Changes in retinal vessel diameter are associated with CVD risk and stroke mortality. This cross-sectional study analysed retinal microvasculature, arterial stiffness, endothelial activation markers and cardiovascular risk factors in 73 young survivors of childhood acute lymphoblastic leukaemia (cALL) and 78 healthy controls. The purpose of the study was to investigate whether retinal vessel analysis can represent a feasible method to assess endothelial health in survivors of cALL. Retinal fundus images were summarized into two different retinal vessel measurements: central retinal artery/vein equivalents (CRAE/CRVE) and arteriolar-venular ratio (AVR).

In comparison with age-matched controls, cALL survivors had higher cardiovascular risk factors and more retinal signs of microvascular damage (higher CRVE, lower AVR). Their arterial stiffness and endothelial activation markers (sVCAM-1) were also increased. The authors suggest that retinal vessel changes could predate overt clinical abnormalities such as obesity or metabolic abnormalities. Indeed, 10 survivors demonstrated arterial changes despite having normal triglyceride levels and BMI (although it is well known that BMI underestimates the proportion of fat tissue in cancer survivors). The potential clinical application of retinal vessel analysis, as a noninvasive early screening tool for identifying survivors at increased risk for CVD, is clearly interesting. The cross-sectional design of this study represents a clear limitation, since only serial assessments could clarify to what extent the severity of retinal microvascular changes can predict the development of cardiovascular morbidities.

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9.12. Ultrasound is superior to palpation for thyroid cancer detection in high-risk childhood cancer and BMT survivors

Hess J, Schafernak K, Newbern D, Vern-Gross T, Foote J, Van Tassel D, Jamshidi R, Walsh A

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hess2@phoenixchildrens.com

The development of a second neoplasia is a major concern during the follow-up of childhood cancer survivors (CCS). Secondary cancers account for nearly 50% of non-relapse mortality at 5 years. Thyroid tumours represent 10-20% of subsequent neoplasia cases, due to the high sensitivity of the gland to irradiation. Patients treated with radiotherapy (RT) to the head, neck, upper thorax, or total body irradiation (TBI) are at higher risk for subsequent thyroid cancer. These tumours may occur many years after the completion of cancer treatment, with a dose-dependent response at doses <30 Gy, and an increased risk in younger patients. Current American Children's Oncology Group screening guidelines recommend annual neck palpation as a screening test to detect thyroid nodules.

The purpose of this retrospective chart review was to determine if ultrasound (US) is better than palpation to detect thyroid cancer in high-risk CCS. Inclusion criteria were age <20 years at diagnosis and history of RT to the head, neck, upper thorax, or TBI for a primary malignancy, or as part of a conditioning regimen for hematopoietic stem cell transplantation (HSCT). 225 patients had documented palpation and 144 (64%) also had US evaluation. US identified 14 patients who had a final tissue diagnosis of thyroid cancer, with a sensitivity of 100%, while palpation alone identified only 2 of 16 cases, yielding a sensitivity of 12.5%. Screening US correctly identified cases without thyroid cancer with a specificity of 73.1%, while palpation yielded a specificity of 100%. US negative predictive value was 100%. None of the 95 patients who underwent at least one screening US and were not noted to have a suspected thyroid nodule, developed thyroid cancer in longitudinal follow-up.

The debate on whether the gold standard to detect secondary thyroid tumours is neck palpation or US has been active for a long time. This study has the limitation of neck palpation performed by different endocrinologists, but shows that US has much higher sensitivity. US is able to identify the thyroid neoplasm when it is still undetectable by neck palpation and presumably less invasive, and this should result in earlier and easier interventions. On the other hand, the use of thyroid US does not seem to be associated with a higher frequency of unnecessary invasive procedures, such as fine needle aspiration biopsy (FNAB). These findings, together with the low cost of the procedure, lead to consider US as the gold standard for thyroid nodule detection in CCS population.

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9.13. Hyperthyroidism after radiation therapy for childhood cancer: A report from the childhood cancer survivor study

Inskip PD, Veiga LHS, Brenner AV, Sigurdson AJ, Ostroumova E, Chow EJ, Stovall M, Smith SA, Leisenring W, Robison LL, Armstrong GT, Sklar CA, Lubin JH

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inskippeter@gmail.com

Childhood cancer survivors (CCSs) are at increased risk to develop thyroid disease, in particular hypothyroidism, nodular disease and thyroid cancer. Hyperthyroidism is less common but may occur after ionizing radiation exposure, even if the causative mechanisms are not completely clarified.

This study analysed the Childhood Cancer Survivor Study's cohort of 5-year survivors of childhood cancer diagnosed at hospitals in the US and Canada between 1970 and 1986. The occurrence of hyperthyroidism was ascertained among 12 183 survivors who responded to serial questionnaires. Radiation doses to the thyroid and pituitary gland and chemotherapy exposures were estimated from medical records; 179 self-reported cases of hyperthyroidism were identified, 148/179 were diagnosed 5 or more years after cancer diagnosis.

A significantly higher adjusted incidence rate was associated with female sex, recent calendar year of follow-up, and specific types of cancer (Hodgkin lymphoma, followed by central nervous system neoplasms and leukaemia). However, the most important result of this study is the direct correlation between thyroid radiation dose and prevalence of self-reported hyperthyroidism 5 years after cancer diagnosis. Neither radiation dose to the pituitary gland nor chemotherapy was associated with hyperthyroidism. Even if the relatively small number of patients with hyperthyroidism did not allow the identification of a threshold dose, a linear relation described the thyroid radiation dose-response for hyperthyroidism prevalence 5 years after cancer, with an estimated relative risk of 1.06 per +1 Gy. The next step could be the identification of a cut-off dose, in order to discriminate at risk patients needing a careful and prolonged follow-up. Radiation-induced thyroid damage may cause the expression of both stimulatory and blocking antibodies directed against TSH receptor. It is unclear whether radiation-related hyperthyroidism and radiation-related primary hypothyroidism result from distinct (even if sometimes overlapping) autoimmune pathways. Radiation-related primary hypothyroidism can also result from a direct radiation-induced damage to thyroid cells that does not involve an autoimmune process.

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Cancer Treatment and Bone Health

9.14. High impact physical activity and bone health of lower extremities in childhood cancer survivors: A cross-sectional study of SURfit

Zürcher SJ, Jung R, Monnerat S, Schindera C, Eser P, Meier C, Rueegg CS, von der Weid NX, Kriemler S

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susi.kriemlerwiget@uzh.ch

Childhood cancer survivors (CCS) can experience impaired bone health as a consequence of the disease itself, the specific treatments and/or associated chronic conditions. This cross-sectional study analyses baseline data from the single-centre randomized-control SURfit trial – which is testing a physical activity (PA) intervention in adult and adolescent survivors of childhood cancer that is ongoing in Switzerland. The purpose of this analysis was to define the prevalence of BMD *Z*-score < -1 SDS (reduced bone health) in 161 young adult childhood cancer survivors (CCS) and to analyse the relationship between mechanical impact loading induced by PA and bone health. Mechanical loading of bone results from bending or torsional forces through muscles, or from bone compression through impact forces (running, jumping).

An elevated frequency of reduced bone health in CCS was confirmed by both DXA and pQCT, and was associated with increased risks of fracture and osteoporosis. As in the general population, bone health was better in physically active patients. CCSs exercising with high duration and frequency impact loadings showed

healthier bone characteristics (higher trabecular bone density and conserved cortical geometry) compared to their sedentary counterparts.

The authors propose a relatively simple solution to the problem of bone impairment, because they demonstrate that just few minutes of feasible impact loading of the lower body are associated with better microstructural and densitometric bone characteristics and reduced risk of bone pathologies. The better results might be obtained with fast running or jumping, which have a high impact on bone. These results should be confirmed in younger and still growing CCS, in order to define whether regular PA is able to restore a normal BMD and promote the achievement of an appropriate bone mass peak.

9.15. Efficacy and safety of denosumab therapy for low bone mineral density in childhood cancer survivors: A report of preliminary experience

Huang TH, Liu HC, Hou JY, Chang CY, Sun FJ, Yeh TC
Pediatr Blood Cancer. 2019 Oct;66(10):e27927.
yeh@mmh.org.tw

This monocentric observational study analysed the efficacy of denosumab in addition to calcium and vitamin D supplementation on bone mineral density (BMD) in 113 young childhood cancer survivors (CCS) with initial BMD *Z*-score < -1.5.

Low BMD is common in CCS, due to cancer-related factors affecting bone health both directly (cancer-induced osteolysis) and indirectly (nutrition, physical activity, corticosteroid and/or irradiation therapy). The role of denosumab (an inhibitor of receptor activator of nuclear factor kappa-B ligand, RANKL used as antiresorptive medication) is well documented in adults, while it is still rarely used in children. To date, this is the first study to show a significant improvement of BMD *Z*-score in 20 CCS treated with denosumab, after the completion of chemotherapy.

Overall, denosumab treatment was safe, even if 8 patients experienced hypocalcaemia, which was promptly recognized and corrected. Mean height adjusted BMD Z-score at baseline was -2.68 but increased to -2.0, -1.96, and -1.33 after 0.5, 1, and 1.5 years after denosumab treatment, respectively. These results appear encouraging, but they need to be confirmed in larger cohorts with a longer follow-up. In particular, in this cohort, 5/20 patients were >18 years old at the start of denosumab treatment and so information was limited on the effects of denosumab on linear growth and pubertal growth spurt, since this drug acts on bone remodelling. In addition, neither the fracture risk nor the dose-response effect of therapy was analysed and the observation period reached 1.5-year duration only in 8 treated patients. A controlled trial would be desirable to better evaluate the benefits of denosumab on BMD and the risk of adverse effects, in particular in young growing patients.

9.16. Severity of reduced bone mineral density and risk of fractures in long-term survivors of childhood leukemia and lymphoma undergoing guideline-recommended surveillance for bone health

Bloomhardt HM, Sint K, Ross WL, Rotatori J, Ness K, Robinson C, Carpenter TO, Chow EJ, Kadan-Lottick NS
Cancer. 2020;126(1):202–210.
nina.kadan-lottick@yale.edu

While in healthy adults and children low BMD is associated with fracture risk, this relationship still needs to be clarified in childhood cancer survivors (CCS). This cross-sectional study analysed BMD and fracture history in 542 childhood leukaemia/lymphoma survivors, who received guideline-recommended DXA surveillance at 2 years post-therapy. 17% of survivors had low lumbar spine BMD (*Z*-score < -1), and 3.5% had very low BMD (*Z*-score < -2); 29.2% of patients who were 15- to 19-year-old at diagnosis had low BMD and 10.8% had very low BMD. Older age at diagnosis, white race, and being underweight were significantly associated with low BMD. Overall, 116 patients reported ≥ 1 post-therapy non-digit fracture and 25 had multiple fractures. Survivors with low BMD had greater risk of non-digit fractures (odds ratio: 2.2) and specifically long-bone fractures (odds ratio: 2.7).

The study demonstrates a significant association between low lumbar spine BMD and post-therapy fractures, emphasizing the clinical importance of routine DXA surveillance in childhood leukaemia survivors, particularly

those treated in adolescence or young adulthood when peak bone mass is acquired. Interestingly, this study found no difference in the frequency of fractures and low BMD related to vitamin D/calcium status and supplementation, which differs to observations in patients with juvenile rheumatoid arthritis or idiopathic juvenile osteoporosis. Limitations of this study are its cross-sectional design and lack of data on other factors known to negatively affect BMD (sedentary lifestyle, smoking, concurrent hormonal defects as hypogonadism).

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Cancer Treatment, Growth and Growth Hormone

9.17. Final height in growth hormone-deficient childhood cancer survivors after growth hormone therapy

Rodari G, Cattoni A, Albanese A

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giulia.rodari@unimi.it

Growth hormone deficiency (GHD) is the most common endocrine late effect in children with tumours involving the hypothalamus-pituitary (HP) area or exposed to cranial, craniospinal or total body radiotherapy (RT). The location of brain tumours in continuity with the sellar/suprasellar area and HP radiation doses $\geq$18 Gy represent the most important risk factors for GHD.

This retrospective study analysed 87 childhood cancer survivors (CCS) treated with recombinant GH (rhGH). Patients were divided into 2 groups: A) children treated with cranial radiotherapy or affected by non-irradiated tumours of the HP area; B) children treated with craniospinal or total body irradiation. Overall height (HT) gain after 1 and 2 years of rhGH therapy was 0.38 $\pm$ 0.35 SDS and 0.18 $\pm$ 0.30 SDS, respectively. No differences were found between the 2 groups during the first year of treatment, but group B showed a poorer response to treatment during the second year. Mean final height (FH) was in the normal range, but not significantly different from HT SDS at baseline; 67% of children failed to reach their mid parental height (MPH).

Spinal, craniospinal or total body irradiation, precocious or early puberty contribute to the suboptimal growth response to rhGH in CCS. The administration of rhGH after craniospinal RT may result in an exaggeration of the skeletal disproportion often observed also in GH untreated CCS, due to the specific radiation-induced damage to the spine. For all of these reasons, final height of CCS often falls below MPH. In this study, the major determinants of FH were height at rhGH start and lag time between the end of antineoplastic treatments and rhGH start. GHD in these patients tends to present with progressive linear growth deceleration, rather than with significant short stature, which underlines the importance of early initiation of rhGH therapy as soon as height velocity decreases. rhGH therapy aims to prevent further linear growth deceleration, even if it is expected that it cannot normalize height. Treatment, although failing to induce catch-up growth, prevents progressive height loss, leading to a FH within the normal range, even if still below MPH.

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10. Type 1 Diabetes Mellitus

Thomas Kapellen, Sabine Klamt, Heike Bartelt, Elena Sergeyev, Vivien Bach, Wieland Kiess
Hospital for Children and Adolescents, Centre for Pediatric Research, University of Leipzig, Leipzig, Germany

10.1. Congenital infections as contributors to the onset of diabetes in children: A longitudinal study in the United States, 2001–2017

Rogers MAM, Kim C
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For many years, infections have been thought to trigger the onset of type 1 diabetes (T1DM) or even to be one of the causes of autoimmune processes that eventually lead to the destruction of the pancreatic beta-cell. In particular, maternal rubella virus infections during pregnancy are reported to increase the risk of T1DM in children, and fetal rubella infection leads to higher risk of T1DM in those affected. Widespread rubella vaccination has decreased the number of infants with congenital rubella syndrome in many countries, although it remains a problem in developing countries.

This paper describes the association between congenital infections and subsequent diabetes risk in children in the United States using data from a nationwide private health insurer for the years 2001–2017. In total 1 475 587 infants were followed for on average 3.9 years. Information was obtained regarding congenital infections (rubella, cytomegalovirus, other congenital infections) perinatal infections, and the development of diabetes mellitus and diabetic ketoacidosis. 781 infants with congenital infections and 73 974 with perinatal infections were identified. Diabetes developed in 3334 children. The odds of developing diabetes for children with history of congenital rubella infection was 12-fold higher ($P = 0.013$) and for children with congenital cytomegalovirus infection were 4-fold higher ($P = 0.011$) than for those without infection. Children with other congenital infections had 3-fold higher odds of developing diabetes ($P = 0.044$). Results were similar for diabetes ketoacidosis. Children with history of other perinatal infections had a 49% higher odds of developing diabetes ($P < 0.001$).

It is to be concluded that this insurance register-based study shows that congenital and other perinatal infections are associated with higher risks of developing diabetes mellitus during childhood. Hence, vaccination against rubella remains an important preventive action to reduce the incidence of diabetes in children.

From earlier studies prior to vaccination, we know that especially congenital rubella infection increased the risk of development of T1DM in children. Rubella remains an important public health issue, especially in developing countries. Many other congenital infections cannot be prevented by vaccination as yet. Even for preventable infections, vaccine hesitancy is not uncommon, especially in US and Europe. In this US study, even if the absolute numbers of congenital infections were low, the increased risk of diabetes was significant and sizeable. Interestingly also perinatal infections increased this risk (with a lower effect). The authors recommend a vaccination program for women of childbearing age, especially regarding rubella.

10.2. Lower incidence rate of type 1 diabetes after receipt of the rotavirus vaccine in the United States, 2001–2017

Rogers MAM, Basu T, Kim C
Sci Rep. 2019 Jun 13;9(1):7727

As outlined in paper 10.1, intrauterine and early neonatal infections with a number of viruses are thought to contribute to the incidence of type 1 diabetes (T1DM) later in life. Amongst the viruses to be considered, enteroviruses have been found to be the most important. It is therefore important to know whether vaccination against enteroviruses would reduce the incidence of autoimmune disease, and in particular of childhood T1DM.

The association between rotavirus vaccination and the incidence of childhood T1DM was tested in this cohort of 1 474 535 US infants from 2001 to 2017 using data from a nationwide health insurance company. Completion of rotavirus vaccine series was associated with a 33% (95% CI: 17%, 46%) lower risk of childhood T1DM compared to unvaccinated children. Full completion of the pentavalent vaccine series was associated with a 37% (95% CI: 22%, 50%) lower risk of T1DM, but partial or incomplete vaccination showed no association. There was a 31% (95% CI: 27%, 35%) reduction in hospitalizations during the 60-day period after vaccination compared to unvaccinated children. Overall, there was a 3.4% decrease in annual incidence of T1DM in children aged 0–4 in the USA from 2006 to 2017, which coincides with introduction of rotavirus vaccination in 2006. In conclusion (here and in paper 10.3), rotavirus vaccination was associated with a reduced incidence of T1DM. Thus, and importantly, rotavirus vaccination may be the first practical measure that could play a role in the prevention of T1DM (and potentially other autoimmune diseases).

10.3. Association of rotavirus vaccination with the incidence of type 1 diabetes in children

Perret KP, Jachno K, Nolan TM, Harrison LC
JAMA Pediatrics 2019, 173, 280–282

Amongst the enteroviruses that have been associated with the development of type 1 diabetes (T1DM) in children, rotavirus infection is the most common. Using publicly available data from Australian national registries, the authors performed interrupted time-series analyses to compare the incidence of newly diagnosed childhood T1DM during the 8 years before the national introduction in 2007 of oral rotavirus vaccine for all infants aged 6 weeks and older versus the 8 years afterwards. National uptake of rotavirus vaccine was high and estimated to be 84%. Between 2000 and 2015, 16 159 new cases of T1DM in children aged 0 to 14 years were recorded by the National Diabetes Services (24.4 cases [95% CI, 22.4–26.7] per 100 000 children).

Interestingly, in the youngest age group, 0 to 4 years, the incidence of T1DM decreased by 15% (rate ratio, 0.85 [95%CI, 0.75–0.97]; $P = 0.02$) after the introduction of oral rotavirus vaccine in 2007. However, in older children, aged 5–9 years and 10 to 14 years, there was no change in T1DM incidence cases or any temporal differences during the entire 16-year period.

These data (here and in paper 10.2) add evidence that introduction of oral rotavirus vaccination into a national immunization program with a high coverage leads to a decline in the incidence T1DM in young children.

In contrast to data from Finland (comprising a smaller number of cases and shorter time frame of observation), these Australian data showed a reduction of T1DM cases in the youngest age group but not in older children. In the meantime, the overall incidence of T1D in Australia is no more rising and seems to have levelled off. Whether this is an effect of the rotavirus vaccination should be verified by a case control linkage study, as the authors have planned. In other countries, such as Germany, RV vaccination has also been implemented in the national vaccination plan, however without this having led to a decrease in the incidence rate in T1D until now.

10.4. Association of gluten intake during the first 5 years of life with incidence of celiac disease autoimmunity and celiac disease among children at increased risk

Aronsson CA, Lee H-S, Hårdaf Segerstad EM, Uusitalo U, Yang J, Koletzko S, Liu E, Kurppa K, Bingley PJ, Toppari J, Ziegler AG, She J-X, Hagopian WA, Rewers M, Akolkar B, Krischer JP, Virtanen SM, Norris JM, Agardh D, for the TEDDY Study Group
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Some children have a high genetic risk to develop type 1 diabetes (T1DM) and/or celiac disease. However, environmental factors may modify such risks. One arm of the TEDDY study assessed the influence of high gluten intakes on the development of celiac disease in genetically high risk children.

Between 2004 and 2010, 8676 newborns carrying HLA antigen genotypes associated with increased risks of T1DM and celiac disease were enrolled. Screening for celiac disease with tissue transglutaminase autoantibodies was performed annually in 6757 children from age 2 years. Data on gluten intake were

available in 6605 children (98%), estimated using 3-day food records collected at ages 6, 9, and12 months and biannually thereafter up to age 5 years.

The children (49% females) were followed-up for median 9.0 years [interquartile range, 8.0–10.0]), and of these 1216 (18%) developed celiac disease autoimmunity and 447 (7%) developed celiac disease. The peak incidence for both outcomes was at age 2 to 3 years. Gluten intake was associated with higher risk of celiac disease autoimmunity (hazard ratio [HR], 1.30 per gram/day [95% CI, 1.22–1.38]). By age 3 years, the absolute risk of celiac disease autoimmunity in those with the reference gluten intake levels was 28.1%, and was 34.2% in those with 1 gram/day higher intakes (absolute risk difference: 6.1% [95% CI, 4.5%–7.7%]). For celiac disease itself, gluten intake was associated with a HR, 1.50 (95% CI, 1.35–1.66) higher risk per 1 gram/day; absolute risk of coeliac disease by age of 3 years was 20.7% in the reference intake group and 27.9% with 1 gram/day higher intakes (absolute risk difference: 7.2% [95% CI, 6.1%–8.3%]).

In conclusion, higher gluten intake during the first years of life was associated with a higher risk of celiac disease autoimmunity and also celiac disease among these genetically predisposed children of mixed ancestry/ethnicity in Australia.

For dietary recommendations in genetically high risk infants, the estimated cut point for gluten intake was estimated to be >2 gram/day of gluten at ~2 years of age, which is equivalent to one slice of white bread or one portion of pasta. However, it was found in this study that a reduction of gluten or even a gluten free diet in the first year of life has no major impact on the development of T1DM.

10.5. Association of cereal, gluten, and dietary fiber intake with islet autoimmunity and type 1 diabetes

Hakola L, Miettinen ME, Syrjälä E, Akerlund M, Takkinen M-H, Korhonen TE, Ahonen S, Ilonen J, Toppari J, Veijola R, Nevelainen J, Knip M, Virtanen SM
JAMA Pediatr 2019; 173:953–960.

Dietary proteins, such as gluten, have been suggested to serve as triggers of the autoimmune process that leads to type 1 diabetes (T1DM). These authors studied the potential associations of cereal, gluten, and dietary fiber intake with the development of islet autoimmunity (IA) and T1DM.

The prospective birth cohort Finnish Type 1 Diabetes Prediction and Prevention Study initially recruited children with known genetic susceptibility to T1D from 1996 to 2004 from two university hospitals in Finland and followed up every 3–12 months up to 6 years for dietary intake (cereals, gluten, and dietary fiber from repeated 3-day diaries), islet autoantibodies (IA), and manifestation of T1DM. 6081 infants (78% of those invited) participated. Dietary data were available for 5714 (94.0%) and dietary and IA (defined as repeated positivity for islet cell antibodies and at least one of three autoantibodies analyzed, or the presence of T1D) for 5545 (91.2%), of whom 3762 (68%) had IA data up to age 6 years. Data on incident T1DM were available on all children from the Finnish Pediatric Diabetes Register, updated in 2015.

During the 6-year follow-up, 246/5545 (4.4%) children developed IA and 90/ 5714 (1.6%) developed T1DM. For IA, the intakes of oats (hazard ratio [HR] per +1 g/MJ: 1.08; 95% CI, 1.03–1.13), wheat (HR, 1.09; 95% CI, 1.03–1.15), rye (HR, 1.13; 95% CI, 1.03–1.23), gluten-containing cereals (HR, 1.07; 95% CI, 1.03–1.11), gluten without avenin from oats (HR, 2.23; 95% CI, 1.40–3.57), gluten with avenin (HR, 2.06; 95% CI, 1.45–2.92), and dietary fiber (HR, 1.41; 95% CI, 1.10–1.81) were all associated with higher risks. Also for T1DM, the intakes of oats (HR, 1.10; 95% CI, 1.00–1.21) and rye (HR, 1.20; 95% CI, 1.03–1.41) was associated with higher risks. After multiple testing correction, the associations with IA remained statistically significant.

Higher intakes of oats, gluten-containing cereals, gluten, and dietary fiber were associated with an increased risk of IA. This confirms the notion that, beneath substantial genetic components (that do not change over short time periods), environmental factors (that can change quickly) particularly dietary factors are likely to explain the increases in childhood T1DM seen in many countries. Cereals are the most important carbohydrate source in most populations. There is a known link between IA and e.g. the dietary intake of gluten. However, many studies report that gluten free diets do not prevent T1DM in children with high genetic susceptibility (1). In the current highly selected high risk population from Finland, energy adjusted intakes of oats and rye were associated with study development of both IA and T1DM in this. The findings might not be extrapolated to

lower risk populations or different HLA risk cohorts, but they do serve as a model for disease development. Whole grain cereals with high fibre content are usually a good dietary choice in the general population but might not be so in those at high risk for T1DM.

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10.6. Association of type 1 diabetes with standardized test scores of Danish school children

Skipper N, Gaulke A, Möller Sildorf S, Eriksen TM, Nielsen NF, Svensson J
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Type 1 diabetes (T1DM) is clearly associated with higher risks for cardiovascular disease and late complications, such as retinopathy and nephropathy. A putative impact of T1DM on neurocognitive development is less clear. For example, it is unclear whether T1DM is associated with school performance in children.

This study compared standardized reading and mathematics test scores of school children with and without T1DM, using population-based retrospective data from January 1, 2011, to December 31, 2015 on Danish public school children attending grades 2, 3, 4, 6, and 8. Mathematics test scores were accessible in 524 764 children, and reading performance scores in 1 037 006 (mean (SD) age: 10.31 (2.42) years; 51% male). In linear regression models outcomes with and without adjustment for socioeconomic characteristics were analyzed. Primary outcomes were defined as pooled test scores in mathematics and reading.

T1DM was diagnosed in 2031 children. They had similar combined mathematics and reading scores (mean 56.56) to children without diabetes (56.11). No significant difference in combined scores was found between groups in a linear regression model fully adjusted for grade, test topic, year, and socioeconomic status (mean difference: 0.45 (95% CI, −0.58 to 1.49)).

In summary, there was no difference in standardized reading and mathematics test scores of children with T1DM compared to children without diabetes. These data are truly important, considering the plausible risks of both glycopenia on the brain during hypoglycemic events as well as chronic and short-term hyperglycemia on cognitive performance. Some previous studies had shown lower academic performance in children with T1DM, while others had shown no difference. Therefore, the issue remains controversial. Differences between study findings could be explained by effects of hypoglycemia on working memory and language processing. Furthermore, in the current large retrospective Danish cohort, while there was no difference between children with and without T1DM, there was an albeit nonsignificant association of lower test scores with an HbA1c above 7.5%. Also, children with HbA1c $<7.5\%$ scored higher in the tests than the population mean. There was no association with DKA at onset nor with diabetes duration of more than 4 years. However, children with diabetes had missed more tests than their healthy school peers and this points to a possible susceptibility of not reaching their true potential simply through lower school/test attendance rather than through direct neuroglycopenic events.

10.7. Real world hybrid closed-loop discontinuation: Predictors and perceptions of youth discontinuing the 670G system in the first 6 months

Messer LH, Berget C, Vigers T, Pyle L, Geno C, Wadwa PR, Driscoll KA, Forlenza GP
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Dissatisfaction with technologies, discontinuation of use and inappropriate adjustments of insulin pump settings pose important areas of concern in adolescents using diabetes technologies. This study searched for predictors of hybrid closed loop (HCL) discontinuation and perceived barriers to use in 92 adolescents with type 1 diabetes

(T1DM) (age 8–25 years) who had initiated the Minimed 670G HCL system. Participants were followed prospectively for only 6 months and data collected on demographic, glycemic (time-in-range, HbA1c), and psychosocial variables [Hypoglycemia Fear Survey (HFS); and Problem Areas in Diabetes (PAID)]. In addition and importantly, participants who discontinued HCL (<10% HCL use at clinical visit) completed a questionnaire on perceived barriers to HCL use.

Ninety-two participants (15.7 ± 3.6 y, HbA1c 8.8 ± 1.3%, 50% female) initiated HCL, and 28 (30%) discontinued it, the majority (64%) after 3–6 months. Odds of discontinuing HCL was 2.7-fold higher (95% CI: 1.12, 6.28; $P = 0.026$) for each 1% higher baseline HbA1c. Youth who discontinued HCL rated difficulty with calibrations, number of alarms, and too much time needed to make the system work as the biggest problems of HCL. Qualitatively derived themes included technological difficulties (error alerts, not working correctly), too much work (calibrations, finger sticks), alarms, disappointment in glycemic control, and expense (cited by parents).

Youth with higher baseline HbA1c are at higher risk of discontinuing HCL within 6 months and should be the target of new interventions to support device use. The primary reasons for discontinuing HCL relate to the workload required to use HCL. This echoes evidence from an obesity intervention trial where the major reason for non-participation was lack of time, cumbersomeness and effort to attend the program (1).

HCL systems were implemented to improve the time in glucose target range in T1DM patients and to possibly make care easier especially in younger patients. There is evidence that, even in patients with poor metabolic control, such systems can improve time in range. However, with the 670G system the workload will increase (at least for the first months) for a patient transferring from the 640G or other insulin pumps due to need for multiple calibration finger sticks. On the other hand, parents and patients will have to let the system control the child. This in itself is not always easy and may represent an additional psychologic barrier to use. These authors described their intensive efforts at patient education after initiation of HCL. Those patients who discontinued had worse metabolic control and reported using the system and CGM significantly less already in the first month. They disliked the workload due to extra blood glucose measurements and attending to alarms. It is recommended that diabetes teams should develop a system to better identify patients/families who will possibly find HCL to burdensome and focus on education before starting to discuss the use of HCL. Such education should include the potential workload involved and possible problems compared to more traditional treatments. The 670G is the first available HCL system and upcoming systems should be improved regarding ease of calibration and kicking off on auto mode.

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10.8. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes

Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, Gitelman SE, Gottlieb PA, Krischer JP, Linsley PS, Marks JB, Moore W, Moran A, Rodriguez H, Russell WE, Schatz D, Skyler JS, Tsalikian E, Wherrett DK, Ziegler AG, Greenbaum CJ, Type 1 Diabetes TrialNet Study Group

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Type 1 diabetes (T1DM) is a chronic autoimmune disease that leads to destruction of insulin producing beta cells, which leaves the patient dependent on exogenous insulin for survival. Some interventions have delayed the loss of insulin production in T1DM, but interventions to prevent clinical progression before T1DM onset are not yet available.

This phase 2, randomized, double-blind, placebo-controlled trial tested a single 14-day course of teplizumab (an Fc receptor–nonbinding anti-CD3 monoclonal antibody, over) in non-diabetic but high-risk relatives of patients with T1DM. Follow-up for progression to T1DM was performed with 6-monthly oral glucose-tolerance testing.

In total 76 participants (55 [72%] ≤18 years of age) underwent randomization, 44 to teplizumab, and 32 to placebo. The median time to T1DM diagnosis was 48.4 months with teplizumab versus 24.4 months with placebo; T1DM was diagnosed in 19 (43%) of the teplizumab group and in 23 (72%) of the placebo group

(adjusted hazard ratio: 0.41, 95% CI, 0.22 to 0.78; $P = 0.006$). The annualized incidence of T1DM was 14.9% per year with teplizumab and 35.9% with placebo. Rash and transient lymphopenia were expected adverse events on teplizumab. KLRG1 + TIGIT + CD8 + T cells were more common in the teplizumab group versus placebo. Among the participants who were HLA-DR3–negative, HLA-DR4–positive, or anti–zinc transporter 8 antibody–negative, fewer participants in the teplizumab group than in the placebo group developed T1DM. Despite the small size of the trial, it can be concluded that teplizumab delayed progression to T1DM in high-risk participants.

Teplizumab seems a hopeful candidate to reduce or prevent autoimmune beta cell destruction. The strongest effect in this high-risk population was seen in the first year after teplizumab administration. However, and sadly, not every person on the way to T1DM seemed to have benefited in the same way and many different factors may influence the progression of the disease. Especially persons with high anti-ZnT8 antibodies may be less responsive. This study tested a single course of teplizumab. A repeat application may further increase its efficacy. However, in other studies 55% of patients developed antibodies against teplizumab, which may limit repeat treatment cycles. The potential of teplizumab to prolong insulin secretion even after the T1DM onset in children and adolescents is also being explored currently. A trial in younger high-risk patients identified through screening programs should also be designed.

10.9. Improving emergency department management of diabetic ketoacidosis in children

Baumer-Mouradian SH, Gray MP, Wolfgram PM, Kopetsky M, Chang F, Brousseau DC, Frenkel MM, Ferguson CC
Pediatrics 144, number 4, October 2019:e20182984

Diagnostic delays in the pediatric emergency department (ED) can lead to unnecessary interventions, prolonged length of hospital and emergency department stay (LOS) and even death. This is especially true in diabetic patients presenting with possible diabetic ketoacidosis (DKA). At one institution, DKA determination time (from arrival to diagnosis) was 86 minutes, and 61% of patients did not meet the criteria for DKA. As a result, intravenous (IV) placement occurred in 85% of patients without DKA. This study evaluated the implementation of four key interventions (point-of-care (POC) testing, order panels, provider guidelines, and nursing guidelines) to reduce DKA determination time from 86 minutes to a target 30 minutes, and IV placements in patients without DKA from 85% to target level 20% over 18 months.

Between 2015 and 2018, 783 patients with diabetes mellitus were evaluated for DKA. After all four interventions, DKA determination time decreased from 86 to 26 minutes ($P < 0.001$). In patients without DKA, IV placement decreased from 85% to 36% ($P < 0.001$). ED LOS decreased from 206 to 186 minutes ($P = 0.009$) in patients discharged from the hospital after DKA evaluation. POC testing and order panel use increased from 0% to 98% and 90%, respectively. This study shows that using quality-improvement methodology (plan-do-study-act cycles), a meaningful and clinically relevant reduction in DKA determination time, percentage of IV placements, and ED LOS can be achieved.

However, this study also shows that improving quality of care in the ED can be difficult. POC testing of pH and bicarbonate was one crucial step to improve management. However, possibly the data should not be extrapolated to smaller European pediatric diabetes centers, where ketone POC measurements in diabetic patients with typical symptoms of DKA might be sufficient. Written standardised guidelines and repeated training and education of ED staff would most likely improve DKA management and reduce complications and mortality in most health care settings around the world.

10.10. 'I'm essentially his pancreas': Parent perceptions of diabetes burden and opportunities to reduce burden in the care of children < 8 years old with type 1 diabetes

Commissariat PV, Harrington KR, Whitehouse AL, Miller KM, Hilliard ME, Van Name M, DeSalvo DJ, Tamborlane WV, Anderson BJ, DiMeglio LA, Laffel LM
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Across all age groups, management of type 1 diabetes (T1DM) places substantial workload, responsibility and emotional burden, such as worries and stress, on families. This study explored parent perceptions of the burdens of caring for very young children with T1DM. Semi-structured qualitative interviews were conducted with parents (85% mothers) of 79 children with T1DM, aged 1 to <8 years old, in 4 pediatric diabetes clinical centers. Interviews were transcribed, coded, and analyzed using hybrid thematic analysis to derive central themes.

Children (77% white) had diabetes for ≥ 6 months: mean $\pm$ S.D. age was 5.2 $\pm$ 1.5 years, diabetes duration 2.4 $\pm$ 1.3 years, HbA1c 63 $\pm$ 10 mmol/mol (7.9 $\pm$ 0.9%); 66% using insulin pump, and 61% using CGM. Three major themes emerged related to diabetes burden: (a) the emotional burden on the parents and their children, (b) the burden of finding, training, and trusting effective secondary caregivers to help manage the child's diabetes, and (c) suggestions for more comprehensive, personalized diabetes education from healthcare providers for parents and secondary caregivers to reduce parental burden and worry.

In families with very young children with T1DM, parental perceptions of the burden of managing diabetes are common and could be mitigated by tailored and structured education programs that increase parent knowledge, bolster parents' self-confidence, and increase trust in their secondary caregivers to manage diabetes. Reduced parental burden and increased caregiver knowledge may positively impact child's glycemic control, as well as improve parent and child quality of life.

This study addresses very well one of the major burdens of parental care for young children with diabetes. Parents have to explain repeatedly basic knowledge about diabetes management to their children and, even more problematic, to those persons providing secondary care for their child. The education of these secondary caregivers, e.g. in kindergarten or primary school, has to be done in a personised manner. Therefore, parents need the help of diabetes educators and the willingness of those persons who are responsible for children outside the home. Both are time consuming and have to be delivered with care. Even in the US setting described here, a lack of personnel with medical background in kindergartens is described. A recent American Diabetes Association statement demands that all childcare staff responsible for young children should have a basic knowledge of diabetes and its management and know how to get medical help if needed. Staff who are more directly and more often involved in the child's care may even require advanced education, such as regarding insulin administration, carbohydrate counting, and more.

10.11. Screening for retinopathy in children with type 1 diabetes in Denmark

Herskin CW, Olsen BS, Madsen M, Kjaersgaard P, Fredheim S, Johansen A, Kristensen K, Birkebaek NH, Svensson J, Pilgaard KA, Johannesen J

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Children with type 1 diabetes (T1DM) should be screened regularly for signs of retinopathy by fundus photography in order to prevent visual impairment. According to Danish national guidelines, screening should take place at ages 12, 15, and 18 years after a minimum of 3 years of diabetes duration. As glycemic control has improved over time, the prevalence of retinopathy is expected to have decreased.

This study investigated the prevalence, degree, and progression of retinopathy in children with T1DM, to inform the question whether screening at age 12 years is currently still indicated in Denmark. Data on all Danish children with T1DM onset between 2003 and 2013 ($n=2943$) were collected from the 'DanDiabKids' registry. 2382 children had registered screenings.

The prevalence of retinopathy at ages 12, 15, and 18 years was 0.9%, 2.3%, and 3.1%, respectively. Only minimal background retinopathy was detected in over 90% overall, and in 100% at 12 years. On subsequent rescreening, retinopathy had resolved spontaneously in 87.5% of cases overall, and in 100% of cases detected at 12 years.

Thus, the prevalence of retinopathy in Danish T1DM children is low. At 12 years, the prevalence was 0.9%, all with minimal background retinopathy, and this resolved in 100% on rescreening. Retinopathy screening at age 12 years in Denmark does not seem to have any clinical relevance.

Even with improved glycemic control due to intensive insulin treatment, after 30 years diabetes duration 20% of all T1DM patients suffer from proliferative retinopathy. ISPAD guidelines recommend screening for retinopathy from age 11 years onwards and/or after 5 years of diabetes duration. The Danish national diabetes registry provides data on all children with T1DM up to age 18 years. Older Danish data from the 1990s showed evidence of diabetic retinopathy in 17.7%. However, in recent years metabolic control has improved and diabetic retinopathy in this age group seems to have become rare. This is well supported by the data in this study; all patients aged <15 years had only minimal background retinopathy that was completely reversible. Other recent studies have reported even lower prevalence of diabetic retinopathy. Screening recommendations for diabetic retinopathy in children should therefore be revised.

10.12. Glucose-responsive insulin patch for the regulation of blood glucose in mice and minipigs

Jicheng Yu, Jinqiang Wang, Yuqi Zhang, Guojun Chen, Weiwei Mao, Yanqi Ye, Anna R Kahkoska, John B Buse, Robert Langer, Zhen Gu

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Glucose-responsive insulin delivery systems that mimic pancreatic endocrine function could enhance health and improve quality of life for people with type 1 and type 2 diabetes with reduced β-cell function. However, insulin delivery systems with rapid *in vivo* glucose-responsive behaviour typically have limited insulin-loading capacities and cannot be manufactured easily. A single removable transdermal patch, bearing microneedles that had been loaded with insulin and a non-degradable glucose-responsive polymeric matrix, were manufactured by in situ photopolymerization. The device was shown to regulate blood glucose in insulin-deficient diabetic mice and minipigs (for minipigs >25 kg, glucose regulation lasted >20 h with patches of ~5 cm^2).

Under hyperglycaemic conditions, phenylboronic acid units within the polymeric matrix reversibly form glucose-boronate complexes that - owing to their increased negative charge - induce swelling of the polymeric matrix and weaken the electrostatic interactions between the negatively charged insulin and polymers, promoting the rapid release of insulin. This proof-of-concept study may aid the development of other translational stimuli-responsive microneedle patches for drug delivery.

10.13. Six-month randomized, multicenter trial of closed-loop control in type 1 diabetes

Brown SA, Kovatchev BP, Raghinaru D, Lum JW, Buckingham BA, Kudva YC, Laffel LM, Levy CJ, Pinsker JE, Wadwa RP, Dassau E, Doyle FJ 3rd, Anderson SM, Church MM, Dadlani V, Ekhlaspour L, Forlenza GP, Isganaitis E, Lam DW, Kollman C, Beck RW, iDCL Trial Research Group.

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Despite advances in diabetes care, attaining good glycemic outcomes in patients with type 1 diabetes (T1DM) remains challenging and often is not achieved. For example, the targets set by the American Diabetes Association are met in only a minority of patients. It is hoped that the use of a closed-loop system (also referred to as an 'artificial pancreas') that automates aspects of insulin delivery might offer the potential to attain the desired glycemic outcomes. Meta-analyses have suggested that closed-loop systems are effective. Currently, one closed-loop system, the Medtronic MiniMed 670G, is in commercial use in the USA, but randomized trials are needed to assess its efficacy and safety. A system that modulates basal insulin delivery but does not administer automated boluses, is referred to as a "hybrid" closed-loop system.

This 6-month randomized, multicenter trial, a parallel-group, unblinded, randomized trial was conducted at 7 university centers in the USA. Patients with T1DM were assigned in a 2:1 ratio to receive a closed-loop system (Control-IQ, Tandem Diabetes Care) or a sensor-augmented pump (control group). The closed-loop system used an algorithm with a dedicated hypoglycemia safety module, automated correction boluses, and overnight intensification of basal insulin delivery designed to consistently target near-normal glycemia each morning.

168 T1DM patients (age range 14–71 years) were randomized (112 to closed-loop; 56 controls). Baseline HbA1c ranged from 5.4 to 10.6%. All completed the trial. The primary outcome (% time glucose level in target range, 70–180 mg/dl [3.9–10.0 mmol/L] by continuous glucose-monitoring) increased in the closed-loop group from (mean ± S.D.) 61 ± 17% at baseline to 71 ± 12% after 6 months, but remained unchanged at 59 ± 14% in controls (mean adjusted absolute difference, −11%; 95% CI, −9 to −14; $P < 0.001$). Differences between closed-loop and control were larger during the nighttime (midnight to 0600 h), with % time in target 76% and 59%, respectively.

All main secondary outcomes all met the prespecified criterion for significance, favoring the closed-loop system. The mean absolute difference in % time with glucose < 70 mg/dl was −0.88% ($P < 0.001$). The mean adjusted absolute difference in HbA1c after 6 months was −0.33% ($P = 0.001$). In the closed-loop group, the median %time spent in closed-loop mode was 90% over 6 months.

Importantly, no serious hypoglycemic events occurred in either group. 17 adverse events were reported in 16 patients in the closed-loop group, and 2 adverse events in 2 patients in the control group ($P = 0.05$). Diabetic ketoacidosis occurred in 1 participant on closed-loop due to pump infusion set failure; 13 other hyperglycemia or ketosis events occurred in 12 patients on closed-loop, and 2 events in 2 patients in controls; almost all these events were adjudicated as due to infusion set failures. There were 3 other serious adverse events in the closed-loop group (hospitalizations for concussion, otitis, and cardiac bypass surgery) and none in the control group.

Of note, in March 2019 use of the Control-IQ software used by the closed-loop group was temporarily suspended as a precaution after a software error was found. No serious adverse events occurred, but in some instances this led to erroneous discontinuation of insulin delivery for up to several hours, or an erroneous bolus when insulin delivery restarted. Patients continued to use the system in open-loop mode until a software update was remotely deployed to patients via a Web-based software updater. This suspension affected 33 patients on closed-loop for up to 4 weeks (median, 14 days). The analyses included all data recorded during this period, even if the closed-loop mode was not in use.

Strengths of the trial include the inclusion of patients across a wide range of baseline characteristics, 100% patient retention, and good adherence to both intervention and control devices. Continuous glucose monitoring was used to assess outcomes, with minimal reliance on blood glucose measurements. The trial was conducted without remote monitoring by investigators, to reflect real-world use (8, 9, 10, 11). The authors rightly point out limitations of the trial. There were more unscheduled contacts in the closed-loop group, attributed to the use of a new device, and the insulin pumps used by the controls did not have a feature to suspend insulin infusion for predicted hypoglycemia, which is now available on some pumps and has been shown to avoid hypoglycemia. Interpretation of the results must be viewed in the context of the characteristics of the participants and the university-based clinics setting. At trial enrollment, 70% had already been using CGM and 79% an insulin pump, which are substantially more than in typical T1DM patients, and could reflect a strong interest in and willingness to use a closed-loop system among patients who were already familiar with such devices (4, 5, 6, 7).

In conclusion, over a 6-month period, this closed-loop system increased % of time that glucose levels were in target, and reduced hyperglycemia, hypoglycemia, and HbA1c compared to a sensor-augmented pump.

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10.14. Yield of a public health screening of children for islet autoantibodies in Bavaria, Germany

Ziegler AG, 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

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doi: 10.1001/jama.2019.21565. PMID: 31990315

It is unclear how many children in the general population have features of anti-islet cell autoimmunity without later developing type 1 diabetes (T1DM). This public health screening program determined the population prevalence of islet cell autoantibodies (ICA) and the risk for progression to T1DM.

Within this research setting, ICA screening was offered to all children aged 1.75 to 5.99 years in Bavaria, Germany, between 2015 and 2019 by primary care pediatricians during routine well-baby visits. Families of children with multiple ICA were invited to participate in a program of diabetes education, metabolic staging, assessment of psychological stress associated with diagnosis, and prospective follow-up for progression to clinical T1DM until July 31, 2019.

The primary outcomes were presymptomatic T1DM, defined by two or more ICA and categorized into stage 1 (normoglycemia) or stage 2 (dysglycemia), and clinical T1DM (stage 3). Secondary outcomes were diabetic ketoacidosis and parental psychological stress, assessed by the Patient Health Questionnaire-9 (range, 0–27; ≤ 4 indicates no/minimal depression; >20 indicates severe depression).

Of 90 632 children screened (median [interquartile range {IQR}] age, 3.1 [2.1–4.2] years; 48.5% girls), 280 (0.31%; 95% CI, 0.27–0.35) had presymptomatic T1DM, including 196 (0.22%) with stage 1, 17 (0.02%) with stage 2, 26 (0.03%) with clinical T1DM (and 41 who were not staged). After a median (IQR) follow-up of 2.4 (1.0–3.2) years, another 36 children developed clinical T1DM. The 3-year cumulative risk for clinical T1DM in the 280 children with presymptomatic T1DM was 24.9% ([95% CI, 18.5%–30.7%]; 54 cases; annualized rate 9.0%). Two children had diabetic ketoacidosis. Median (IQR) psychological stress scores were increased at the time of metabolic staging in mothers of children with presymptomatic T1DM (3 [1–7]) compared with mothers of children without ICA (2 [1–4]) ($P = 0.002$), but declined after 12 months of follow-up (2 [0–4]) ($P < 0.001$).

Among children aged 2–5 years in Bavaria, Germany, a program of primary care–based screening showed the prevalence of multiple ICA was 0.31%. These findings have implications for population-based screening of children for ICA in other settings and also for future prevention programs. Unfortunately, previous similar large-scale screening campaigns have not yet led to any real and practical prevention strategy, which implies that measuring ICA alone does not reveal the full picture of prediabetes nor does it necessarily inform prevention strategies and their principles.

11. Obesity and Weight Regulation

Martin Wabitsch, Daniel Tews, Julia von Schnurbein, Melanie Schirmer, Eleni Giannopoulou, Christian Denzer

Division of Pediatric Endocrinology and Diabetes and Endocrine Research Laboratory, Department of Pediatrics and Adolescent Medicine, University of Ulm, Ulm, Germany

Preface

We have been very happy to get around 1,500 papers out of our established search strategy in PubMed which have been saved in our 2020 yearbook EndNote database. We have then selected 16 papers (1%) which in our mind have been the most exciting ones. The highlights in this year chapter are publications about new gene variants with major effects on weight regulation, updates on pharmacological and surgical treatment of obesity in adolescents. The Yearbook chapter 2020 on obesity and weight regulation comprises further exciting articles covering a broad research area.

Corona and Obesity

11.1. Obesity could shift severe COVID-19 disease to younger ages

Kass DA, Duggal P, Cingolani O

Johns Hopkins University School of Medicine, Johns Hopkins University, Baltimore, Maryland 21205, USA

dkass@jhmi.edu

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This study tested the association between age and BMI using data from patients ($n = 265$) with COVID-19 infection treated at the intensive care units at 6 US University Hospitals. Results show a significant inverse correlation between age and BMI in this sample. Interestingly, adolescents and young adults were more likely to be obese than older COVID-19 patients. This observation leads to the assumption that in populations with a high prevalence of obesity, COVID-19 will affect younger populations more than previously reported.

Risk factors for critical illness with COVID-19 in children include obesity, as found in 2143 pediatric patients in China (1). Earlier reports from China have already shown that obesity was associated with a 142% higher risk of the occurrence of severe pneumonia in patients with COVID-19 infection in patients treated in Shenzhen (2). BMI >40 kg/m^2 was one of the strongest risk factors for hospitalization in 5,279 patients with COVID-19 infection as reported by the health system in New York City <(3). These data which are further supported by data published in the past weeks clearly indicate that obesity and the obesity-related comorbidities not only predispose to a severe course of COVID-19 disease but also increase the risk for a severe COVID-19 disease in youth.

Even children and adolescents with obesity often have respiratory dysfunction, which is characterized by alterations in respiratory mechanisms, increased airway resistance, impaired gas exchange and low lung volume and muscle strength (4). In patients with abdominal obesity, pulmonary function is also compromised by decreased diaphragmatic excursion. Obese people are therefore predisposed to hypoventilation-associated pneumonia and pulmonary hypertension (5). Furthermore, metabolic disturbances in obesity, mainly insulin resistance and chronic subclinical inflammation, are considered to result in increased vulnerability to pneumonia-associated organ failures. Increased inflammatory cytokines present in patients with obesity contribute to a more severe course of the infection.

It is interesting to remember the H1N1 influenza virus epidemic 10 years ago. The CDC estimated that 41–85 million people were infected, between 180 000 and 370 000 were hospitalized and 8000 to 17 000 died due to the infection (6). Also, here obesity was shown to be a major risk factor for a severe course of the disease.

In conclusion, obesity is a main risk factor for a severe course of COVID-19 infection and increases the risk for critical illness at young age. Measurement of anthropometric and metabolic parameters is crucial to better estimate the risk of complications in patients with COVID-19.

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New Genetic Findings

11.2. Loss-of-function mutations in MRAP2 are pathogenic in hyperphagic obesity with hyperglycemia and hypertension

Baron M, Maillet J, Huyvaert M, Dechaume A, Boutry R, Loiselle H, Durand E, Toussaint B, Vaillant E, Philippe J, Thomas J, Ghulam A, Franc S, Charpentier G, Borys JM, Lévy-Marchal C, Tauber M, Scharfmann R, Weill J, Aubert C, Kerr-Conte J, Pattou F, Roussel R, Balkau B, Marre M, Boissel M, Derhourhi M, Gaget S, Canouil M, Froguel P, Bonnefond A
NRS UMR 8199, European Genomic Institute for Diabetes, Institut Pasteur de Lille, University of Lille, Lille, France;
Department of Metabolism, Section of Genomics of Common Disease, Imperial College London, London, UK. p.froguel@imperial.ac.uk

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These authors sequenced the gene for melanocortin-2 receptor accessory protein (*MRAP2*) in 9418 blood DNA samples from several population studies. They detected 23 rare heterozygous variants, which were significantly associated with an increased risk of obesity (OR 3.8 in children and 2.9 in adults). More so, functional analysis revealed a loss of function for 6 variants, with significantly decreased cAMP-PKA signalling *in vitro* in response to α-MSH and ACTH, and these variants showed a complete penetrance for obesity/overweight. In addition, affected carriers also showed a high rate of hyperglycemia and hypertension – in contrast to carriers of other forms of monogenic obesity. None of the carriers showed hypoadrenalism.

Data in rodents and humans have previously indicated that gene coding variants in *MRAP2* are associated with obesity (1, 2). With an increasing worldwide prevalence of obesity, it is of great interest to understand the genetic background of obesity. Rare variants in over 15 genes have been identified so far, which are believed to be causal for monogenic obesity. However, evidence especially for autosomal dominant variants are equivocal. Functional testing of new variants even in well-established obesity genes is hence mandatory, to ascertain their causal role in the development of obesity. This is illustrated best by variants in the MC4 receptor gene where proven loss of function variants confer an increased risk for obesity, gain of function variants are protective against obesity (see paper 11.3), and some rare non-synonymous variants have no measurable effect at all. Hence this thorough

examination of the functional impact of *MRAP2* variants helps to ascertain their causal role in the development of obesity. Very interesting is also the detailed comparison to reported rates of co-morbidities in other forms of monogenic obesity which indicates that variants in *MRAP2* might influence more than just body-weight.

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11.3. Human gain-of-function MC4R variants show signaling bias and protect against obesity

Lotta LA, Mokrosinski J, Mendes de Oliveira E, Li C, Sharp SJ, Luan J, Brouwers B, Ayinampudi V, Bowker N, Kerrison N, Kaimakis V, Hoult D, Stewart ID, Wheeler E, Day FR, Perry JRB, Langenberg C, Wareham NJ, Farooqi IS
University of Cambridge MRC Epidemiology Unit, Welcome Trust-MRC Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK
luca.lotta@mrc-epid.cam.ac.uk.
Cell 2019;177 (3):59—607.e9.
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https://pubmed.ncbi.nlm.nih.gov/31002796/

A recent GWAS showed that the heritability of thinness was comparable to that of obesity (1). Some loci showed effects across the entire BMI distribution. This is also true for variants in *MC4R*. The present study analyzed data on ~0.5 million people from UK Biobank, with a focus on 61 nonsynonymous variants identified in *MC4R*. Of these 61 variants, 12 were nonsense/frameshift variants and 49 were missense variants.

Two functional read outs were investigated in a cellular model: i) canonical Gas-mediated cAMP production and ii) the recruitment of b-arrestin to MC4R. In contrast to most previous studies of human *MC4R* variants, which measured the accumulation of cAMP, the majority of *MC4R* variants investigated here affect both cAMP production and the recruitment of b-arrestin-2 to MC4R. Interestingly, the vast majority of the variance (88%) in the association of BMI with the different *MC4R* variants was explained by their functional effects on b-arrestin recruitment.

Moreover, the investigators found that, in UK Biobank, ~1 in 16 participants (6%) were carriers for gain-of-function (GoF) *MC4R* alleles that exhibited signaling bias, preferentially increasing b-arrestin recruitment rather than cAMP production. These GoF mutants which resulted in an increased b-arrestin recruitment showed an enhanced signaling via the MAPK pathway.

Accordingly, individuals with a GoF allele had significantly lower BMI and up to 50% lower risk of obesity, type 2 diabetes, and coronary artery disease.

To provide some illustrative data, the investigators calculated that the BMI of carriers of one GoF *MC4R* allele was on average 0.39 kg/m^2 lower than noncarriers. Those who carried two GoF alleles had a BMI which was on average 0.88 kg/m^2 lower. In an individual with a height of 1.7 m this would correspond to a body weight reduced by 2.5 kg.

In contrast to previous studies showing that the accumulation of cAMP is critical for *MC4R* signaling, these data demonstrate that *MC4R*signaling through b-arrestin is responsible for its regulation of body weight. These results may be the basis for the development of b-arrestin-biased *MC4R* agonists to induce weight loss as well to treat obesity-associated metabolic comorbidities. The authors make the point impressively and suggest that the clinical effects of genetic variants found in populations that exhibit natural signaling bias for a given pathway can serve as a 'blueprint' for modulating that pathway pharmacologically with a biased agonist.

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Basic Science

11.4. Omega-3 fatty acids activate ciliary FFAR4 to control adipogenesis

Hilgendorf KI, Johnson CT, Mezger A, Rice SL, Norris AM, Demeter J, Greenleaf WJ, Reiter JF, Kopinke D, Jackson PK
Baxter Laboratory, Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, California 94305, USA
pjackson@stanford.edu
Cell. 2019;179, 1–17.
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https://pubmed.ncbi.nlm.nih.gov/31761534/

The current study demonstrates that primary cilia, which are present on all adult mesenchymal stem cells including preadipocytes, play a major role in adipogenic differentiation. Using a transgenic mouse model where all preadipocytes were devoid of cilia, they could elegantly show that cilia are crucial for adipose tissue development. Further, adipogenesis was dependent on a cilia-located G-protein coupled receptor, FFAR4, which senses ω3 fatty acids.

Adipose tissue mass is flexibly controlled in response to nutritional status by altering adipose volume (hypertrophy) and adipocyte number (hyperplasia). As excessive expansion of the adipose tissue by hypertrophy is associated with metabolic complications (1), the adipogenic potential of preadipocyte is a determinant of metabolic health by balancing between hypertrophy and hyperplasia. Understanding the mechanisms which regulate this adipogenic potential might be helpful to develop novel strategies to counteract the metabolic syndrome.

Mechanistically, FFAR4 activation leads to increase of cAMP levels, and downstream activation of the CREB and EPAC signaling pathways. Analysis by transcriptomics and investigating the accessibility of DNA for transcription factors, further revealed the implication of chromatin remodeling in the process of the FFAR4-driven transcriptional adipogenic program. Of note, FFAR4 could not be activated by saturated fatty acids.

Previous studies have demonstrated that dietary supplementation with ω3 fatty acids improves insulin sensitivity and inhibits adipose tissue inflammation in both mice and humans (2). It could be thus speculated that such diets shift adipose expansion towards hyperplasia, by inducing adipogenic potential of preadipocytes. The impact of ω3 fatty acids on adipogenesis *in vitro* is however controversial, warranting further research.

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Body Weight and Appetite/Energy Regulation

11.5. Appetite control is improved by acute increases in energy turnover at different levels of energy balance

Hägele FA, Büsing F, Nas A, Hasler M, Müller MJ, JE Blundell, Bosy-Westphal A
Department of Human Nutrition, Institute of Human Nutrition and Food Sciences, Christian-Albrechts University, Kiel, Germany; Department of Applied Nutritional Science and Dietetics, Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany
abosyw@nutrition.uni-kiel.de.

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PMID 31305927.
https://pubmed.ncbi.nlm.nih.gov/31305927/#affiliation-1

In this randomized crossover study, 16 healthy adults were examined in a metabolic chamber during four different conditions of energy balance (ad libitum energy intake, zero energy balance, 25% caloric restriction, and 125% overfeeding) and at three levels of energy turnover (ET): Physical activity level (PAL) 1.3 low, 1.6 medium, and 1.8 high ET, achieved by walking on a treadmill.

Interestingly, compared to high ET, low ET was associated with increased subjective appetite ratings assessed by visual analog scales, a higher desire to eat and a positive energy balance during subsequent ad libitum food intake. Low ET resulted also in a decrease in circulating GLP-1 and an increase in ghrelin. On the other hand, high ET was associated with decreased appetite, high GLP-1 serum levels and low ghrelin serum levels.

The control of body weight is achieved by influencing energy intake (EI) through food intake and energy expenditure (EE) through physical activity. Energy turnover (ET) consisting of EI and EE should be kept in a balance. Easy in thinking and from a simple teleological point of view, endocrine signals, e.g. from adipose tissue and the gastrointestinal tract, report the current state of EB to key brain regions and EE should directly impact the control of appetite and EI. However, it has been hypothesized that the control of body weight might be easier in conditions when energy turnover (ET) is high as compared to conditions with low ET.

This study in healthy individuals now demonstrates two important physiological results: i) appetite is regulated more effectively at a high level of ET and ii) high level physical activity not only helps to control body weight by increasing energy expenditure but results impressively in an improved appetite sensation. Therefore, these results contrast with some paradigms related to the concept of body weight control.

There have been observations in the past – and every one of us reflecting on their lifestyle and doing more exercise may have noticed this subjectively – indicating that there might be an asymmetric control of EB with a 'regulated zone' at high EE and an 'unregulated zone' at low EE (1).

In addition, it has been hypothesized that there exists an 'exercise-induced anorexia' mediated by lowered concentrations of acylated ghrelin and elevated concentrations of GLP-1 and PYY [reviewed in (2)].

This study is the first to prove an asymmetric control of appetite, with a high ET that improves appetite control. The study was even able to show that this is probably causally associated with increased GLP-1 and decreased ghrelin concentrations independent of EB.

It is worth noting that the investigators chose low-intensity physical activity to increase the PAL. This simulates daily activities without the effects of vigorous exercise on metabolism and avoids fatigue or pain in persons with very low fitness. For sure, increasing physical activity in daily life may be easier for most people than reducing food consumption while maintaining an equal EB.

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11.6. Leptin's hunger-suppressing effects are mediated by the hypothalamic–pituitary–adrenocortical axis in rodents

Perry RJ, Resch JM, Douglass AM, Madara JC, Rabin-Court A, Kucukdereli H, Wu C, Song JD, Lowell BB, Shulman GI
Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215USA

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, USA
blowell@bidmc.harvard.edu
gerald.shulman@yale.edu

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In this paper, Perry *et al.*studied several animal models to disentangle the mechanism by which leptin suppresses hunger. In rats, the hyperphagia induced by a 48 h fast, or a hypoglycemic hyperinsulinemic clamp, or uncontrolled diabetes, was completely suppressed by treatment with a glucocorticoid receptor antagonist, despite low leptin levels. In contrast, external administration of corticosterone overcame the food suppressive effects of leptin rescue. In mice, overexpression of the cortisol inactivating enzyme 11β-hydroxysteroid dehydrogenase type 2 in agouti-related peptide producing neurons suppressed both fasting and corticosterone-induced hyperphagia. Both fasting and corticosterone administration increased firing rate in electrophysiological studies.

From their results, the authors conclude that hyperphagia in fasting and poorly controlled diabetes is dependent upon hypercorticosteronemia, and leptin's effect on food intake is mediated via suppression of the hypothalamus-adrenocortical axis.

With these observations, the authors add an important piece of information to the complex regulation of satiety in very thorough experiments, thus returning to the old model that glucocorticoids are at the centre of energy balance. However, they are not the first group to examine this interaction, and previous results are contradictory. While one group also found that leptin administration can suppress raised corticosterone levels in rats with uncontrolled diabetes (1), in contrast others observed an increase in corticosterone production by leptin treatment in rats both *in vivo* (2) and also *in vitro* (3). Also, in a previous study in adrenalectomized *ob/ob* mice, leptin reduced food intake and body weight independently of absence or presence of concomitant corticosterone substitution (4). This complexity is highlighted by the fact that even in the current study, the reduction of elevated corticosterone levels in fasted rats by leptin substitution did not reach significance.

Therefore, the conclusion of these authors is maybe too bold – suppression of an activated hypothalamus-adrenocortical axis might be one pathway through which leptin suppresses hunger in rodents, but most likely not the only one.

How much of a role this mechanism of leptin action plays in humans remains even less clear. While one study in humans with congenital leptin deficiency (CLD) found increased cortisol levels in the leptin-naïve state (5), no other study replicated this finding (6, 7). Furthermore, leptin substitution in CLD patients did not reduce cortisol levels (6) but even increased them (8).

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11.7. Leptin mediates postprandial increases in body temperature through hypothalamus–adrenal medulla–adipose tissue crosstalk

Perry RJ, Lyu K, Rabin-Court A, Dong J, Li X, Yang Y, Qing H, Wang A, Yang X, Shulman GI
Yale School of Medicine, New Haven, Connecticut, USA
Gerald.shulman@yale.edu
J Clin Invest 2020;130(4):2001–16.
https://pubmed.ncbi.nlm.nih.gov/32149734/

In this study, Perry et al used their well-established animal model of fasted rats to unravel how feeding induces hyperthermia and energy expenditure.

They showed that intracerebroventricular injection of leptin normalized fasting induced low plasma epinephrine and norepinephrine levels and body temperature mimicking postprandial conditions. The rise in body temperature seems to be mediated via β-adrenergic stimulation of adipose tissue triglyceride lipase, mostly in brown adipose tissue (BAT), as antagonism with atenolol fully abrogated the thermogenic responses to both refeeding and to leptin, whereas infusing epinephrine replicated leptin's effect of raising body temperature. Inhibition of adipose triglyceride lipase abrogated the increase in body temperature observed after epinephrine infusion, which was also reduced by removal of the BAT. The capability of feeding to stimulate leptin production depended on time restriction, as continuous refeeding could not raise leptin, catecholamines levels, or body temperature.

Meal thermogenesis contributes to daily energy expenditure and many (1, 2), although not all (3), studies have shown that meal thermogenesis is diminished in obese individuals, which might contribute to the pathogenesis of obesity. Hence, regulation of food induced thermogenesis is of special interest.

Serum catecholamine concentrations are known to increase after meal ingestion (4), therefore β-adrenergic activity is an interesting potential mediator of postprandial increases in body temperature. Fittingly, it is now well known that in rats intravenous infusion of leptin, (5–7) selective injection of leptin into the brain, (8–11) or site-specific activation of leptin-receptors in the dorsomedial hypothalamus (12) increases sympathetic nervous activity in various tissues including the BAT (6, 8–11).

How readily can we translate these results into humans? So far, it has not been possible to demonstrate an increase in catecholamine levels during leptin substitution in patients with congenital leptin deficiency (13), although leptin might still increase regional sympathetic nervous activity. Of special interest for obese patients is the possibility that feeding stimulates leptin production, catecholamines levels, and body temperature depending on time restriction, which might explain some of the benefits of intermittent fasting (14).

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Body Weight Regulation and Insulin Sensitivity

11.8. Brain insulin sensitivity is linked to adiposity and body fat distribution

Kullmann S, Valenta V, Wagner R, Tschritter O, Machann J, Häring HU, Preissl H, Fritsche A, Heni M
Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Tübingen, Germany
martin.heni@med.uni-tuebingen.de
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This study by Kullmann *et al.* investigated the impact of brain insulin resistance on medium and long-term changes in body weight and body fat in adults at high risk for T2DM, undergoing a 24-months lifestyle intervention program ($n = 28$ at the 24-months follow-up, $n = 15$ at the 9-years follow-up). Study participants underwent two hyperinsulinemic-euglycemic glucose clamps with cerebrocortical activity assessed by magnetoencephalography before and during the clamps. Subjects with high brain insulin sensitivity lost significantly more body weight (ø -7.2 kg) after two years of lifestyle intervention and also showed a pronounced reduction of visceral adipose tissue (ø -1.3 l) compared to subjects with brain insulin resistance. Importantly, baseline brain insulin sensitivity was associated with lower regain of body weight and smaller increases in total and visceral fat mass in the long-term follow-up spanning a period of 9-years. To further test for the role of CNS insulin responsiveness as a potential determinant of body fat distribution, the investigators obtained data on brain insulin sensitivity using fMRI of the hypothalamic region with administration of intranasal insulin in a cross-sectional cohort ($n = 112$) also participating in the aforementioned lifestyle intervention program. Better hypothalamic insulin responsiveness was significantly associated with less visceral adipose tissue independent of age, sex, and BMI. No association could be found for hypothalamic insulin sensitivity and subcutaneous adipose tissue. Hypothalamic insulin response was furthermore associated with lower HbA1c, lower fasting glucose and lower HOMA-IR.

Insulin resistance at the level of skeletal muscle or the liver is a hallmark feature of the pathogenesis of type 2 diabetes mellitus and the name-giving component of the metabolic or *insulin resistance* syndrome. Over the past three decades, detailed knowledge has been amassed about the pathophysiology of impaired insulin signaling in many types of tissues under conditions of overfeeding and obesity, and its consequences for substrate flux and development of obesity-associated comorbidities (1). But what about the brain? Glucose utilization in the CNS does not depend on insulin, but this does not imply that brain functioning is unaffected by insulin signaling. Indeed, insulin receptors are expressed abundantly in the brain with highest expression rates in humans seen in cortical and subcortical regions, the cerebellum and also prominently in the hypothalamus (2). Insulin exhibits anorexigenic properties when administered to rodent brains. Experimental data suggests that brain insulin in humans affects lipid metabolism in liver and visceral adipose tissue (3, 4) and also improves whole body insulin sensitivity by stimulating peripheral glucose uptake and suppressing endogenous glucose production (5–7). Interestingly, brain sensitivity to insulin shows considerable inter-individual variability. Obese subjects seem to be more often affected by 'brain insulin resistance', although it remains unclear whether this observation can be interpreted as a consequence of obesity or as a potential causal mechanism that promotes weight gain and ectopic fat accumulation (8).

In conclusion, the results of Kullmann *et al.* add an important piece to the puzzle of obesity pathogenesis, underscoring the role of brain insulin sensitivity as a determinant of body fat distribution and predictor of long-term changes in subcutaneous and visceral fat accumulation, which will very likely have important implications for an individual's cardiometabolic risk.

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11.9. Chronic mirabegron treatment increases human brown fat, HDL cholesterol, and insulin sensitivity

O'Mara AE, Johnson JW, Linderman JD, Brychta RJ, McGehee S, Fletcher LA, Fink YA, Kapuria D, Cassimatis TM, Kelsey N, Cero C, Sater ZA, Piccinini F, Baskin AS, Leitner BP, Cai H, Millo CM, Dieckmann W, Walter M, Javitt NB, Rotman Y, Walter PJ, Ader M, Bergman RN, Herscovitch P, Chen KY, Cypess AM

Diabetes, Endocrinology, and Obesity Branch, Intramural Research Program, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH, Bethesda, Maryland, USA

aaron.cypess@nih.gov

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This clinical study addressed the chronic effects of the β3-adrenergic receptor agonist mirabegron on BAT activity, blood parameters, and insulin sensitivity in a small cohort of healthy women ($n = 14$). Participants were treated for 4 weeks orally with mirabegron 100 mg per day. BAT activity was assessed by 18F-FDG PET/CT at baseline and after the treatment period. Additionally, resting energy expenditure (REE), plasma metabolites, as well as glucose and insulin metabolism were investigated.

Attempts to treat obesity by behavioral interventions alone have been largely unsuccessful, thus complementary pharmacological treatment might be necessary to improve outcomes. In rodent models, targeting β3-adrenergic receptors seemed to be an attractive target as agonists improved energy expenditure and glucose homeostasis (1). However, in humans, limited bioavailability and cardiovascular side effects hampered consideration of β3 agonists a useful therapy (2). Recently, mirabegron, a β3 agonist used to treat overactive bladder, was reported to improve glucose homeostasis and insulin sensitivity in mice (3), by inducing brown adipose tissue (BAT) activity.

In accordance with previous evidence for acute effects of mirabegron in male subjects (4), here, chronic mirabegron treatment increased BAT activity and REE, but had no effect on body weight or body composition. Additionally, treatment improved insulin sensitivity and glucose tolerance as well as insulin secretion. These data demonstrate that chronic mirabegron treatment can activate BAT and may be useful to improve glucose metabolism. However, it still must be addressed whether the improved glucose homeostasis is a direct result of

BAT activation. One interesting aspect could be the increase in plasma bile acid levels seen in this study and a previous one (5). Bile acids have been demonstrated to have multiple beneficial effects on metabolism via the liver, intestine, BAT, and microbiome. Further studies might address whether bile acids contribute to the metabolic effects of chronic β3-adrenergic treatment.

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11.10. The β3-adrenergic receptor agonist mirabegron improves glucose homeostasis in obese humans

Finlin BS, Memetimin H, Zhu B, Confides AL, Vekaria HJ, El Khouli RH, Johnson ZR, Westgate PM, Chen J, Morris AJ, Sullivan PG, Dupont-Versteegden EE, Kern PA

College of Medicine, University of Kentucky, Lexington, Kentucky, USA

philipkern@uky.edu

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This clinical study assessed the effect of the β3-adrenergic agonist mirabegron on glucose homeostasis in obese individuals.

Chronic activation of β3-receptors in mice leads to the appearance of brown-like (e.g. 'beige') adipocytes in white adipose tissue, a process referred to as 'browning'. Activation of brown adipose tissue (BAT) as well as browning has proved beneficial in rodent models (1), including improved glucose homeostasis. Mirabegron, a β3-receptor agonist approved for the treatment of overactive bladder, was recently reported to acutely activate BAT and increase resting energy expenditure in humans (2).

Here, the authors investigated whether treatment with mirabegron is beneficial for glucose homeostasis in adipose tissue and skeletal muscle of obese individuals. The study was performed in $n = 13$ subjects (age 35–65, BMI>27) who received mirabegron 50 mg/day for 12 weeks. Before and after treatment, body composition (DEXA), OGTT, euglycemic clamp, and biopsies from muscle and s.c. adipose tissue was assessed. Additionally, PET/CT scans after cold exposure were performed to measure BAT activity. Overall, mirabegron treatment significantly improved glucose tolerance, insulin sensitivity and HbA1c levels. At the adipose level, mirabegron stimulated lipolysis, reduced fibrotic fiber protein expression, and increased the number of alternatively activated macrophages. In skeletal muscle, where β3-receptors are absent, triglyceride levels were reduced, and type-I fibers increased.

This study demonstrates for the first time that treatment with mirabegron in an FDA-approved dose is effective at improving glucose homeostasis in obese individuals. Probably because of the low mirabegron dosage, treatment had no cardiac side effects in contrast to previous studies using acute high doses (2). Interestingly, mirabegron also induced marked changes in skeletal muscle, which is devoid of b3-receptors, indicating that these changes are secondary to those in adipose tissue. However, mirabegron did not reduce body weight and failed to induce BAT activation as shown in studies with mice and humans (2–4) This might be a result of low BAT presence in

obesity or due to the low dose of mirabegron used here. Larger studies including placebo controls might reveal if the effects mirabegron persist in the long run.

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11.11. Steroid metabolomic signature of insulin resistance in childhood obesity

Gawlik AM, Shmoish M, Hartmann MF, Wudy SA, Hochberg Z
Department of Pediatrics and Pediatric Endocrinology, School of Medicine in Katowice, Medical University of Silesia, Upper Silesia Children's Care Health Centre, Katowice, Poland
agawlik@mp.pl
Diabetes Care. 2020;43(2):405–410.
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Here, Gawlik *et al.*describe the urinary steroid metabolomic profile associated with insulin resistance (IR) as assessed in a cohort of 87 non-syndromic obese children and adolescents.

To explore the previously defined novel concept of a disease-specific steroid metabolomic signature (1), these authors investigated the steroid metabolomic profile (31 steroid metabolites) using gas chromatography-mass spectrometry in urine samples from obese children. 24-h urine collection is recognized as a stable and reproducible method for steroid metabolite assessment. Interestingly, the metabolomic profile of the children with IR (20/87 children) showed significantly higher levels of steroids from all three adrenal pathways (adrenal androgens, glucocorticoids and mineralocorticoids) compared to children without IR. In addition, children with IR presented higher 5α-reductase and 21-hydroxylase activities, and lower 11βHSD1 activity.

It is well accepted that glucocorticoids can induce IR. The findings here demonstrate convincingly that IR and hyperinsulinemia are associated with higher adrenal steroid production. Based on these findings, the authors suggest that the adrenal gland per se is a target of IR or hyperinsulinemia. The underlying regulation is complex and one obvious regulatory mechanism is direct stimulation by insulin of the expression of steroidogenic factor-1 and steroidogenetic genes, independent of the CRH-ACTH-MC2R-PKA pathway. Thereby, hyperinsulinemia in IR may directly increase generation of adrenal hormones. Given the fact, that glucocorticoids induce IR, the authors propose a vicious cycle involving these factors.

Due to the small sample size, more studies are needed to verify these exciting results and the innovative underlying concept. Identifying the IR-specific steroid metabolomic profile of obese children may facilitate the design of effective and personalized therapeutic strategies. It would be interesting to investigate whether children with IR may benefit from e.g. a pharmacological intervention to reduce their exaggerated adrenal steroidogenesis.

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11.12. A randomized, controlled trial of liraglutide for adolescents with obesity

Kelly AS, Auerbach P, Barrientos-Perez M, Gies I, Hale PM, Marcus C, Mastrandrea LD, Prabhu N, Arslanian S; NN8022-4180 Trial Investigators

Department of Pediatrics and Center for Pediatric Obesity Medicine, University of Minnesota Medical School, Minneapolis, USA

kelly105@umn.edu

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Kelly *et al.*randomly assigned (1:1) $n=251$ obese adolescents (age 12 to <18 years) to receive either liraglutide (3.0 mg), a long-acting glucagon-like peptide-1 agonist, or placebo subcutaneously once daily in addition to lifestyle therapy. Liraglutide was superior to placebo in terms of reducing BMI z-score at week 56 from baseline (–0.22; 95% confidence interval –0.37 to –0.08). Liraglutide reduced BMI by –4.64 percentage points, and body weight by –4.50 kg, more than placebo. The most commonly reported adverse events were – as expected from clinical trials in adults – typical gastrointestinal side effects of GLP-1 analogue therapy (nausea, vomiting, diarrhea) which became less frequent over the course of treatment. Only very few participants in both groups had serious adverse events ($n=3$ vs. $n=5$).

The prevalence of obesity in children and adolescents has stabilized at a high level in recent years (1,2). The question of effective therapy strategies remains an important challenge with, among other factors, relevant health economic dimensions due to the high number of individuals affected.

Evidence-based guidelines for the treatment of obesity in children and adolescents recommend the implementation of multimodal lifestyle interventions with the aim of a long-term changes in dietary and exercise habits (3). However, the short- and medium-term changes in weight status that can be achieved with such therapy programs appear to be only small, both in clinical studies and under the conditions of regular medical care, and there is also a lack of data on desired (and possibly also undesired) long-term effects (4,5). Furthermore, the arsenal of available pharmacotherapeutic options for obesity in pediatric patients is more than limited, with no EMA-approved drugs in Europe, and only two FDA-approved substances (orlistat, phentermine) available in the US (6,7).

Although liraglutide treatment reduced BMI *z*-score and body weight, which are clinically meaningful according to current evidence (8), no significant change in any traditional cardiometabolic disease risk marker was observed between the intervention and placebo group. Therefore, more research is needed to develop risk-adapted, multi-modal treatment strategies for childhood and adolescent obesity. Given the here presented promising results and its overall good safety profile, liraglutide will hopefully find its place as a welcome addition to such tailor-made intervention approaches.

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11.13. Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss

Day EA, Ford RJ, Smith BK, Mohammadi-Shemirani P, Morrow MR, Gutgesell RM, Lu R, Raphenya AR, Kabiri M, McArthur AG, McInnes N, Hess S, Paré G, Gerstein HC, Steinberg GR
Center for Metabolism, Obesity and Diabetes Research, McMaster University, Hamilton, Ontario, Canada
gsteinberg@mcmaster.ca
Nat Metab 2019;1:1202–1208.
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Metformin has become a mainstay in the treatment of T2DM in over six decades of clinical use and is today one of the world's most commonly prescribed drugs. Anti-diabetic properties of metformin rely on an inhibition of hepatic glucose output through AMPK-dependent, but also AMPK-independent effects (reviewed in (1)). Nevertheless, proposed mechanisms explaining the glucose-lowering, insulin-sensitizing actions of metformin do not explain its moderate weight loss effects (2). Recent observational data identified growth differentiation factor 15 (GDF15) as a novel biomarker for metformin treatment, with GDF15 levels reflecting the dose of metformin in T2DM adults participating in the ORIGIN trial (3). GDF15 is a peptide hormone produced as part of the organism's stress response, and levels increase after exercise, and tissue injury (4, 5). GDF15 levels are also elevated in obese subjects (6) and seem to persistently increase following bariatric surgery, correlating with weight loss achieved by this surgical intervention (7). In rodents, GDF15 exerts its anorectic effects through the GDNF family receptor α-like (GFRAL) complex, which is solely expressed in the hindbrain (6).

Here, Day *et al.* hypothesized that metformin decreases body weight by inducing secretion of a hepatocyte-derived endocrine factor that communicates with the CNS. Unbiased transcriptomics of metformin-stimulated mouse hepatocytes and of serum proteomes from patients with T2DM treated with metformin revealed that, of the upregulated gene products in mouse hepatocytes, the most significantly upregulated corresponding protein in human serum was GDF15. Further experiments in cultured primary mouse hepatocytes demonstrated that metformin increased GDF15 expression by 55% and GDF15 secretion in a dose-dependent manner, independent of the AMPK pathway, and through stress response pathways involving ATF4 and CHOP. Next, the authors generated GDF15-knock out mice and assessed the effects of metformin on energy homeostasis in these animals compared to wildtype mice. In wildtype animals, metformin not only increased serum GDF15 and improved glucose tolerance, but also reduced food intake and weight gain under conditions of a high fat-diet (HFD). These metabolically beneficial effects were absent in GDF15-KO-mice on a HFD, but similar to wildtype animals when chow-fed. This finding points to a diet-specific effect of GDF15-induced suppression of food-intake. Interestingly, there were no differences in physical activity and energy expenditure between wildtype and knock out-animals.

Further support for these exciting findings comes from another very recent publication by Coll *et al.*(8). These authors demonstrate in their own series of experiments that in wildtype mice serum GDF15 levels are increased by oral metformin through increased GDF15 expression in the intestine and kidneys. As in Day *et al.*, Coll *et al.* report that metformin ameliorated weight gain on high fat-diets in wildtype mice, but not in GDF15-KO mice or in mice lacking the GDF15-receptor GFRAL. Treatment with a GFRAL-antagonist antibody also reversed the weight-lowering effects of metformin in obese mice.

Taken together, these results confirm the need for intact GDF15-GFRAL-signaling for metformin-induced weight loss. As GDF15-mediated weight loss seems to be independent of leptin and GLP-1 signaling, as observed in respective mouse loss-of-function models (6), the recent studies by both Day *et al.* and Coll *et al.* open up interesting and new perspectives on the use of metformin to promote weight loss and as a potential cornerstone of future combination therapy regimens, e.g. together with GLP-1 agonists and other weight loss-drugs.

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New Insight into Bariatric Surgery

11.14. Five-year outcomes of gastric bypass in adolescents as compared with adults

Inge TH, Courcoulas AP, Jenkins TM, Michalsky MP, Brandt ML, Xanthakos SA, Dixon JB, Harmon CM, Chen MK, Xie C, Evans ME, Helmrath MA
Department of Surgery, Children's Hospital Colorado, Colorado, USA
thomas.inge@ucdenver.edu.
The New England journal of medicine. 2019;380(22):2136–45.
https://pubmed.ncbi.nlm.nih.gov/31116917/

Here, Inge *et al.* explored whether the outcomes of Roux-en-Y gastric bypass (RYGB) differ according to the age of the patient at the time of surgery. They analysed retrospective data on the 5-year outcomes of RYGB in 161 adolescents from the Teen-LABS-study (1) and in 396 adults with a history of obesity dating back to age 18 years, enrolled in the LABS-study (2), regarding weight, coexisting conditions, as well as mortality, abdominal reoperations and selected micronutrition levels.

Most importantly, there was no significant difference between adolescents and adults in the percent weight change (-26% vs. -29%, $P = 0.08$) and mortality (1.9% vs. 1.8%) 5 years after RYGB surgery. However, the rates of remission of type 2 diabetes and hypertension were significantly higher in adolescents vs. adults (86% and 68% vs. 53% and 41%, respectively), whereas the rate of abdominal reoperations (mainly cholecystectomies) was significantly higher in adolescents (19 vs. 10) during 5 years after surgery. The proportion with low ferritin and low vitamin D levels markedly increased among adolescents (48% and 38%), whereas low ferritin levels slightly increase and low vitamin D levels decrease in adults (29% and 24%) 2 years after surgery, most probable because of a decrease in adherence to supplementation after surgery among adolescents. Improvements in cholesterol and triglyceride levels were observed in both cohorts.

Although accumulating evidence supports bariatric surgery as an efficacious and safe intervention also for severe obese adolescents (3–6), providers still are reluctant to refer them for bariatric surgery (7). Thus, the present findings are an important contribution to an early intervention and in line with other publications, showing better results at an earlier timepoint of surgery (8–10).

However, due to the paucity of long-term data on bariatric surgery in adolescents, it is still not impossible to determine a definitive risk-benefit analysis of bariatric surgery in this population. It is indispensable that all adolescents are cared for by multidisciplinary teams in centers with expertise in adolescent extreme obesity and bariatric surgery. Measures to improve patient understanding of the procedure and lifestyle changes, as well as compliance, need to be implemented in a preoperative treatment program. Risk-benefit ratios must be assessed on a case-by-case basis, keeping in mind that full information on long-term risks is not yet available.

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11.15. 5-year mental health and eating pattern outcomes following bariatric surgery in adolescents: a prospective cohort study

Järvholm , Bruze G, Peltonen M, Marcus C, Flodmark CE, Henfridsson P, Beamish AJ, Gronowitz E, Dahlgren J, Karlsson J, Olbers T

Childhood Obesity Unit, Skåne University Hospital, Malmö, Sweden; Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

kajsa.c.jarvholm@skane.se.

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In this non-randomized matched-control study, 81 obese adolescents undergoing Roux-en-Y gastric bypass surgery (RYGB) who participated in the Adolescent Morbid Obesity Surgery (AMOS) study (11) were compared with 80 obese controls from the Swedish Childhood Obesity Treatment Register (BORIS) who received conventional obesity treatment (12).

Concerning psychiatric drug treatment, no significant between-group difference was found at baseline or during 5-year follow-up. However, both groups showed increases over time in psychiatric medication. Whereas there was no between-group difference in treatment for psychiatric disorder at baseline, RYGB adolescents received significant more mental health treatment than controls during the 5 years post-surgery.

Self-reported mental health and binge eating were assessed in the surgical group by questionnaires, showing small but significant improvements in self-esteem and activation, and moderate improvements in binge eating 5 years after surgery, but no changes in overall mood, pleasantness or calmness. Patients with a better overall mood and higher self-esteem at 2-year follow-up had a higher percentage reduction in BMI at 5-year follow-up.

Although limited by a relatively small sample size and non-randomized groups, this study gives important insights in the mental health of adolescents over a long time after bariatric surgery, thereby reflecting the importance of ongoing assessments of mental status in this patient group. There is an unmet need for mental health services for adolescents with severe obesity whether they receive bariatric surgery or not. The suggestion that surgical weight management would improve mental health outcomes requires caution.

In contrast to physical and metabolic health benefits of bariatric surgery (1–4) and mental health problems of obese adolescents (5–7), little is known about the long-term course of mental health in adolescents with severe obesity who have undergone bariatric surgery (8–10). Therefore, the current paper, depicting specific mental health outcomes during 5 years follow-up in adolescents after bariatric surgery, is of great importance.

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Consensus Statement

11.16. Joint international consensus statement for ending stigma of obesity

Rubino F, Puhl RM, Cummings DE, Eckel RH, Ryan DH, Mechanick JI, Nadglowski J, Ramos Salas X, Schauer PR, Twenefour D, Apovian CM, Aronne LJ, Batterham RL, Berthoud HR, Boza C, Busetto L, Dicker D, De Groot M, Eisenberg D, Flint SW, Huang TT, Kaplan LM, Kirwan JP, Korner J, Kyle TK, Laferrère B, le Roux CW, McIver L, Mingrone G, Nece P, Reid TJ, Rogers AM, Rosenbaum M, Seeley RJ, Torres AJ, Dixon JB

King's College London, Department of Diabetes, School of Life Course Science, London, UK

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https://pubmed.ncbi.nlm.nih.gov/32127716/

More than 100 medical and scientific organizations supported this international consensus statement, which describes impressively how unscientific public narratives of obesity cause weight stigma and stimulates discrimination of obese people. Consequently, the statement calls for strong policies and legislation to prevent weight-based discrimination.

The consensus group reviewed published evidence and developed statements using a Delphi process. Weight stigma occurs everywhere in our societies and unfortunately quite severely also in healthcare settings. There is clear scientific evidence that this weight stigma results in psychological as well as physical harm. This is observed in the workplace, during education, and in healthcare settings. It has been shown that obese individuals are less likely to seek and to receive adequate care and social support.

The main contents of the statement are as follows:

- Weight stigma is frequent amongst healthcare providers. The authors demand professional teaching by academic institutions and professional bodies on the causes, mechanisms, and treatments of obesity, including stigma-free skills and practices.
- Obesity stigma in our societies is based on unproven assumptions which say that obesity is the cause of a lack self-discipline and personal responsibility. Patients are blamed for a failure. Current scientific evidence however shows that the regulation of body energy homeostasis and body weight is not entirely under volitional control, and that biological, genetic and environmental factors contribute substantially to obesity.
- The influence of media on the stigmatization and discrimination of obese people is relevant. Media are able to shape public attitudes and beliefs. The consensus group demands media to produce fair, scientifically accurate, and non-stigmatising descriptions of obese people.
- This consensus article is an excellent basis for education, training and contains the relevant scientific data as an basis for discussions, innovative projects and action. The final aim of this statement is to stimulate the development of educational and policy initiatives to end discrimination against people with obesity, as well as to facilitate access to care for people with obesity who are in need of it.

12. Type 2 Diabetes, Metabolic Syndrome and Lipid Metabolism

Orit Pinhas-Hamiel MD

Pediatric Endocrine and Diabetes Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat-Gan, and Juvenile Diabetes Center, Maccabi Health Care Services, Tel-Aviv University, Sackler School of Medicine, Israel

Preface

This is the year of COVID-19. Although fortunately children and adolescents with diabetes are not reported as a high risk group, the course of disease in adults serves as an important lesson of the importance of good glycaemic control in acute illness (12.1).

Studies on the prevalence of type 2 diabetes (T2DM) in children and adolescents continue to be published, thus verifying that T2DM is a global problem. A study on the prevalence of T2DM from Hungary shows alarming numbers; the prevalence of T2DM among European populations is similar to that in high-risk ethnic groups in the USA (12.2). T2DM in adolescents has been established as more aggressive than in adults. This year we learn about the impact of T2DM on bone health, as well as the pathway between chronic hyperglycaemic and osteoporosis (12.3).

Regarding treatments for T2DM in children, lifestyle intervention showed little success (12.4). However, liraglutide demonstrated effectiveness in improving glycaemic control (12.5). Moreover, metabolic bariatric surgery resulted in a striking attenuation in diabetic kidney disease (12.6). Dissecting racial bias in the healthcare system, including diabetes care, is discussed (12.7).

A new concept is introduced, intraindividual variability in cardiovascular disease risk factors, independent of their absolute values (12.9). Non-alcoholic fatty liver disease is recognized as a risk factor for prediabetes and diabetes (12.10). Ten-hour time-restricted eating showed a beneficial effect on the metabolic syndrome (12.11).

Results of 20-years follow-up on the treatment with statins of familial hyperlipidaemia have been published (12.13), as well as a Cochrane review that revealed low rates of complications of this treatment in children (12.14). Inclisiran is a promising new medication for treatment of familial hyperlipidaemia (12.15).

And on the lighter side, where in the world do researchers work on weekends and holidays? Read the article (12.17) on work-life balance.

Type 2 Diabetes

12.1. Outcomes in patients with hyperglycemia affected by covid-19: Can we do more on glycemic control?

Sardu C, D'Onofrio N, Balestrieri ML, et al.
Diabetes Care. 2020.
doi: 10.2337/dc20-0723

Short summary: In this observational cohort of hospitalised COVID-19 patients in Italy, patients who had hyperglycaemia, with or without diabetes, were at higher risk for severe COVID disease than normoglycaemic patients. Insulin infusion was effective for achieving glycaemic targets and reducing mortality in patients with COVID-19.

Comment: Hyperglycaemia and insulin resistance are common in critically ill patients, even in those without diabetes. Twenty years ago Van den Berghe *et al*. first reported that intensive insulin therapy to maintain blood glucose at or below 110 mg/dl (6.1 mmol/l) reduces morbidity and mortality among critically ill patients in the surgical intensive care unit.[1] The recent epidemic of COVID-19 enabled reassessment of these findings.

In December 2019, clusters of pneumonia cases of unknown etiology emerged in Wuhan, Hubei Province, China. A novel coronavirus was identified as the causative agent, which was named severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), and the disease it causes was called COVID-19. On March 11, 2020, the World Health Organization declared COVID-19 as a pandemic.

Among patients with COVID-19, hyperglycaemia is found among those without a previous diagnosis of diabetes, as well as in those with diabetes. To determine whether tight glycaemic control is beneficial in patients with COVID-19 with moderate disease, patients with COVID-19 with moderate pneumonia were stratified to 4 groups based on diabetes status and on normoglycaemia or hyperglycaemia (an admission plasma glucose level >7.7 mmol/l) at admission: 1. Prior diabetes, with normoglycaemia, $n = 18$; 2. prior diabetes, with hyperglycaemia n = 8; 3. No prior diabetes, with normoglycaemia $n = 26$; 4. No prior diabetes with hyperglycaemia $n = 7$.

At admission, mean IL-6 and D-dimer levels were significantly and persistently higher in the hyperglycaemia than normoglycaemia group, despite the same treatment for COVID-19. One week after admission, pneumonia had progressed in 40% of those with hyperglycaemia compared to only 9% of those with normoglycaemia. Death occurred in 20% of the patients with hyperglycaemia compared to 5.9% of those with normoglycaemia. Furthermore, hyperglycaemic patients treated with insulin infusion ($n = 15$) had lower IL-6 and D-dimer levels, and less severe lung disease than those not treated with insulin infusion ($n = 10$). The composite end point (admission to intensive care, invasive ventilation, or death) occurred in 33% of hyperglycaemic patients treated with insulin infusion compared with 80% of hyperglcaemic patients without insulin infusion. Death occurred in 0% in the hyperglycaemic group treated with insulin infusion and 50% in the hyperglcaemic group without insulin infusion.

These findings suggest that in the context of COVID-19, elevated blood glucose, with or without diabetes, may cause an inflammatory response associated with increased severity of disease and higher risk of death. These findings highlight the extreme importance of tight glycaemic control for patients with diabetes.

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12.2. Changes in the incidence and prevalence of type 1 and type 2 diabetes among 2 million children and adolescents in hungary between 2001 and 2016 - a nationwide population-based study

Barkai L, Kiss Z, Rokszin G, et al.
Arch Med Sci. 2020;16(1):34–41.
doi: 10.5114/aoms.2019.88406

Short summary: Alarming numbers are reported here on the high prevalence of T2DM in children and adolescents in Hungary. In this nationwide population-based study, the prevalence of T2DM in children aged 0–18 years rose between 2001 and 2006 from 20/100 000 to 36/100 000 and decreased thereafter to 22/100 000 by the end of 2016. Prevalent cases showed significant female predominance in every year.

Comment: The increasing prevalence of T2DM in children and adolescents is recognized as a serious public health issue. However, its prevalence varies across geographic regions and populations. In the U.S.A., the overall prevalence of T2DM is reported as 24/100 000, but differs widely by ethnicity: 63/100 000 in American Indians, 56/100 000 in non-Hispanic blacks, 40/100 000 in Hispanics, 19/100 000 in Asian Pacific Islanders, and only 9/100 000 in non-Hispanic whites.[1]

Studies of T2DM prevalence in European children and adolescents also show a magnitude gap: 0.21/100 000 in England, and 2.3/100 000 in Germany, and now 22/100 000 in Hungary, which is as high as among U.S. Asian Pacific Islanders.

The reasons for the much higher prevalence of T2DM in Hungary compared to the rest of Europe are unknown and should be investigated. The optimistic message of the article is evidence of a decline over recent years in the incidence of T2DM among children and adolescents, from 8/100 000 per year to 5/100 000 per year.

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12.3. NIPA2 regulates osteoblast function by modulating mitophagy in type 2 diabetes osteoporosis

Zhao W, Zhang W, Ma H, Yang M
Sci Rep. 2020;10(1):3078.
doi: 10.1038/s41598-020-59743-4

Short summary: This study describes a specific mechanism of reduced bone formation secondary to hyperglycaemia. In hyperglycaemic states, advanced glycation end products (AGEs) downregulate the highly selective magnesium transporter expression of *NIPA2*in osteoblasts. This results in magnesium deficiency, which is associated with osteoporosis.

Comment: A meta-analysis of studies among T2DM populations showed a higher risk of hip fractures among younger than older persons, among women than men, among those using insulin than non-insulin users, and among those with disease duration of more than 10 years compared to shorter duration.[1] Increased risk of fracture was associated with insulin treatment, hypoglycaemic episodes, microvascular complications and loss of bone mass.[2] Further, chronic hyperglycaemia favours non-enzymatic reactions between glucose and proteins, producing advanced glycation end products (AGEs), which affect bone formation. Among young girls, insulin resistance and chronic inflammation were associated with lower total bone mineral content.[3]

Magnesium is a crucial mineral; approximately 50–60% of total body magnesium is stored in bones. Its influx and efflux in mammalian cells are mediated by metal ion transporters. Magnesium deficiency is associated with osteoporosis and several studies found an association between T2DM and magnesium deficiency.

The *NIPA2* gene (non-imprinted in Prader-Willi/Angelman syndrome), which is located adjacent to the imprinted domain in the Prader-Willi syndrome deletion region of chromosome 15, encodes a highly selective magnesium transporter that is involved in the transport of magnesium ions into cells.

This group of researchers previously reported that AGEs regulate the expression of *NIPA2* in osteoblasts in a concentration-dependent manner.[4] Here, they showed that *NIPA2* expression was reduced both in db/db mice and in-vitro models of osteoporosis in T2DM. Further, overexpression of *NIPA2* increased osteoblast function. Mitophagy (a process that selectively degrades damaged mitochondria) accelerated the osteogenic dysfunction. Vice versa, inhibition of mitophagy rescued the function of osteoblasts, such that the level of mitophagy in the high glucose environment was negatively regulated by *NIPA2*.

Taken together, these results suggest that *NIPA2* positively regulates the osteogenic capacity of osteoblasts via the mitophagy pathway in T2DM.

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12.4. Evaluation of the longitudinal change in health behavior profiles across treatment groups in the TODAY clinical trial

Kaar JL, Schmiege SJ, Drews K, et al.
Pediatr Diabetes. 2020;21(2):224–32.
doi: 10.1111/pedi.12976

Short summary: This high-quality, longitudinal lifestyle intervention, with ample funding, had little effect on improving health behaviour profiles of adolescents with T2DM.

Comment: The first sweeping recommendation for adolescents with obesity, and certainly for those with T2DM, is lifestyle change. This study challenged the benefit of this approach. A total of 699 adolescents with T2DM aged 10–17 years were enrolled in a family-based behavioural lifestyle intervention program targeted to promote weight loss. Each family was assigned a personal activity and nutrition leader. The intervention was divided into three cascading stages. In the first stage, the personal leader met with the family weekly for the first 6 to 8 months, and meetings were devoted to increasing physical activity, individual calorie intake goals, self-monitoring and problem-solving. In the second stage, the meetings took place every other week for 12 to 16 months. In the third stage, meetings were monthly for 24 to 28 months. These meetings were devoted to maintenance of healthy lifestyle targets. I believe this is the best possible program one can ask for: professional, personal, longitudinal and funded. Adherence to the program decreased with time, (92% completed relevant assessments at baseline, 76% at 6 months, and 65% at 24 months). Changes that occurred in lifestyle behaviour did not persist at 24 months.

Clinicians need to be aware of the serious and disappointing findings of this study, which reflect the futility of our recommendations for lifestyle changes, and the limited measures we currently have to treat this growing problem.

12.5. Liraglutide in children and adolescents with type 2 diabetes

Tamborlane WV, Barrientos-Perez M, Fainberg U, et al.
N Engl J Med. 2019;381(7):637–46.
doi: 10.1056/NEJMoa1903822

Short summary: Liraglutide is superior to placebo in improving glycaemic control in children and adolescents with T2DM.

Comment: Liraglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist, also known as an incretin mimetic. It causes glucose-dependent stimulation of insulin secretion, inhibits glucagon release, delays gastric emptying and suppresses appetite. Liraglutide was approved for adults with T2DM one decade ago. This industry-funded, international, multicenter, randomized, controlled, phase III trial (the Ellipse study) studied the safety and effectiveness of Liraglutide in children and adolescents with T2DM.

In total, 135 children with T2DM (aged 10–17 years), treated with diet, metformin or insulin, were randomized to receive either subcutaneous liraglutide (escalating dose from 0.6 to 1.8 mg per day) or placebo. After 26 weeks, the mean glycated haemoglobin (HbA1c) level decreased by 0.64% in the liraglutide group, but increased by 0.42% in the placebo group; after 52 weeks, this difference was even greater (-0.50% vs. 0.80%). Furthermore, a higher proportion of adolescents achieved an HbA1c $<7\%$ on liraglutide vs. placebo (63.7% vs, 36.5%; $P<0.001$). BMI-Z score did not differ between the groups (please see also paper 11.12); the authors hypothesized that this might be because only $\sim 50\%$ of the liraglutide group received the full dose of 1.8 mg per day during the trial (per study protocol, doses were not increased if the average of fasting plasma glucose measurements on 3 consecutive days preceding the dose-escalation visit was <110 mg/dl or 6.1 mmol/l). Gastrointestinal complaints and hypoglycaemic events were more common in the liraglutide group.

Clinicians should be aware that liraglutide is available in our tool-box for management of children and adolescents with T2DM.

12.6. Effect of surgical versus medical therapy on diabetic kidney disease over 5 years in severely obese adolescents with type 2 diabetes

Bjornstad P, Hughan K, Kelsey MM, et al.
Diabetes Care 2020;43(1):187–95.
doi: 10.2337/dc19-0708

Short summary: Over 5 years follow-up, bariatric surgery compared with medical treatment resulted in dramatic attenuation of diabetic kidney disease (DKD) in adolescents with T2DM and severe obesity, beyond the impact of improved glycaemic control and weight loss.

Comment: DKD is the leading cause of end-stage renal disease and dialysis in the Western world. Adolescents with T2DM have significantly higher rates of DKD than adults with T2DM.[1] Renal hyperfiltration, as well as elevated urinary albumin excretion (UAE, i.e. microalbuminuria) are considered strong risk factors for the progression to end-stage renal disease, and may predict progressive DKD prior to the loss of renal function.

This study compared rates of DKD between two US cohorts over a period of 5 years. Thirty T2DM patients were participants of the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS), 24 underwent Roux-en-Y gastric bypass and 6 underwent vertical sleeve gastrectomy procedures. They were compared to 63 T2DM participants of the Treatment Options of Type 2 Diabetes in Adolescents and Youth (TODAY), who had received only medical treatment.

Hyperfiltration decreased from 21% to 18% in the surgical group (Teen-LABS), but increased from 7% to 48% in the medical treatment group (TODAY). Elevated UAE decreased from 27% to 5% in Teen-LABS, but increased from 21% to 43% in TODAY. TODAY participants had much higher odds of hyperfiltration and elevated UAE (15.7 and 27.3, respectively) at 5 years follow-up, after adjustment for baseline age, sex, body mass index and HbA1c. Teen-LABS participants showed a 23% decrease in high blood pressure, whereas TODAY participants showed a 40% increase. Mean HbA1c decreased from 6.8% to 5.9% at year 5 in Teen-LABS, but increased from 6.2% at baseline to 8.8% at year 5 in TODAY. Interestingly, participants in Teen-LABS had a higher mean BMI at baseline; however, at 5 years, BMI did not differ significantly between the groups.

Despite the obvious benefits of bariatric surgery, the authors also present its downside. This includes the requirement for lifelong nutrient supplementation to prevent or treat dietary deficiencies, deleterious implications on bone health, potential impacts on offspring, and the mental health burden. Indeed, 23% of Teen-LABS participants experienced complications that required subsequent operations and readmissions related to their bariatric surgery.

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12.7. Dissecting racial bias in an algorithm used to manage the health of populations

Obermeyer Z, Powers B, Vogeli C, et al.
Science 2019;366(6464):447-53.
doi: 10.1126/science.aax2342

Short summary: Dissecting racial bias in health care systems revealed that a type of software program, which determines who receives access to high-risk health care management, routinely accepts healthier whites ahead of blacks who are less healthy.

Comment: The year 2020 will be remembered also by the death of George Floyd in police custody, which powered a global movement against racial injustice.

In the USA, large health systems and payers use algorithms to target patients with complex diseases to specially trained health providers. Such algorithms have been built to improve the care of patients with complex health, such as diabetes, by providing additional resources. Due to the high costs of the additional resources, health

systems rely extensively on algorithms to identify patients who will benefit the most. While 'the algorithms by themselves are neither good nor bad', developers who build these algorithms rely on past data to design predictors of future health care needs.

The current study was set in a large academic hospital. All patients who were enrolled in a high-risk management program based on their health risk over a two-year period were identified and categorized according to their race. The current use of algorithms that determine who receives access to high-risk health care management programs was found to routinely accept healthier whites into the programs ahead of less healthy blacks. Using a different setting, an algorithm that specifically excluded race as a predictor more than doubled the number of black patients eligible to be enrolled in high risk management programs.

In other places in the world, algorithms in health care systems may also entail structural inequalities according to sex or ethnicity; these should be addressed and corrected.

Metabolic Syndrome

12.8. Metformin treatment in young children with fragile X syndrome

Biag HMB, Potter LA, Wilkins V, et al.
Mol Genet Genomic Med. 2019;7(11):e956.
doi: 10.1002/mgg3.956

Short summary: Nine boys with Fragile X syndrome (FXS), aged between 2 to 7 years old, received off-label treatment with metformin for at least 3 months. Language development and behaviour improved in the majority of children. A controlled trial of metformin in very young children with FXS, whose brains are in a critical developmental window, is needed.

Comment: Fragile X syndrome (FXS) is the leading cause of inherited intellectual disability, affecting about 1 in 2500 to 5000 males, and 1 in 4000 to 6000 females. The physical features of FXS include a long face, prominent ears, hyperextensible finger joints and macro-orchidism in males. Overweight and obesity are common in FXS for 3 main reasons. Firstly, overeating is commonly associated with anxiety and obsessive-compulsive behaviours. Secondly is the common use of atypical antipsychotics. Thirdly, in about 10% of those affected, the Prader-Willi phenotype (PWP) of FXS is found. The FXS-PWP is associated with hyperphagia, morbid obesity, delayed puberty, and lowered cytoplasmic FMRP interacting protein CYFIP1 (OMIM: 606322) expression.[1] Treatment with metformin led to clinical improvements in eating behaviours and weight loss in those with and without FXS-PWP, and is now recommended for the treatment of obesity in FXS.

Metformin administration in a knockout mouse model of FXS reduced the high testicular weights and improved several behavioural phenotypes.[2] A controlled trial of metformin for individuals with FXS aged 6–25 years is ongoing. However, evidence suggests the existence of a critical period of development during which targeted interventions may have significant and durable effects on the developmental trajectory and outcomes in FXS. That was the rationale in the current study for the treatment of metformin in young children aged 2–7 years.

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12.9. Variabilities in childhood cardiovascular risk factors and incident diabetes in adulthood: The Bogalusa Heart Study

Du T, Fernandez C, Barshop R, Fonseca V, Chen W, Bazzano LA
Diabetes Care.2019;42(9):1816–23.
doi: 10.2337/dc19-0430

Short summary: In this longstanding cohort study ($n = 1718$), high intraindividual variability over time in BMI and in HDL-C during childhood, independent of their mean levels, conferred an increased risk of later-life diabetes.

Comment: Intraindividual variabilities in cardiovascular disease risk factors (CVRFs) such as BMI, blood pressure and the atherogenic lipids profile, have recently triggered interest as potential novel markers that can contribute to risk prediction, in addition to the absolute levels of CVRFs. Among adults, long-term follow-up data show consistent associations of higher variability in BMI, blood pressure and lipids with future adverse outcomes such as cardiovascular disease events, cognitive dysfunction and mortality. However, in adults, CVRF variability itself could result from common confounding risk factors such as older age, pre-existing comorbidities and medications. Childhood, a period during which few confounding risk factors or comorbidities exist, is the perfect time to assess the impact of intraindividual variabilities on CVRFs.

The Bogalusa Heart Study (BHS) is unique in having repeatedly measured and prospectively collected measurements of CVRFs from childhood through young adulthood to midlife. Between 1973 and 2016, 9 cross-sectional surveys of children aged 4–19 years and 11 surveys of adults aged 20–58 years, who had been previously examined as children, were conducted approximately every 3–4 years.

During the 20.5-year follow-up period, 133 of 1718 participants developed diabetes. Increased variability in BMI and in HDL-C in childhood were associated with greater diabetes risk. This applied to only high BMI variability or only high HDL-C variability. Interestingly, other measures of the metabolic syndrome such as systolic/diastolic blood pressure, total cholesterol, triglycerides and LDL-C were not significantly associated with diabetes.

The authors suggest a number of pathophysiological mechanisms that could underlie the observed associations of intraindividual childhood BMI or HDL-C variability with diabetes. Among these possibilities, increased variabilities in BMI and HDL-C could reflect unhealthy lifestyle, or they could reflect an essential trait of responses or adaptations to alterations in neuroendocrine signals. Previous data demonstrated that fluctuations in BMI causes an unfavourable body composition characterized by more fat mass and less lean mass. In summary, dysfunction in CVRF homeostasis impacts the development of diabetes later in life.

12.10. Prevalence of prediabetes and diabetes in children and adolescents with biopsy-proven non-alcoholic fatty liver disease

Nobili V, Mantovani A, Cianfarani S, et al.
J Hepatol. 2019;71(4):802–10.
doi: 10.1016/j.jhep.2019.06.023

Short summary: Prediabetes is highly prevalent in Caucasian children and adolescents with well-characterized, biopsy-proven non-alcoholic fatty liver disease (NAFLD).

Comment: NAFLD is considered the liver presentation of the metabolic syndrome. NAFLD encompasses a wide spectrum of liver abnormalities, ranging from simple liver steatosis to steatohepatitis, fibrosis, cirrhosis and end-stage liver disease. Multiple factors affect NAFLD development and progression, including obesity, environmental factors and genetic background (e.g. variants in the patatin-like phospholipase domain-containing 3 gene, *PNPLA3*).[1] NAFLD has emerged as the most common chronic liver disease in children and adolescents in Western countries.

The study cohort comprised 599 Caucasian children and adolescents with NAFLD (301 girls), aged 5–17 years; compared with 118 children and adolescents with obesity (49 girls) without NAFLD of the same age. The strongest predictor of NASH was increased waist circumference, together with the presence of the risk allele (G) of rs738409 in *PNPLA3*.

Among children with biopsy-proven NAFLD, the prevalence of prediabetes was 19.8% and of newly diagnosed diabetes, 0.8%. This compared with 11% and 0%, respectively, among children with obesity but without NAFLD. Of note, children/adolescents with prediabetes/diabetes had higher degrees of hepatic steatosis and lobular inflammation than did those with normal glucose tolerance. The presence of prediabetes/diabetes was significantly associated with an almost 2-fold increased risk of having both severe hepatic steatosis and severe

lobular inflammation, and was marginally associated with ballooning degeneration ($P = 0.069$), but not with significant hepatic fibrosis.

These findings suggest that children with NAFLD need close follow-up evaluation of glucose metabolism, in addition to long-term monitoring for progression of liver disease.

Reference

1. Bruschi FV, Tardelli M, Claudel T,*et al*. PNPLA3 expression and its impact on the liver: current perspectives. Hepat Med 2017;9:55–66. doi: 10.2147/HMER.S125718.

12.11. Ten-Hour time-restricted eating reduces weight, blood pressure, and atherogenic lipids in patients with metabolic syndrome

Wilkinson MJ, Manoogian ENC, Zadourian A, et al.
Cell Metab. 2020;31(1):92–104 e5.
doi: 10.1016/j.cmet.2019.11.004

Short summary: Ten-hour time-restricted eating (TRE) for 12 weeks in adults with metabolic syndrome treated with standard medical care resulted in weight loss, decreased waist circumference, and lower blood pressure and levels of cardiovascular disease-promoting lipids.

Comment: Erratic eating patterns and eating over an extended period of time during the 24-h day can disrupt circadian rhythms. Chronic circadian disruption is associated with increased risk for components of obesity, hypertension, insulin resistance, inflammation and dyslipidemia. Objective longitudinal monitoring of human eating habits over several days has found that >50% of people eat within a window of >15 h.

Time-restricted eating (TRE) is an emerging dietary intervention that aims to maintain a consistent daily cycle of feeding and fasting to support robust circadian rhythms.

In this pilot study, 19 participants (13 men), mean age 59 $\pm$ 11 years, diagnosed with the metabolic syndrome, self-reported eating during a time window >14 hours per day. Their mean baseline weight was 98 $\pm$ 20 kg and their mean BMI 33.06 $\pm$ 4.76 kg/m^2. The participants received a validated app, myCircadianClock, to log their caloric intake during the 2-week baseline and 12-week intervention periods, and used continuous glucose monitors.

During the 12-week intervention, eating windows reduced by on average 28.75% (-4.35 ± 1.32 h). Despite no recommendations to change dietary quantity or quality, mean daily caloric intakes reduced by 9%$\pm$14%. This was associated with a significant reductions from baseline in body weight (-3.30 ± 3.20 kg [-3%], $P = 0.00028$), BMI (-1.09 ± 0.97 kg/m^2 [-3%], $P = 0.00011$), and accompanied by desirable reductions in percent body fat, waist circumference and blood pressure levels. Trends were observed to improvements in fasting glucose, fasting insulin and HbA1c. Most importantly, TRE was accompanied by significant reductions in total cholesterol (-13 ± 24 mg/dl [-7%], $P = 0.03$), and LDL-C (-12 ± 19 mg/dl [-11%], $P = 0.016$). These changes were not explained by the changes in weight.

The high level of adherence, lack of reported adverse effects, and low dropout rates suggest that a self-selected 10-h window for TRE may be a feasible treatment option for individuals with the metabolic syndrome. Larger studies with longer follow-up are needed.

Hyperlipidemia

12.12. GPR146 Deficiency protects against hypercholesterolemia and atherosclerosis

Yu H, Rimbert A, Palmer AE, et al.
Cell. 2019;179(6):1276–88 e14.
doi: 10.1016/j.cell.2019.10.034

Short summary: Deficiency in the orphan G protein coupled receptor 146 (GPR146) decreases blood lipid levels and protects against atherosclerosis in mice, independent of LDL receptor activity.

Comment: Monogenic hypercholesterolemia is a heterogeneous group of disorders, characterized by marked increases in LDL-C or triglycerides, or both, and a very high risk of premature atherosclerotic disease. Known causes include mutations in the LDL receptor (*LDLR*), apolipoprotein B-100 (*APOB*), *PCSK9* and the LDLR adaptor protein 1 (*LDLRAP1*) genes.

In large population studies, over 300 gene loci have been shown to be associated with plasma lipid levels. The current study assessed the function of GPR146, an orphan G protein-coupled receptor, as a regulator of plasma cholesterol levels. In a series of experiments, GPR146 was shown to regulate plasma lipid levels in mice through activation of extracellular signal regulated kinase (ERK) signalling in hepatocytes, upon feeding or after a short period of fasting. The activation of ERK signalling enhances activity of hepatic sterol regulatory element binding protein 2 (SREBP2) and of very-low-density lipoprotein secretion. This in turn increases circulating LDL-C and triglyceride levels.

Hence, the depletion of GPR146 in mice substantially reduces circulating LDL-C and triglyceride levels and protects mice against atherosclerosis, reducing lesion areas by up to 90%, in an LDL receptor-independent manner.

To the best of our knowledge, human gain-of-function and loss-of-function *GPR146* mutation have not been described. As deficiency protects against hypercholesterolaemia, the identification of small molecules that inhibit *GPR146* is potentially an effective strategy to treat hypercholesterolaemia and atherosclerosis.

12.13. 20-Year Follow-up of statins in children with familial hypercholesterolemia

Luirink IK, Wiegman A, Kusters DM, et al.
N Engl J Med. 2019;381(16):1547–56.
doi: 10.1056/NEJMoa1816454

Short summary: Twenty years of treatment of statin therapy in children with Familial Hypercholesterolemia (FH) slowed the progression of carotid intima–media thickness and reduced the risk of cardiovascular disease in adulthood. By age 39 years, the cumulative incidence of cardiovascular disease events in untreated parents with FH was 26% compared with only 1% in treated children.

Comment: FH is an autosomal dominant disorder. Recent genetic epidemiological studies suggest a frequency of about 1 in 250. Coronary atherosclerosis occurs prematurely and lifelong treatment, from early childhood, is needed. In 2008, the American Academy of Paediatrics issued revised recommendations for the management of hypercholesterolemia in children. Within days after publication, the new policy statement had elicited a firestorm of controversy.[1] Questions about the evidence base for statins emerged mainly in regard to the robustness of long-term evidence in children, and the possible harms of statins.

The current study investigated the long-term benefits of initiating early statin therapy in children with FH. The cohort comprised 184 individuals with FH and 77 of their unaffected siblings who were followed for 20 years. Statin therapy in this group was initiated at a mean age 14.0 $\pm$ 3.1 years. Overall, 79% of individuals with FH reported continued use of lipid-lowering medication. Their mean LDL-C levels decreased by 32%, from 237 mg/dl at baseline to 161 mg/dl at follow-up. In contrast, in their unaffected siblings, LDL-C levels increased by 24%, from 98 mg/dl to 122 mg/dl. At baseline, mean carotid intima–media thickness was greater in FH children than in their unaffected siblings. However, during the 20-year period, the mean rate of thickening was similar in both groups. Most importantly, by age 39 years, the cumulative incidence of cardiovascular disease events was 26% in the parents and 1% in treated children; death from cardiovascular causes was 7% in parents and 0% in children. No episodes of rhabdomyolysis or other serious adverse events were reported.

These findings support the notion that atherogenesis is the product of both the magnitude and the duration of exposure of the arterial wall to LDL-C, and reinforces clinical guideline recommendations to initiate statin therapy by age 8–10 years in individuals with FH. The authors concluded that this makes a strong case not only for ‘the lower the better’ but also for ‘the younger the better.’

Reference
1. de Ferranti S, Ludwig DS. Storm over Statins — The Controversy Surrounding Pharmacologic Treatment of Children. 2008;359(13):1309–12. doi: 10.1056/NEJMp0805953.

12.14. Statins for children with familial hypercholesterolemia

Vuorio A, Kuoppala J, Kovanen PT, et al.
Cochrane Database Syst Rev. 2019;2019(11).
doi: 10.1002/14651858.CD006401.pub5

Short summary: This systematic review shows that statin treatment is an effective lipid-lowering therapy in children with Familial Hypercholesterolemia (FH). Few or no safety issues were identified.

Comment: Cochrane is an international charitable organisation that was formed to inform health decision-making about treatments. A global independent network of researchers provide systematic reviews and meta-analyses that facilitate evidence-based choices about health.

The current study assessed the effectiveness and safety of statin use in children with heterozygous FH. Nine randomized placebo-controlled studies with data on 1177 participants were included. There is high-quality evidence that statins reduce mean LDL-C concentration at all time points. The important take-home message is that differences in frequency of adverse effects, such as myopathy and impaired liver function, are small or non-existent between the treatment and placebo groups. None of the studies reported rhabdomyolysis or clinical adverse events.

The reviewers noted that the intervention and follow-up periods of included studies were short (median 24 weeks; range of 6 weeks to 2 years). Nevertheless, a few documentations reported improvement in the function of the arterial endothelium in children with FH, as well as in carotid intima-media thickness. This review should encourage paediatricians to screen children for hyperlipidaemia and refer them for treatment if needed.

12.15. Inclisiran for the treatment of heterozygous familial hypercholesterolemia

Raal FJ, Kallend D, Ray KK, et al.
N Engl J Med. 2020;382(16):1520–30.
doi: 10.1056/NEJMoa1913805

Short summary: Inclisiran (a small interfering double-stranded RNA against PCSK9) is superior to placebo in reducing LDL-C among individuals with heterozygous Familial Hypercholesterolemia who are already on statins and ezetimibe.

Comment: Proprotein convertase subtilisin kexin type 9 (PCSK9) is synthesized primarily in the liver, and enters the circulatory system, where it binds to, and accelerates the degradation of hepatic LDL receptors. This process reduces the capacity of the liver to remove LDL-C from the circulation. Pharmacologic inhibition of PCSK9 by monoclonal antibodies against PCSK9 have been shown to reduce LDL cholesterol levels by more than 50%. However, monoclonal antibodies require administration every 2 to 4 weeks.

Inclisiran is a long-acting, small interfering double-stranded RNA agent, which reduces the production of PCSK9 in the liver. The current study examined the safety and efficacy of inclisiran in lowering LDL-C among individuals with heterozygous Familial Hypercholesterolemia.

Individuals with LDL cholesterol levels of at least 100 mg/dl (2.6 mmol/l), despite receiving a maximally accepted dose of statin therapy with or without ezetimibe, were randomized to receive either inclisiran 300 mg ($n=242$) or matching placebo ($n=240$). The study drug was administered as a subcutaneous injection on day 1, and then at 3, 9 and 15 months (The ORION-9 trial).

Two months after the last injection, LDL-C levels decreased by 39.7% from baseline in the inclisiran group and increased by 8.2% in the placebo group (between-group difference −47.9%). The LDL-C levels at 9 months decreased by 59.0 mg/dl in the inclisiran group and increased by 9.9 mg/dl in the placebo group (between-group difference −68.9 mg/dl). PCSK9 levels decreased by 60.7% in the inclisiran group and increased by 17.7% in the placebo group. Inclisiran was associated with lower levels of total cholesterol, non–HDL cholesterol, apolipoprotein B and triglycerides than the placebo, together with higher HDL cholesterol levels. Apart from

higher rates of injection-site reaction in the inclisiran group (17.0% vs. 1.7%), the frequency of adverse events was similar in the two groups, as assessed according to system organ class.

The short-term findings of this study are very promising. We now need to wait for the results of cardiovascular disease outcome trials.

12.16. Identification of ALK in thinness

Orthofer M, Valsesia A, Magi R, et al.
Cell. 2020.
doi: 10.1016/j.cell.2020.04.034

Short summary: In this study, rather than focusing on genes associated with obesity, the authors studied genetic variants associated with thinness (BMI $<$6th percentile for age). In a genome-wide association study from the Estonian Biobank, 881 people were classified as thin, and 3173 individuals with normal weight (BMI 30–50th percentile) served as controls. Five genomic loci (2 intergenic and 3 intronic loci) were identified as associated with thinness, among them the *ALK* gene.

Comment: The *ALK* locus has been found to be associated with multiple metabolic traits including BMI, plasma triglyceride levels, plasma LDL-C levels, glucose homeostasis, plasma adiponectin levels and HbA1c. Knockdown of the *ALK* gene in Drosophila results in reduced triglyceride levels, on normal as well as high-sucrose diets, and exhibited a mild reduction in lifespan, *ALK* knockout mice were born with normal weight. They developed a thin phenotype at age 5 weeks, which persisted into adulthood. Their body adiposity was reduced and their length was normal. Mice on a standard chow diet exhibited a thin phenotype, elevated adiponectin levels and improved glucose homeostasis, while having unaltered food intake and activity. On challenging adult mice with a high fat diet for 16 weeks, *ALK* knockout mice were significantly protected against obesity. *ALK* knockout mice have an elevated daily energy expenditure compared to wildtype mice, suggesting excess catabolism as the potential cause of their adiposity-resistant phenotype.

ALK is a member of the insulin receptor superfamily that was first described as an oncogene. Based on the expression of its mRNA throughout the nervous system during embryogenesis and also in the adult brain, mammalian *ALK* is thought to play a role in the development and function of the nervous system. *ALK* is not expressed in the liver, muscle, white adipose tissue and brown adipose tissue.

The researchers suggested that *ALK* expression in neurons of the hypothalamus regulates energy expenditure by affecting adipose tissue lipolysis.

So now we need *ALK* inhibitors to be skinny.

12.17. Working 9 to 5, not the way to make an academic living: Observational analysis of manuscript and peer review submissions over time

Barnett A, Mewburn I, Schroter S
BMJ. 2019;367:l6460.
doi: 10.1136/bmj.l6460

Short summary: There is wide variation around the world in how researchers manage their work-life balance.

Comment: The Christmas edition of *The BMJ* published this observational study that examined more than 49 000 manuscripts and 76 000 peer review online submissions to *The BMJ* and *The BMJ* Open. They assessed whether the submissions were made on weekends, national holidays or late at night. Clear and consistent differences were seen between countries. Chinese researchers most often worked on weekends and after midnight; whereas researchers in India and Scandinavian countries, which have a greater focus on work-life balance, were more likely to submit their papers during 9 to 5 hours on weekdays.

What is the right way? It will be interesting to assess the rate of burnout of researchers in China compared with other countries. As for now, we recommend you stop reading and go have a great afternoon outdoors.

13. Global Health for the Paediatric Endocrinologist

Jean-Pierre Chanoine
Endocrinology and Diabetes Unit, British Columbia Children's Hospital and University of British Columbia

Preface

Welcome to the 5th edition of this chapter on Global Health in Pediatric Endocrinology and Diabetes. As usual, the selected articles cover most aspects of pediatric endocrinology and diabetes. However, I particularly recommend the first 5 papers that are presented in the first section. They make us reflect on more philosophical aspects of our work as pediatric endocrinologists interested in global health.

Advocacy, History and Society

13.1. Corruption in global health: the open secret *(personal opinion)*

García PJ
School of Public Health, Cayetano Heredia University, Lima, Peru
patricia.garcia@upch.pe
Lancet 2019; 394: 2119–24.
doi: 10.1016/S0140-6736(19)32527-9

- The author is a physician and has served as Health Minister in her home country, Peru.
- She reviews the role of leaders in low-resource settings in the development of corruption and extends her comments to high-resource environments.
- She discusses 6 types of corruption: absenteeism, informal payments from patients, theft of money, supplies and medications, corruption in service provision, favouritism, and manipulation of data.

This opinion article emphasizes the magnitude and cost of corruption in global health and raises several important issues that we are all facing, sooner or later, knowingly or unknowingly, in our daily practice. How can paediatric endocrinologists ensure that they are not part of the problem and, in addition, that they are part of the solution?

This opinion given by Dr Garcia in her article brings to my mind two examples of difficult situations often faced by paediatric endocrinologists. A first example is the uneasy relationship between the pharmaceutical industry and the paediatric endocrinologist. Over the last 25 years, in order to decrease the conflict of interest between the pharmaceutical industry and the trainees/staff members, ethical rules in North America have prevented perks that used to be common, such as invitations to the annual meeting of professional societies (including flights, meals, registration, and accommodation). While the system is not perfect, in high-income countries, strong institutions and other sources of funding such as universities, non-industry grants or personal resources have made it possible to keep attending many of these precious annual conferences. In low-income countries, where alternative sources of funding are not available and physician's salaries are low, rejecting industry support is much more difficult and leads to difficult choices. How can we ensure that paediatric endocrinologists in low- and high-income countries benefit from the same opportunities? A second example is the lack of access to medicines in many low-income countries (and sometimes also in high-income countries). Why is the medicine that was just prescribed to our patient unavailable, unaffordable or of unsuitable quality? A lack of transparency in the many steps of the process that brings the medicine from the manufacturer to the patient (i.e. cost of production, registration and distribution, tender process, quality control, contracts between various players) plays a major role. Paediatric endocrinologists could and should play a major advocacy role in getting full transparency. While we may think that poor access to medicines is exclusively limited to low-income countries, this is not the case. For instance, a recent BMJ article reports that in August 2019, ASPEN UK admitted taking

part in an anticompetitive arrangement by illegally paying two competitors to secure a monopoly for the distribution of fludrocortisone in UK. This resulted in an 1800% increase in the price paid by the National Health Services (NHS) in UK for fludrocortisone (from 1.5 to 30 GBP for 30 tablets of fludrocortisone). In addition to paying a fine of 8 million GBP, 'Aspen has promised to ensure that in the future there will be at least two suppliers of fludrocortisone in the UK to help the NHS obtain better prices' (reference). As proposed by Dr Garcia in her article, we need to develop 'new models that could work to fight against corruption in global health, and to funders to support this effort'.

Reference

1. Iacobucci G. Drug firms colluded to hike fludrocortisone price by 1800%, says watchdog. *BMJ* 2019;367:l5881.

13.2. The age of paediatrics

Sawyer SM, McNeil R, Francis KL, Matskarofski JZ, Patton GC, Bhutta ZA, Esangbedo DO, Klein JD

Centre for Adolescent Health, Royal Children's Hospital, Parkville, Victoria, Australia; Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia

Murdoch Children's Research Institute, Parkville, Victoria, Australia; Centre for Global Child Health, The Hospital for Sick Children, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Centre of Excellence in Women and Child Health, Aga Khan University, Karachi, Pakistan; Division of Paediatrics, Providence Hospital Ikoyi, Lagos, Nigeria; Department of Pediatrics, University of Illinois at Chicago, Chicago, IL, USA

susan.sawyer@rch.org.au

Lancet Child Adolesc Health 2019; 3: 822–30.

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- The upper age limit of paediatric care varies markedly from country to country.
- The authors surveyed 1372 paediatricians in 115 countries and found that, based on their personal experience, the upper age limit of pediatric services had increased over the last 20 years of their practice, reflecting greater awareness of adolescent health.
- A greater focus on adolescent health during training is recommended.

Between September and December 2018, the authors sent an invitation to participate in an online survey to multiple regional pediatric organisations, asking them to distribute it to their members. The survey focused on the upper age limit of pediatric services in their country and on the respondents' perceptions of the upper age limits of pediatric services 10 years and 20 years ago. The survey was completed by 1372 pediatricians in 115 countries. The highest mean upper age limit of pediatric patients was 19.5 years in the USA. The lowest mean upper age limit was 11.5 years in South Africa. Within a country, replies from pediatricians vary and in 14 countries, the upper age limit varied by more than 10 years. The 600 pediatricians who had practiced for over 20 years reported that the mean upper age limit of inpatients had increased from 16.2 years two decades ago, to 17.4 years now. The main reason for the rising age over time was felt to be a greater awareness of adolescent health and leadership by professional associations. The authors suggest that a greater focus on adolescent health is required within pediatrics to ensure that the future pediatric workforce is appropriately equipped to respond to the changing disease pattern across childhood and adolescence.

Overall, the study shows that there is little agreement on the upper age limit for pediatric care. But should there be an international consensus on the age range of young patients seen by a pediatrician? Interestingly, WHO does not presently have a common definition for the upper age limit of pediatrics. For instance, the Essential Medicine List for children (EMLc) defines a child as younger than 12 years. This means that contraceptives are listed only in the adult EML, not in the EMLc. In the HIV/AIDS section of the WHO website (www.who.int/hiv/pub/guidelines/arv2013/intro/keyterms/en/ adolescence), an adult is a person older than 19 years of age while a child and an adolescent are defined as persons aged <10 and <19 years, respectively. For the WHO growth curves, an adult is a person older than 20 years of age, children as <5 years and school age children $+$ adolescents as 5–19 years. Practically, younger children are commonly seen by a pediatrician, and young adults (after high school) are commonly seen by an adult physician. It can be argued that an international age limit is not realistic and that the age of adolescence should be based on maturation (puberty, independence): age at which adolescents leave home to go to college, work independently or marry; age at which pubertal development and adult height are achieved (for instance, in British Columbia, Canada, the age of transition from

pediatric to adult care is officially flexible, from 17 up to 21 years). There may also be practical reasons that influence the age at which a pediatric patient is cared for by a pediatrician or by an adult physician, such as the availability of pediatric in- and outpatient care (for instance, in francophone Africa, the lack of pediatric endocrinologists means that most children and adolescents are followed by adult endocrinologists). Overall, an important message is that physicians who care for adolescents, whatever the definition is, should receive appropriate specialty and sub-specialty training.

13.3. Effective coverage measurement in maternal, newborn, child, and adolescent health and nutrition: Progress, future prospects, and implications for quality health systems

Marsh AD, Muzigaba M, Diaz T, Requejo J, Jackson D, Chou D, Cresswell JA, Guthold R, Moran AC, Strong KL, Banerjee A, Soucat A, on behalf of the Effective Coverage Think Tank Group
Department of Maternal, Newborn, Child and Adolescent Health and Ageing; Department of Health Systems Governance and Financing; Department of Sexual and Reproductive Health and Research including the UNDPUNFPA-UNICEF-WHO-World Bank Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, Geneva, Switzerland; Division of Data, Analysis, Planning and Monitoring and Health Division, United Nations Children's Fund, Headquarters, School of Public Health, University of the Western Cape, Cape Town, South Africa
tdiaz@who.int
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doi: 10.1016/S2214-109X(20)30104-2

- Sustainable Development Goals (SDG) were adopted by United Nations Member States in 2015. Universal health coverage is at the centre of SDG #3 but lacks metrics that make it possible to assess how effective the provided healthcare is.
- WHO and UNICEF convened a group of experts, the Effective Coverage Think Tank Group, to develop a consensus on the definition and measurement of effective health coverage for maternal, newborn, child, and adolescent health and nutrition.
- The Group developed a 7-step standardised cascade for the measure of effective coverage that can be applied to various practical situations.

In addition to providing clinical care for patients (and families) with endocrine diseases and with diabetes, pediatric endocrinologists often need to serve as advocates for their patients and their families, especially in settings where universal health coverage does not exist. This is often a difficult task that goes beyond the subspecialty training they receive. The 7-step cascade developed by the Think Tank Group in this article is useful to guide pediatric endocrinologists in their journey as an advocate for their patients. Several practical examples are developed by the authors. Using Type 1 diabetes care as an example, the cascade could be described as follows. Step 1 (target population) consists in identifying the population of children with diabetes (for instance, by improving the diagnosis and by the development of a registry); Step 2 (service contact coverage) consists in determining the proportion of children with diabetes who actually come in contact with the (relevant) health service (for instance, the patient may be known but may not have access to a diabetes team for various reasons such as travel distance, lack of education or poverty); Step 3 (input adjusted coverage) investigates whether the team accessed by the patient is ready to provide the expected care (for instance, there may be insufficient staffing or suboptimal training); Step 4 (intervention coverage) ensures that patients with Type 1 diabetes who come in contact with a diabetes team actually receive the service (for instance follow up may not be available in a timely fashion or language barriers may prevent provision of care); Step 5 (quality-adjusted coverage) determines whether the care is provided according to the expected standards (for instance, the team may not provide care according to internationally-recognized standards such as the ISPAD guidelines or to locally available standards developed for the specific environment of the patient); Step 6 (user adherence-adjusted coverage) assesses whether children with diabetes adhere to provider instructions; Step 7 (outcome-adjusted coverage) investigates whether the provision of optimal care and good adherence to the recommendations actually result in the expected health outcome (for instance, prevention of diabetic complications, decrease in admissions for diabetes keto-acidosis). This framework will be useful to pediatric endocrinologists trying to define a clear process to optimize support for their patients and families with Type 1 diabetes and can be applied to many of our patient groups.

13.4. From dwarves to giants: South American's contribution to the history of growth hormone and related disorders

Boguszewski CL, da Silva Boguszewski MC, de Herder WW
Department of Internal Medicine, Endocrine Division (SEMPR), University Hospital, Federal University of Parana, 80030-110 Curitiba, Brazil; Department of Pediatrics, Endocrine Division (SEMPR), University Hospital, Federal University of Parana, 80030-110 Curitiba, Brazil; Department of Internal Medicine, Sector of Endocrinology, Erasmus Medical Center, Rotterdam, the Netherlands
clbogus@gmail.com (Boguszewski CL); w.w.deherder@erasmusmc.nl (de Herder WW)
Growth Hormone & IGF Research 2020; 50: 48–56.
doi: 10.1016/j.ghir.2019.11.004

- The authors summarize the historical aspects of the discovery of mechanisms underlying short and tall stature in South America.
- Tall stature stories include gigantism in Patagonia, acromegaly and the relationship between glucose metabolism and GH excess.
- Short stature reports include the discovery of GHRH receptor and GH receptor mutations (Laron Syndrome).

This interesting paper mixes history, clinical description and science. It is beautifully illustrated. The first part describes the (tragic) interaction between Spanish sailors, surprised and intimidated by the large size of two populations in Patagonia where they landed during their discovery travels in the 15th and 16th centuries. These populations are now extinct, likely because of diseases brought by the sailors, a stark reminder of the dolorous colonial history in South America, and the cause of the tall stature is unclear. More recently, in the 19th and 20th centuries, the description of tall individuals (most of them with a pituitary adenoma) and the consequences of the disease on the quality of their life serve as a reminder that in all cultures, being different is a cause of discrimination. Many of these giants died early following a life as boxers or circus workers. Finally, at the other hand of the spectrum, the authors describe extremely short people suffering from two medical conditions well-known to pediatric endocrinologists: isolated GH deficiency secondary to an inactivating mutation in the GH-releasing hormone receptor gene, and inactivating mutation of the GH receptor gene (Laron syndrome). The article is also a teaching opportunity and the authors compare the clinical findings of these two conditions found in small communities in Brazil and Ecuador, respectively. Of note, the Ecuadorian patients, who, as research subjects, markedly contributed to our understanding of the growth hormone axis and to the development of recombinant IGF-1, did not benefit from longterm treatment with the expensive medicine that they contributed to develop. It was not provided by the pharmaceutical company and was not paid for by the health system. This situation, which infuriated Dr Guevara-Aguirre (Ecuador) who spent many years following these patients, is a reminder of our duty to protect the patients who participate in the clinical studies they are involved in.

13.5. Branding of subjects affected with genetic syndromes of severe short stature in developing countries

Guevara-Aguirre J, Guevara C, Guevara A, Gavilanes AA
College of Medicine, Universidad San Francisco de Quito, Quito, Pichincha, Ecuador; Department of Pediatrics, Maastricht University, Maastricht, Limburg, The Netherlands; Instituto de Endocrinologia y Metabolismo, IEMYR, Quito, Pichincha, EcuadorInstituto de Endocrinologia y Metabolismo, IEMYR, Quito, Pichincha, Ecuador
jguevara@usfq.edu.ec
BMJ Case Rep 2020;13:e231737.
doi: 10.1136/bcr-2019-231737

- The authors report 2 cases of severe short stature, Laron Syndrome and Cornelia-Delange-like syndrome, followed in Ecuador, a country with limited resources.
- They highlight the tendency of a society to discuss children with severe short stature with derogatory terms to automatically assume that these children have developmental delay.
- They also describe the shortcomings in diagnosis and management faced by these patients in low-resource settings.

This article is an opportunity to reflect on our role as clinicians, researchers but also advocates. It mixes history, science and clinical care. It focuses on two issues: the first one is the derogatory terms by which short patients are called ('dwarfs', 'midgets') and the second is the lack of resources in many low income countries that lead to suboptimal assessment and management. The first issue is unfortunately not limited to low income countries. Being different is often a reason for discrimination, and extreme short stature is a clear example. In our work as pediatric endocrinologists, short stature is a very common reason for referral, reflecting the perceived importance of height in the society. The authors highlight the consequences, not of the short stature per se, but of the discrimination associated with the short stature on the quality of life of patients. This should lead us to reflect on our role as advocates for our patients. The second issue is more specific to low-resource settings, where expensive diagnostic tests and treatments are often difficult to obtain. Again, as pediatric endocrinologists, we need to learn how to collaborate, communicate and advocate for access to diagnostic tests and to medicines. Our task also consists in ensuring that we make the best use of limited resources.

Diabetes

13.6. Management of diabetes during Ramadan fasting in children and adolescents: A survey of physicians' perceptions and practices in the Arab Society for Paediatric Endocrinology and Diabetes (ASPED) countries

Elbarbary A, Deeb A, Habeb A, Beshyah SA
Ain Shams University, Cairo, Egypt; Paediatric Endocrinology Department, Mafraq Hospital, Abu Dhabi, United Arab Emirates; Prince Mohammed bin Abdulaziz Hospital for National Guard, Madinah, KSA; Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates; Dubai Medical College, Dubai, United Arab Emirates
nancy_elbarbary@med.asu.edu.eg
Diabetes Res Clin Pract 2019; 150: 274–281.
doi: 10.1016/j.diabres.2018.12.014

- An online survey was sent to physicians registered with the Arab Society for Paediatric Endocrinology and Diabetes (ASPED) to assess the attitudes of health professionals in the management of Type 1 diabetes during Ramadan.
- There were 167 responders (86% were pediatricians, 14% were adult physicians).
- Almost 80% of the physicians would allow their patients to fast. Attitudes varied markedly among the health professionals surveyed (mainly pediatricians) regarding the prevention and management of both hypo- and hyperglycemia.

The results of the survey show that close to 80% of the surveyed physicians would allow patients to fast. Only a small majority (61%) emphasized the importance of providing education before fasting. Those with hypoglycemia unawareness were considered as high-risk patients for fasting by 47% of physicians. A majority (62%) felt that fasting should be broken if symptomatic hypoglycemia develops. In terms of management, a majority of respondents would decrease basal insulin by 25%, would recommend several dietary adjustments and would use rapid-acting insulin analogs and carbohydrate counting. This article is relevant to pediatric endocrinologists all over the world who care for Muslim children and adolescents with Type 1 diabetes. It complements another article (reference) that provides guidelines for the management of Type 1 (and Type 2) diabetes during Ramadan fasting. The survey shows that the attitudes towards Ramadan fasting vary widely between health professionals not only regarding the criteria required for permission to fast safely but also regarding the management guidelines. I had the opportunity of discussing this interesting issue with a Muslim colleague who also has Type 1 diabetes in order to better understand her point of view. First, she mentioned that, although Ramadan fasting is a pillar of Islam, it is very clearly written that fast can be broken if it may lead to self-harm, such as with diabetes. It is a sin to harm yourself in these circumstances. However, not fasting, or eating food in public, even for permitted reasons, can result in bullying of the children and can lead to discrimination, emphasizing the importance of social norms and of education. Second, it is clearly taught that injecting insulin is not regarded as breaking the fast. In contrast, injecting glucagon or IV glucose is considered

as breaking the fast, although it is, as mentioned above, permitted in patients with Type 1 diabetes. Finally, the survey appropriately mentions that monitoring of Type 1 diabetes must be more stringent during fasting. However, for patients in resource-limited settings who have access only to human insulin, or for those who cannot afford to pay for more than 1–2 strips a day, optimizing diabetes management is more challenging than for those who have access to insulin pump, long-acting analogues, appropriate number of glucose strips or glucagon. Thus, proposing the most appropriate options for Ramadan fasting needs to take into account the individual circumstances of each patient and family.

Reference

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13.7. Smartphone-based, rapid, wide-field fundus photography for diagnosis of pediatric retinal diseases

Patel TP, Kim TN, Yu G, Dedania VS, Lieu P, Qian CX, Besirli CG, Demirci H, Margolis T, Fletcher DA, Paulus YM
University of Michigan, Kellogg Eye Center, Department of Ophthalmology and Visual Sciences, Ann Arbor, MI, USA; New York University School of Medicine, Department of Ophthalmology, New York, NY, USA; University of Montreal, Department of Ophthalmology, Montreal, Canada; Washington University School of Medicine in St. Louis, Department of Ophthalmology and Visual Sciences, St. Louis, MO, USA; University of California, Berkeley, Department of Bioengineering and Biophysics Program, Berkeley, CA, USA; Chan-Zuckerberg Biohub, San Francisco, CA, USA; University of Michigan, Department of Biomedical Engineering, Ann Arbor, MI, USA
ypaulus@med.umich.edu
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- This study investigates the feasibility of acquiring diagnostic quality fundus photographs in children using a child-friendly smartphone.
- Photographs were acquired in 43 patients (mean age 6.7 years) with i.e. retinoblastoma, Coats' disease, commotio retinae and optic nerve hypoplasia.
- There was 96% agreement between image-based diagnosis and the treating clinician's diagnosis. This device, which can acquire fundus photos in 2.3 minutes and is well-tolerated, brings the possibility of easily assessing retinal disease in children with diabetes.

This article shows us what the future could look like for children and adolescents with diabetes living in low resource-settings. The concept of 'point of care testing' (POC), where investigations are performed directly where the patient lives (instead of having the patient travel to a center where equipment is present) is developing fast. The authors developed a portable handheld smartphone-based retinal camera (like the one used in an i-phone). The device captures high-quality fundus images, stores them, and transmits them via the wireless communication system of the device for remote evaluation. This is a welcome innovation in countries where children with Type 1 diabetes experience early and severe diabetic complications and insufficient screening for these complications. It has many potential applications in pediatric endocrinology and diabetes. One of the most attractive is the use of POC testing for the neonatal screening for congenital hypothyroidism. In many low-resource settings, mothers deliver at home, time-sensitive shipment of samples is not feasible, central laboratories are non-existent, and recall of patients is difficult. POC testing could be performed by a visiting allied health professional who could then contact a specialist in case of positive results. It is hoped that in the coming few years, a variety of POC tests will be available for large scale use, bringing to children in developing countries the care they deserve.

13.8. Diabetic microvascular complications among children and adolescents in northwestern Tanzania: A cross-sectional study

Msanga D, Reis K, Kayange N, Bakalemwa R, Kidenya B, Hau D, Mwanansao C, Mahamba D, Ottaru S, Kwiyolecha E, Peck R
Weill Bugando School of Medicine, Mwanza, TZ; Center for Global Health, Division of Medicine, Weill Cornell Medical College, New York, NY, US; University of Dodoma, Dodoma, TZ
kgr4001@med.cornell.edu

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- The authors assessed the prevalence of diabetic microvascular complications in 155 children and adolescents with Type 1 diabetes in northwestern Tanzania.
- They observed poor diabetes control in 69% of the patients and a high rate of diabetic nephropathy (32.9%), retinopathy (10.3%) and neuropathy (13.6%).
- Innovative initiatives are needed to optimize glycemic control.

Over the last 10–20 years there has been a marked increase in capacity in pediatric endocrinology in sub-Saharan Africa. This has led to the understanding that, like other parts of the world, Type 1 diabetes is not uncommon in African children and adolescents. Unfortunately, microvascular complications appear early in a high percentage of patients. Not surprisingly, in their study, prevalence of microvascular complications was significantly associated with older age (adolescence), poor glycemic control (HbA1c >12.5%) and longer duration of diabetes (>5 years), although the latter association was only statistically significant in univariate analysis. While a genetic predisposition of African patients cannot be ruled out, it is likely that the high rate of early microvascular complications is due to a combination of environmental factors such as insufficient number of trained allied health professionals, lack of access to insulin (although organisations such as Changing Diabetes in Children and Life for a Child support access to insulin and supplies for children with Type 1 diabetes in Tanzania and elsewhere) or high price of glucose strips resulting in poor blood glucose monitoring. These aspects of diabetes care are unfortunately not discussed by the authors. The authors also make the interesting point that pre-existing renal disease (such as renal disease secondary to schistosomiasis) may contribute to the high rate of nephropathy, highlighting the need for guidelines that meet the specific needs of a population. To my knowledge, such guidelines are not commonly available for children and adolescents with diabetes living in low resource settings.

Endocrinology

13.9. People are taller in countries with better environmental conditions

German A, Mesch G, Hochberg Z
Pediatric Department, Bnei-Zion Medical Center, Haifa, Israel; Rappaport Family Faculty of Medicine, Technion—Israel Institute of Technology, Haifa, Israel; Department of Sociology, University of Haifa, Haifa, Israel
rprzeev@technion.ac.il
Front. Endocrinol. 2020; 11:106.
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- The authors assessed the relationship between markers of a stressful environment and final height in adult men and women in 71 countries (including 31 countries that are members of the Organisation for Economic Co-operation and Development [OECD]).
- They found that the more stressful the environment, the shorter the adult height.
- By order of decreasing importance, the relationship between markers of a stressful environment and height were income inequality > air pollution > growth domestic product > corruption perception index > homicide rate > life expectancy > unemployment.

Overall, this interesting article suggests that the better the environment (defined by 7 equally weighted indicators of a stressful environment: homicide rates, growth domestic product per capita, income inequality, corruption perception index, unemployment rate, urban air pollution and life expectancy), the greater the final adult height. The authors consider the relationship between environment and height at the global level and rank final height according to published characteristics of each country. Several comments come to mind. First, they mention that the within-country variability is similar to the between-country variability. However, published data do not make it possible to determine whether the within-country variability is affected by the same stressors as the between-country variability. For instance, within a country, are children less affected by an adverse environment growing faster then those more affected by these stressors? Second, it is important to understand the relationship between qualitative and quantitative nutrition, a key determinant of linear growth, and the various environmental markers

used in this study. For instance, is income inequality affecting growth because poorer people have less money to buy food or because they live in less desirable areas where air pollution is higher, chronic diseases more common and psychological stress due to lack of safety greater? The most likely assumption is that it may be a combination of several factors. Third, the study focuses on final height as a marker for growth in childhood and adolescence, but it is also conceivable that this growth is affected by prenatal factors (maternal environment) and opens the door to the concept of transgenerational effect of stress. Finally, at the individual level, short stature is one of the most common reasons why patients are referred to a pediatric endocrinologist. Although pediatric endocrinologists are asked to rule our hormonal deficiency or excess as a cause of short stature, they are well aware that height variation results from a complex interaction between genetic, environmental, socioeconomic, and cultural factors. An example relevant to this article is psychosocial short stature, which is caused by stress and emotional deprivation in children (usually within the family), is associated with low height velocity (that increases back to normal once the stressors are removed) and, provided that the stressors are removed early enough in life, with normal or near normal final height. It is thought to be mediated at least in part by functional, reversible growth hormone deficiency secondary to hypopituitarism and is mostly independent from nutrition. At the clinical practice level, this article is a reminder to have a broad approach when assessing a patient for short stature.

13.10. Timing of the infancy-childhood growth transition in rural Gambia

Bernstein RM, O'Connor GK, Vance EA, Affara N, Drammeh S, Dunger DB, Faal A, Ong KK, Sosseh F, Prentice AM, Moore SE Growth and Development Lab, Department of Anthropology, Institute of Behavioral Science, Laboratory for Interdisciplinary Statistical Analysis (LISA), Department of Applied Mathematics, University of Colorado, Boulder, CO, United States; Department of Pathology, University of Cambridge, Cambridge, United Kingdom; MRC Unit The Gambia, London School of Hygiene and Tropical Medicine, Banjul, Gambia; Department of Pediatrics, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom; MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom; Department of Women and Children's Health, King's College London, London, United Kingdom
robin.bernstein@colorado.edu
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- A delayed transition from the infancy to the childhood growth stage contributes to sub-optimal growth outcomes.
- Using a novel method to assess the timing of infancy-childhood transition (quantification of patterns of adjacent monthly weight-for-age z-score (WAZ) deviation correlations), this transition was found to take place at the age of 12 months in UK. The authors in this study find that the transition takes place earlier in rural Gambia (9 months).
- The authors hypothesize that while a later transition allows maximal extension of the high rates of growth during the infancy, an earlier transition may negatively affect the growth outcomes in childhood but also offers an extended window for later catch-up.

This article is to some extent complementary to the article by German et al. discussed above. The model of Karlberg on which this article is based defines the infant-childhood transition as the period during which the rapid infantile growth decreases towards the more stable state and growth rate plateau of childhood. It is postulated to be associated to a progressive shift from the leading role of insulin and the insulin-like growth factors as mediators of nutritional status in the fetus to the role of the endogenous regulation of growth hormone. At that time, the child enters his/her defined percentile of growth. To assess the timing of this shift, the authors examine the change in the month-to-month correlation of the weight for age *Z* scores (WAZ). The shift in the correlations between adjacent WAZ from positive to negative values is associated with a change from infantile to childhood growth. Because of the importance of early growth for the development of long-term complications in the child, understanding the variation and factors in the timing of transition could be very useful if some of the factors could be modified to positively affect growth. In two UK studies, this shift was found to take place around the age of 12 months. Surprisingly, in the present study, applying the same modeling as the UK studies, the transition in rural Gambia was found to take place earlier, at 9 months. Assuming that the technical limitations of this complicated model do not affect the interpretation of the results, why would the transition from infancy to childhood take place earlier in Gambia compared to UK? It may reflect a trade-off: when energetic resources are limited and are prioritized for immediate, life-saving tasks

(such as fighting infections), rapid growth takes a back seat to ensure that the remaining resources can still support key tasks such as brain development. Catch-up growth may take place later is energetic resources are again plentiful. From a philosophical point of view, it may be that ponderal (and therefore linear) growth should not take place at the detriment of higher functions.

13.11. Worldwide secular trends in age at pubertal onset assessed by breast development among girls: a systematic review and meta-analysis

Eckert-Lind C, Busch AS, Petersen JH, Biro FM, Butler G, Bräuner EV, Juul A
Department of Growth and Reproduction, and The International Research and Research Training Centre in Endocrine Disruption of Male Reproduction and Child Health, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; Section of Biostatistics, Department of Public Health, University of Copenhagen, Copenhagen, Denmark; Division of Adolescent and Transition Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio; Institute of Child Health, University College London Hospital, London, United Kingdom
camilla.eckert-lind@regionh.dk
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- The authors evaluated the change in pubertal onset in healthy girls around the world based on age at thelarche.
- Overall, the age at thelarche decreased 0.24 years per decade from 1977 to 2013.
- This decrease seems to reflect a worldwide trend.

The authors focus on the change in the age of apparition of breast development in girls living in different parts of the world. Despite many limitations that are expected in a meta-analysis of published studies, they show a worldwide decrease in the age of thelarche of about 3 months per decade. This is consistent with the decrease in the age of menarche of 2–6 months per decade reported in many countries around the world over the last 40 years, including India, China, Ghana, and Korea. A major cause for this earlier activation of the hypothalamo-pituitary-gonadal axis is thought to be improved nutrition in children and, in particular in the United States, a major increase in the prevalence of obesity. The common link seems to be an increased production of leptin by the adipose tissue which in turns activates the hormonal cascade leading to the development of puberty. However, the role of endocrine disruptors, rarely measured in low-resource settings, is also postulated. The present article also finds that the mean age at thelarche ranged from 9.8 to 10.8 years in Europe, 9.7 to 10.3 years in the Middle East, 8.9 to 11.5 years in Asia, 8.8 to 10.3 years in the United States, and 10.1 to 13.2 years in Africa. From a clinical point of view, these data have implications on the use of diagnostic and therapeutic agents, in particular in low-resource settings where healthcare funding is limited. Traditional recommendations include determination of basal or stimulated LH and FSH, MRI of the hypothalamo-pituitary region, bone age and pelvic ultrasound to rule out an underlying condition in girls presenting with precocious puberty. However, pediatric endocrinologists know that in the vast majority of the cases, the final diagnosis will be idiopathic central precocious puberty. This suggests that the age at which evaluation of the child with precocious puberty should be considered should be adapted to the local characteristics and that evaluation should rely on clinical examination and follow up, with investigations performed only in children who are at high risk of an underlying condition.

13.12. Global trends in insufficient physical activity among adolescents: A pooled analysis of 298 population-based surveys with 1.6 million participants

Guthold R, Stevens GA, Riley LM, Bull FC
Maternal, Newborn, Child and Adolescent Health and Ageing Department; Department of Non-communicable Diseases; Health Promotion Department, WHO, Geneva, Switzerland; Independent Researcher, Los Angeles, CA, USA; School of Public Health, Imperial College London, UK; Department of Sport and Exercise Science, University of Western Australia, Perth, WA, Australia
gutholdr@who.int
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- The study reports the worldwide prevalence of insufficient physical activity among school-going adolescents aged 11–17 years.
- Data from surveys totalling 1.6 million students aged 11–17 years were analysed.
- The majority of adolescents do not presently meet physical activity guidelines.

WHO set a global target of a 15% relative reduction of insufficient physical activity among adolescents and adults by 2030. Insufficient physical activity was defined as not reaching the current WHO recommendation of 60 min of daily physical activity of moderate-to-vigorous intensity. Indeed, the health benefits of a physically active lifestyle during adolescence are important for the prevention of non communicable diseases through positive effects on weight, on cardiorespiratory and muscular fitness and on cardiometabolic health. Overall, the results of this study show that insufficient physical activity has remained similar or (in boys only) has slightly decreased in adolescents aged 11–17 years between 2001 and 2016. These results, which are not encouraging, need to be interpreted cautiously. First, the authors evaluated physical activity indirectly, through questionnaires, and it is highly possible that simple questions about physical activity may not reflect true physical activity. Second, compared to high-income countries, there was a dearth of data in low-income countries. In fact, data were not available for many countries in sub-Saharan Africa. Finally, culture may affect how questions are understood and answered in different countries. Nevertheless, it seems that few changes in physical activity have taken place over a 15-year period and that the results are similar in all parts of the world. Unfortunately, the study does not provide information on the determinants of physical activity and on how these determinants may differ in different countries. These are arguably key questions for those tasked with developing public health measures. Adolescent activity (or lack thereof) remains a true global issue that needs to be addressed if one hopes to prevent the rapid progression of non-communicable diseases and their cost to fragile health systems.

13.13. Prevalence of vitamin D deficiency in Africa: A systematic review and meta-analysis

Mogire RM, Mutua A, Kimita W, Kamau A, Bejon P, Pettifor JM, Adeyemo A, Williams TN, Atkinson SH
Kenya Medical Research Institute (KEMRI), Centre for Geographic Medicine Research—Coast, KEMRI–Wellcome Trust Research Programme, Kilifi, Kenya; Open University, KEMRI–Wellcome Trust Research Programme Affiliated Research Centre, Kilifi, Kenya; South African Medical Research Council and Wits Developmental Pathways for Health Research Unit, Department of Paediatrics, University of the Witwatersrand, Johannesburg, South Africa; Centre for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; Department of Medicine, Imperial College London, London, UK; and Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine and Department of Paediatrics, University of Oxford, Oxford, UK
satkinson@kemri-wellcome.org
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- The results of 25OH Vit D determination from 129 studies including 21 474 participants in 23 African countries were analyzed.
- Overall, a serum 25(OH)D concentration less than 30 nmol/L was found in 18.5% of the population and less than 75 nmol/l in 59.5% of the population.
- Newborn babies, women and those living in urban areas were found to be at higher risk for low 25OH Vit D.

The article describes the magnitude of 25(OH)vitamin D (25(OH)D) deficiency in many African countries and highlights its role in the high prevalence of rickets reported in Africa. The authors do not comment on the potential reasons for the marked disparities in vitamin D deficiency between countries. For instance, the difference in mean 25(OH)D concentration in Ethiopia (46.5 nmol/l) compared to neighbouring Uganda (82.5 nmol/l) is striking. Is it due to different cultures/lifestyles (the Muslim population represents 12% in Uganda and 34% in Ethiopia), study bias (most of the studies are regional), government policies promoting administration of vitamin D, or to other factors? Many of the risk factors are difficult to modify, i.e. urban living (an increasing proportion of the population lives in cities), female sex (women tend to stay home more than men and, when going out, are more covered than men); geographical location away from the equator (where the sun's UVs are less effective in generating vitamin D) and darker skin (which is less adapted to vitamin D synthesis). It is important to note that while the prevalence of rickets is generally high in Africa, 25(OH)D deficiency may not be the sole culprit. The consumption of milk (a major source of calcium) tends to be low in parts of Africa (for

instance, 62 liters per capita per year in Uganda, which is similar to Europe and North America compared to 20 liters in Ethiopia). Overall, there seems to be enough data to support the development of government policies that promote vitamin D (and calcium) supplementation. The actual process for supplementation needs to be carefully considered: general supplementation vs focus on at-risk population (pregnant mothers and young children), oral vs parenteral administration of vitamin D (the presence of malabsorption may prevent absorption of oral vitamin D), over the counter vitamin supplements vs. food fortification (such as milk).

13.14. Newborn screening in Nigeria: Will incorporating congenital hypothyroidism with sickle cell disease improve neonatal screening programme?

Iroro EY, Tamunopriye J, Datonye B, Lorenzo I
Department of Paediatrics, University of Port Harcourt, Nigeria; Unit of Paediatrics, University of Modena, Italy
lorenzo.iughetti@unimore.it
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- The authors review the present status of newborn screening in Africa and discuss the concept that adding a neonatal screening for congenital hypothyroidism (CH) to an existing sickle cell disease screening would make it easier to implement than developing an independent screening program.
- Structure, funding, political will and an efficient recall system are identified as being key requirements for the development of a successful neonatal screening for CH.

This article considers important aspects around the practical approach towards the development of a successful newborn screening (NBS) program for congenital hypothyroidism (CH) in Nigeria, a resource-limited country. As we all know, NBS for CH is routinely performed in high-resource countries since the 1970's. First, the authors discuss whether there could be a synergy between the NBS for sickle cell disease (SCD) and for CH. This was contemplated in neighbouring Ghana where NBS for SCD has been piloted for many years at Komfo Anokye Teaching Hospital (KATH) in Kumasi. The SCD program is available on the first day of life to the babies born in this tertiary care center, where the samples are analyzed. The program remains active at KATH, but a lack of funding may jeopardize sustainable continuation of the program and its extension to the rest of the country. The addition of an NBS program for CH that would build on the existing infrastructure of the NBS program for SCD has been discussed but is presently not supported by the health authorities (Dr E Ameyaw, personal communication). Second, the development of an NBS for CH needs to consider the specificities of the country and can not be based simply on the protocols used in well-resourced countries. This was addressed in the recent guidelines for development of NBS for CH in India, including the option to measure TSH on a cord blood sample and the use of local laboratories to perform TSH determination. Indeed, several issues are specific to low-resource countries, including lack of a central laboratory, difficulty to trace positive patients, large number of at home deliveries and non-existence of a reliable system to ship samples. Finally, although not mentioned by the authors, point of care (POC) testing for TSH needs to be considered. It is available for NBS for SCD and such a test is being considered for inclusion in the 2020 WHO Model List of Essential In Vitro Diagnostics. Unfortunately, a reliable test is not yet available for TSH, although large-scale testing is being considered by several companies. Such a POC test for TSH would make it possible to have an immediate diagnosis for patients irrespective from where they live or where they were born.

13.15. Adverse outcomes and economic burden of congenital adrenal hyperplasia late diagnosis in the newborn screening absence

Costa de Miranda M, Bertocco de Paiva Haddad L, Madureira G, Bilharinho de Mendonca B, Bachega TASS
Developmental Endocrinology Unit, Laboratório de Hormônios e Genética Molecular/ LIM42, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil 05403-900; Liver and Gastrointestinal Transplant Division, Department of Gastroenterology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil 05403-000
mirelacdm@yahoo.com.br
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doi: 10.1210/jendso/bvz013

- The authors performed a retrospective analysis of the economic burden in a cohort of 195 patients with genetically confirmed CAH born in São Paulo where there is presently no neonatal screening for CAH.
- The cost associated to mortality of undiagnosed patients was estimated to range from $2 239 744 to $10 271 591 per year.
- The mean total cost until 19 years of age (diagnosis, standard treatment, and adverse outcomes) was much higher for a patient with salt wasting CAH ($89 349) compared to a patient with simple virilizing CAH ($5922).

This study is the first step towards a cost-effectiveness analysis that will compare the cost of caring for patients with congenital adrenal hyperplasia (CAH) with and without the implementation of a newborn screening program for CAH. Presently, this screening does not exist in São Paulo. The authors estimate the medical cost of caring for patients with CAH from birth to 19 years. They should be commended for their attempt to include in this analysis as many aspects as possible of treatment cost, including mortality (unrecognized affected patients), dehydration, mental impairment, hospitalization, hormonal determinations, medicines for standard (corticosteroids) and extended (GnRh analogues and growth hormone because of late diagnosis) medical treatment. Their experience brings to mind several comments. First, like many countries where neonatal screening is not available, there is an excess of female compared to male patients, reflecting the greater difficulty to diagnose CAH in male neonates in the absence of genital abnormalities. However, the percent of male neonates diagnosed clinically with CAH has increased markedly from before 1989 (25%) to after 1999 (41.7%) thanks to increased early recognition. Second, sex assignment error (male sex wrongly assigned to female neonates) emphasizes the importance of performing a thorough medical examination of each neonate, an issue common to many low resource settings. Third, the authors could not estimate the effect of sex error assignment or late diagnosis (with more severe virilization) on quality of life and on psychological outcomes. Whether or not, in the Brazilian context, the future financial analysis will show that a neonatal screening would be cost-effective remains to be seen. However, from a human perspective, prevention of neonatal deaths, prevention of mental retardation, less severe virilization and proper sex assignment may be regarded as priceless.

13.16. Assessment of health-related quality of life in Egyptian children and adolescents with congenital adrenal hyperplasia

Musa N, Asem N, Basyony S, Fawaz L
Department of Public Health and Diabetes Endocrine and Metabolism Pediatric Unit, Cairo University, Cairo, Egypt
noha.musa@yahoo.com
J Pediatr Endocrinol Metab 2020; 33(2): 295–304.
doi: 10.1515/jpem-2019-0345

- Using the validated World Health Organization QOL-BREF questionnaire that independently assesses the physical, psychological, social and environmental domains, the authors assessed health-related QOL in 200 Egyptian children and adolescents with CAH.
- Older patients had significantly lower QOL scores ($r=-0.151$, $P=0.033$). The physical domain correlated significantly with the degree of virilisation ($r=-0.491$, $P=0.001$) and frequency of hospitalization ($r=-0.495$, $P<0.001$). The psychological domain was affected by age ($r=-0.157$, $P=0.026$) and timing of genitoplasty ($r=-0.326$, $P=0.001$), while the social domain was affected by age ($r=-0.277$, $P<0.005$) and pubertal stage ($r=-0.195$, $P=0.006$). Salt wasting patients had lower scores at the physical domain ($P=0.001$).
- Health-related QOL worsened with older age, poor hormonal control and high frequency of hospital admissions.

This cross-sectional study included 140 females and 60 males with CAH due to 21-hydroxylase deficiency (mean age 6.6 ± 4.5 years). The WHO questionnaire (WHOQOL-BREF) used by the authors assesses four main domains: physical health (i.e. activities of daily living, dependence on medicines, energy, mobility, pain), psychological health (i.e. body image, negative and positive feelings, self-esteem, learning), social relationships (i.e. social support) and environment (i.e. financial resources, freedom, physical safety, home and physical environments, leisure activities). Overall, children and adolescents with CAH were found to have a lower QOL compared to a general population. Although there was no control group in this study, the version of the

WHOQOL-BREF in Arabic was found to have excellent reliability and validity. Females had lower scores at the psychological domain, whereas males had lower scores at the physical domain. Several aspects of this interesting article deserve comments. First, 77/137 neonates originally assigned to the male sex were later reassigned to the female sex after confirmation of the diagnosis. Although the age at which reassignment was performed is not provided, final diagnosis was obtained at a median age of 4 days, but as late as 10 years. While the questionnaire was administered only to the children (with the help of the parents when needed), it would be interesting to understand the impact of sex reassignment on the parents themselves. Second, in this Egyptian study, clitoroplasty was performed at a young age. The younger the age, the better the psychological component of the QOL. An Arab colleague helped me put this article in the context of her culture. She explained to me the influence of social norms on the timing of the decision of the surgery. Some will argue that surgery should be delayed until the child can make the decision herself. In Egypt (where 85–90% of the population is Muslim), it is important that cosmetic normalization be performed as early as possible regardless of a medical indication. Indeed, in most Arab societies, genital ambiguity affects the family social well-being, sexuality is not openly discussed (and a question about sexual pleasure was removed from the questionnaire), homosexuality is not recognized, assisted reproduction options are limited (*in vitro* fertilization and intrauterine insemination between the spouses are permitted but egg and sperm donation from unrelated donors are not) and women who are unmarried or do not conceive face many social issues. Many of the female patients included in this study were very young, and it would be important to reassess the QOL when they become adults to understand the long term effects of CAH, which can adversely affect fertility and sexual function. This article serves as a reminder that guidelines for the management of CAH need to consider the culture and religion of each population.

14. The Year in Science & Medicine

Ze'ev Hochberg, Ken Ong
Technion-Israel Institute of Technology, IL-31096 Haifa, Israel, rprzeev@technion.ac.il;
Medical Research Council Epidemiology Unit and Department of Paediatrics, Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Cambridge CB2 0QQ, UK, ken.ong@mrc-epid.cam.ac.uk

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14.1. Viral hormones: Expanding dimensions in endocrinology

Huang Q, Kahn CR, Altindis E
Endocrinology 2019;160:2165–2179

This paper characterize viral insulin/IGF-1–like peptides (VILPs), which the authors identified in four members of the Iridoviridae family - viruses with double-stranded DNA genomes, which are found in amphibians, fish, and invertebrates. VILPs can bind to human insulin and IGF-1 receptors and stimulate classic post-receptor signaling pathways. They can stimulate glucose uptake *in vitro* and *in vivo* and stimulate DNA synthesis. DNA sequences of some VILP-carrying viruses have been identified in the human enteric virome.

Mimicry is a common evolutionary principle that occurs to gain an advantage in competing for resources, protection, or survival. Viruses use mimicry to their benefit by expressing molecules that resemble host growth factors or immune response regulators and their receptors. Using a bioinformatics approach, this paper reports that viruses possess the DNA/RNA with potential to encode 16 different peptides with sequence similarity to human peptide hormones and metabolically important regulatory proteins including VILP and α-melanocyte-stimulating hormone. Two of these VILPs bind to the human IGF1 receptor (hIGF1R) with a better affinity than human insulin, but with a less affinity than human IGF-1. VILPs also compete for the human insulin receptor (hIR), but were much weaker affinity than human insulin and even human IGF-1. Consistent with this, all VILPs tested stimulated receptor autophosphorylation with higher potency on hIGF1R than on the human IR. All VILPs stimulated glucose uptake, even if at lower potencies than insulin and IGF-1. Thus, despite the low binding affinity to the hIR, VILPs stimulate biological function, suggesting that these peptides may bind to receptors in some unique way compared with the mammalian hormones.

One mechanism of viral mimicry uses divergent evolution involving the transfer of a gene from a host genome to a viral genome via horizontal gene transfer. Another aspect of viral mimicry generates proteins that affect the host immune system by imitating the function of cytokines such as IL-6, IL-10, and IL-17.

The authors suggest that a potential consequence of VILPs in humans is in the pathogenesis of Type 1 diabetes (T1D). These viral peptides might stimulate or desensitize T-cells and thus contribute to the pathogenesis of T1D by triggering T-cell cross-reactivity with endogenous insulin.

14.2. The dental proteome of homo antecessor

Welker F, Ramos-Madrigal J, Gutenbrunner P, Mackie M, Tiwary S, Rakownikow Jersie-Christensen R, Chiva C, Dickinson MR, Kuhlwilm M, de Manuel M, Gelabert P, Martinón-Torres M, Margvelashvili A, Arsuaga JL, Carbonell E, Marques-Bonet T, Penkman K, Sabidó E, Cox J, Olsen JV, Lordkipanidze D, Racimo F, Lalueza-Fox C, Bermúdez de Castro JM, Willerslev E, Cappellini E
Nature 2020;580:235–238.

These authors applied modern protein analysis to an ancient molar from a male *Homo antecessor* dated to 772–949 thousand years ago (kya) from the Sierra de Atapuerca in Burgos, Spain and also to dentine and enamel from a *Homo erectus* dated to 1770 kya. They found that the composition of these proteomes is similar to that of modern humans, including enamel-specific amelogenin, enamelin, ameloblastin, amelotin and the enamel-specific protease matrix metalloproteinase. Indeed, they had modern-like faces with a considerably deep ancestry in the genus *Homo*, and the cranial morphology of Neanderthals represents a derived form.

Recent developments in the extraction and tandem mass-spectrometric analysis of ancient proteins have made it possible to retrieve phylogenetically informative protein sequences from Early Pleistocene contexts. They show that *Homo antecessor* is tentatively the last common ancestor of Neanderthals and modern humans and is a close sister lineage to subsequent Middle and Late Pleistocene hominins.

For the first time, we hear of protein analysis by mass spectrometry from an 800 kya and 1770 kya fossilized tooth belonging to the hominin species *Homo antecessor*. By comparison, the oldest human DNA recovered so far date to ∼400 kya. This field is called palaeoproteomics and it enables the reconstruction of human evolution from further back in time than ever before. Using palaeoproteomics, they determine the sequence of amino acids within protein remains and compare the ancient protein sequences to those of Neanderthals and *Homo sapiens*. The results suggest that *Homo antecessor* was a sister group to *Homo sapiens*, who first appeared ∼400 kya, Neanderthals, who split from modern humans between 180-800 kya, and Denisovans, who diverged from modern humans and Neanderthals between 780-1,300 kya. *Homo antecessor* was the last common ancestor to modern humans and Neanderthals. His facial features were very similar to those of *Homo sapiens* and the Neanderthals.

14.3. The mutational constraint spectrum quantified from variation in 141,456 humans

Konrad J. Karczewski, Laurent C. Francioli […*et al*...], Daniel G. MacArthur
Nature 2020; 581, 434–443

The Genome Aggregation Database (gnomAD) Consortium https://gnomad.broadinstitute.org compiled data on ~125,000 exomes and ∼15,000 whole genomes from populations around the world. This is one of seven articles (also see Refs 1–6) describing their initial discoveries, showing the power of this vast dataset, and presenting a more complete catalog of loss-of-function variants, which disrupt the encoded proteins. This is an important tool to help inform the diagnosis of rare genetic diseases in our patients. It also provides fundamental insights into which of our genes appear to be essential, by quantifying how many loss-of-function variants are present in each gene in humans.

This is an expansion of the 1000 Genomes Project. The many partners of the Consortium identified more than 443,000 loss-of-function variants, while classifying all protein-coding genes and identifying many duplications, deletions, inversions, and other changes involving larger DNA segments (> 50-100 bases long). At least 25% of all rare loss-of-function variants are structural variants; many people carry these deleterious structural alterations without the expected phenotypes or clinical outcomes.

The physiological function of most genes in the human genome remains unknown. For the discovery of gene function, a common approach is to introduce disruptive mutations into genes in cell or animal models to determine their effects on cellular and physiological phenotypes. Such studies have yielded valuable insight into eukaryotic physiology and have guided the design of therapeutic agents. However, while such model organisms have been crucial in deciphering the function of many human genes, they remain imperfect proxies for human physiology. Using natural loss-of-function variants is supposed to give us a more direct approach to the study of gene function.

Loss-of-function variants are mostly deleterious for health and development and are thus typically maintained at very low frequencies in human populations, but there is wide variation between genes in the numbers of such deleterious variants that we humans carry. These new data describe which genes we can survive without (e.g. loss-of-function variants are frequent in the olfactory genes) and which genes are more essential (e.g. genes that are also associated with embryonic lethality). The latter ‘constrained’ genes are more ubiquitously expressed throughout body tissues and interact with more other proteins – they can be associated with human disease but are more likely de novo mutations than inherited.

The authors suggest the near-future feasibility and considerable value of a human ‘knockout’ project—a systematic attempt to discover the phenotypic consequences of functionally disruptive mutations, in either the heterozygous or homozygous state, for all human protein-coding genes.

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14.4. Insights into human genetic variation and population history from 929 diverse genomes

Bergström A, McCarthy SA, Hui R, Almarri MA, Ayub Q, Danecek P, Chen Y, Felkel S, Hallast P, Kamm J, Blanché H, Deleuze JF, Cann H, Mallick S, Reich D, Sandhu MS, Skoglund P, Scally A, Xue Y, Durbin R, Tyler-Smith C
Science 2020; 367(6484):eaay5012.

These authors sequenced 929 whole-genome sequences from 54 geographically, linguistically, and culturally diverse human populations, as part of the Human Genome Diversity Project, a panel of global populations. The data represent African, Oceanian, and American-Indian populations. They identified 67.3 million single-nucleotide polymorphisms (SNPs), 8.8 million small insertions or deletions (indels), and 40,736 copy number variants (CNVs). These include hundreds of thousands of variants that had not been discovered by previous sequencing efforts, but which are common in one or more population.

Genome sequences from diverse human groups reveals the architecture of genetic variation in our species and also the history of, and relationships between, different populations. They also provide a framework for the design and interpretation of medical genetics studies. Previous large-scale genome-sequencing efforts have been restricted to large, metropolitan populations and used low-coverage sequencing, whereas those sampling human groups more widely have mostly been limited to one to three genomes per population. Here, they present 929 high-coverage genome sequences from 54 diverse populations, only 142 of which were previously sequenced.

Populations in central and southern Africa, the Americas, and Oceania each harbor tens to hundreds of thousands of private, common genetic variants, mostly arising as new mutations. They estimate that the genetic separation between present-day human populations occurred mostly within the past 250,000 years and was shaped by protracted gene flow. Unexpectedly, African populations share many Neanderthal and Denisovan variants that are absent from Eurasia.

The authors conclude that low diversity among the Neanderthal haplotypes in present-day populations indicates that more than one Neanderthal individual contributed genetic material to modern humans, but there was only one major episode of admixture. By contrast, Denisovan haplotype involves more than one episode of admixture.

The results indicate that diversity resulted from differences at the single-nucleotide level rather than copy number variation. They find ancestral genetic variation in African populations that likely predates modern humans and has been lost in most non-African populations.

14.5. The sex with the reduced sex chromosome dies earlier: A comparison across the tree of life

Xirocostas ZA, Everingham SE, Moles AT
Biology Letters 2020;16:20190867

Why do men die younger than women? It is believed that this is because they take bigger risks, have more dangerous jobs, drink, and smoke more, and are poorer at seeking advice from doctors. But maybe it also had something to do with the sex chromosomes that men have. These authors studied sex chromosomes and

lifespans in a wide range of animal species and found shorter lifespan in the sex with reduced or absent chromosome in the heterogametic sex (e.g. the Y chromosome in mammals and the W chromosome in birds).

Sexual dimorphism is seen in many traits and behaviors among animals, including the most fundamental of life-history traits - lifespan. Previous work had showed shorter lifespans of mammalian males and avian females. Here, they took a broader view across the tree of life. They asked whether the heterogametic sex tends to have reduced longevity relative to the homogametic sex. The increased phylogenetic span of this study relative to previous work is important, as it gives greater power to unravel the importance of sex chromosomes.

Across a broad range of species, the heterogametic sex dies 17.6% earlier on average. In birds, butterflies and moths, the males have the homogametic sex chromosomes (usually ZZ) while the females have the heterogametic chromosomes (ZW). In species where males are heterogametic (XY), females live almost 21% longer than males. But in the species where females are heterogametic (ZW), males outlive females by only 7%.

The 'unguarded X hypothesis' suggests that the Y chromosome in male is less able to protect an individual from harmful genes expressed on the X chromosome, which may later expose the individual to health threats.While in an XX individual, a healthy X chromosome can stand in for another X that has deleterious genetic mutations. Another reason might be that the shorter Y is more easily lost with age/environmental damage (1). Some researchers suggested that lifespan differences between sexes are not solely genetic, but are also influenced by a combination of parental investment, exposure to predators, sexual selection and other biotic factors.

The authors suggest three possible explanations for this trend: the degree of degradation of the Y chromosome, telomere dynamics, and side effects of sexual selection.

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14.6. How frequently are articles in predatory open access journals cited

Björk B-C, Kanto-Karvonen S, Harviainen JT
Publications 2020;8:17

Predatory journals, sometimes called write-only publishing or deceptive publishing, follow an exploitive academic publishing business model that involves charging publication fees to authors without checking articles for quality and legitimacy and without providing the other editorial and publishing services that legitimate academic journals provide, whether open access or not. Their numbers keep increasing - 10,332 predatory journals were included on Cabell's blacklist in November 2018. The current study found that 6 of every 10 articles published in a random sample of predatory journals attracted not one single citation over a 5-year period.

Much attention has been focused on the deceitful behavior of such publishers, and on a couple of experiments with flawed or nonsensical manuscripts easily passing a non-existent peer review process in many such journals. Here, the authors measured the actual impact of the articles published in such journals. A random sample of articles published in the ~25,000 peer-reviewed journals had an average of 18.1 citations, with only 9% receiving no citations. The authors studied citation statistics over a 5-year period in Google Scholar for a random selection of 250 articles published in predatory journals in 2014 and found an average of only 2.6 citations per article, and 56% of articles had no citations at all.

They conclude that predatory journals use aggressive marketing tactics and pretend to use peer review but mostly they collect author payments. The authors of this article claim that if they are not read, they cause no harm, but that low-quality or deceptive studies published in these journals are getting undue attention. The harm that such journals cause has mostly to do with the prestige and value of academic publishing in general and your own university in particular. If they are not read, why write them? Such articles are often covered by tax-payers money in the poorest countries – many of such articles come from Africa and South Asia. Only some of the articles in predatory journals contain flawed or directly harmful results, while many describe unexceptional and poorly reported studies. The authors admit it is not straightforward to correctly classify which journals are 'predatory'. Scopus, Web of Science and other widely used citation databases perform some quality control checks on journals, but Google Scholar does not.

One particularly negative aspect of predatory publishing is that it has cast a shadow on the development of more responsible Open Access publishing and has possible slowed down its uptake. Many academic authors have wrongly equated open access and article processing charges with a lack of peer review and assessment quality.

14.7. Dosage analysis of the 7q11.23 Williams region identifies BAZ1B as a major human gene patterning the modern human face and underlying self-domestication

Zanella M, Vitriolo A, Andirko A, Martins PT, Sturm S, O'Rourke T, Laugsch M, Malerba N, Skaros A, Trattaro S, Germain PL, Mihailovic M, Merla G, Rada-Iglesias A, Boeckx C, Testa G.
Science Advances 2019;5:eaaw7908

This paper shows that the craniofacial and cognitive/behavioral phenotypes caused by alterations at the critical gene region for the Williams-Beuren syndrome is caused by changes in the chromatin remodeler *BAZ1B* in neural crest, and can serve as an entry point into the evolution of the modern human face and pro-sociality.

Williams-Beuren syndrome is caused by the hemi-deletion of 28 genes at the 7q11.23 region, and represents a neurodevelopmental condition whose craniofacial dysmorphisms and cognitive/behavioral traits bear directly on domestication-related traits such as facial reduction and retraction, pronounced friendliness, and reduced reactive aggression. Charles Darwin in *The Descent of Man* considered the analogy between modern humans and domesticated species. Anatomically, modern humans display craniofacial and prosocial behaviors that are suggestive of traits that distinguish domesticated species from their wild type. This is known as the self-domestication hypothesis: modern humans went through a domestication process during their evolution, as did cats and dogs. The so-called 'domestication syndrome,' with a set of domestication-related traits was proposed to result from mild neural crest deficits (1).

Here, the authors identified the *BAZ1B* gene at 7q11.23 as a master regulator of the modern human face, based on a molecular and functional dissection in the thus far largest cohort of Williams-Beuren syndrome patients. Then, they utilized the versatility of CRISPR-Cas9 to generate an allelic series of endogenously tagged *BAZ1B* across 7q11.23 dosages to define its dosage-dependent genome-wide occupancy. They defined a pivotal role for *BAZ1B* as an enhancer regulator, consistent with its preferential binding of distal regulatory regions, and to partition its dosage-dependent regulation into bona fide direct and indirect targets. Further on, they provide the first experimental evidence for the cardio-cephalic neural crest syndrome that had been suggested to explain the domestication syndrome and had pointed to *BAZ1B* as one of the candidates underlying this syndrome. It also reports a modern-specific enrichment for regulatory changes both in *BAZ1B* and its experimentally defined downstream targets. Of these genes, *FOXP2*, *ROBO1*, and *ROBO2* have been associated with brain wiring processes critical for vocal learning in several species.

This provides the first empirical validation of the human self-domestication hypothesis and positions *BAZ1B* as a master regulator of the modern human face. It is remarkable that genes implicated in neural crest development also play significant roles in cognitive processes, such as language or the theory of mind (understand of desires and intentions, among others) which are affected in the 7q11.23 syndromes.

Reference

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14.8. Patient-customized oligonucleotide therapy for a rare genetic disease

Kim J, Hu C, Moufawad El Achkar C, Black LE, Douville J, Larson A, Pendergast MK, Goldkind SF, Lee EA, Kuniholm A, Soucy A, Vaze J, Belur NR, Fredriksen K, Stojkovska I, Tsytsykova A, Armant M, DiDonato RL, Choi J, Cornelissen L, Pereira LM, Augustine EF, Genetti CA, Dies K, Barton B, Williams L, Goodlett BD, Riley BL, Pasternak A, Berry ER, Pflock KA, Chu S, Reed C, Tyndall K, Agrawal PB, Beggs AH, Grant PE, Urion DK, Snyder RO, Waisbren SE, Poduri A, Park PJ, Patterson A, Biffi A, Mazzulli JR, Bodamer O, Berde CB, Yu TW.
N Engl J Med. 2019 Oct 24;381(17):1644-1652.
doi: 10.1056/NEJMoa1813279.

This remarkable case report - by authors from Boston Children's Hospital and funded by Mila's Miracle Foundation - describes the development and use of a patient-customised antisense oligonucleotide drug that was tailored specifically against the unique DNA sequence mutation in a 6-year old girl with Batten disease, a degenerative neurological disease due to neuronal accumulation of lipofuscin and characterised by intractable seizures. They first tested the drug *ex vivo*, using cell lines from the patient, then showed that systemic administration reduced the frequency of seizures and halted her previously deteriorating neurological function.

This approach is the ultimate in individualised disease management. First, they used whole genome sequencing to detect her unique compound heterozygous mutation in the *MFSD8* gene, then they designed a unique antisense oligonucleotide to block the effects of her aberrant splice site mutation. The girl's name is Mila, and her drug is named after her. I urge you to read her story at: https://www.milasmiracle.org/milasen.

14.9. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial

Harrison SA, Bashir MR, Guy CD, Zhou R, Moylan CA, Frias JP, Alkhouri N, Bansal MB, Baum S, Neuschwander-Tetri BA, Taub R, Moussa SE
Lancet. 2019 Nov 30;394(10213):2012-2024.
doi: 10.1016/S0140-6736(19)32517-6.

Resmetirom (MGL-3196) is a liver-directed, orally active, selective thyroid hormone receptor-β agonist. This 36-week long randomised, placebo-controlled trial in 348 US adults with biopsy confirmed non-alcoholic steatohepatitis (fibrosis stages 1–3) shows that Resmetirom reduced hepatic fat by -37.3% (compared to -8.5% on placebo). Adverse events were mostly mild or moderate and were balanced between groups.

The effects of thyroid hormones on reducing LDL cholesterol (by suppressing its generation by the liver) are long established. Notably, before the availability of thyroid hormone assays, Lawson Wilkins used changes in plasma cholesterol levels to monitor treatment of hypothyroidism with thyroid extract. Indeed, Resmetirom was originally designed to treat dyslipidamia and reduces circulating LDL cholesterol levels by ~30%. Furthermore, it shows high selectivity for thyroid hormone receptor-β and has no impact on the central thyroid axis.

These findings are exciting as there are currently no effective pharmacological treatments that effectively prevent the progression to fibrosis in non-alcoholic steatohepatitis. Resmetirom is currently in large scale phase 3 trial with this histological endpoint, and another promising option in late stage trials is obeticholic acid, a semi-synthetic bile acid analogue.

14.10. Low-dose aspirin for the prevention of preterm delivery

Hoffman MK, Goudar SS, Kodkany BS, Metgud M, Somannavar M, Okitawutshu J, Lokangaka A, Tshefu A, Bose CL, Mwapule A, Mwenechanya M, Chomba E, Carlo WA, Chicuy J, Figueroa L, Garces A, Krebs NF, Jessani S, Zehra F, Saleem S, Goldenberg RL, Kurhe K, Das P, Patel A, Hibberd PL, Achieng E, Nyongesa P, Esamai F, Liechty EA, Goco N, Hemingway-Foday J, Moore J, Nolen TL, McClure EM, Koso-Thomas M, Miodovnik M, Silver R, Derman RJ, ASPIRIN Study Group
Lancet. 2020 Jan 25;395(10220):285-293.
doi: 10.1016/S0140-6736(19)32973-3.

This randomised, placebo-controlled trial ('ASPIRIN') in 11,976 nulliparous women in six low or middle-income countries (India, Democratic Republic of the Congo, Guatemala, Kenya, Pakistan, and Zambia) found that low-dose aspirin (81 mg daily from early pregnancy) reduced the risk of preterm delivery (before 37 weeks gestational age) to 11.6% compared to 13.1% in controls (Relative Risk 0.89 [95% CI 0.81 to 0.98], $P = 0.012$). There were also significant reductions in perinatal mortality (0.86, $p = 0.048$), fetal loss (0.86, $P = 0.039$), early preterm delivery (<34 weeks (0.75, $P = 0.039$), and early preterm delivery with hypertensive disorders of pregnancy (0.38 [0.17–0.85], $P = 0.015$).

Preterm birth is the leading cause of newborn mortality and childhood disability. Hence, any intervention to avoid preterm delivery has huge potential for clinical and socio-economic benefits. This trial tested the hypothesis that low-dose aspirin would prevent preterm delivery by reducing mechanisms related to pre-

eclampsia during early pregnancy placentation and spiral artery formation. The findings are consistent with that premise, as a larger effect was seen particularly for early preterm delivery associated with hypertensive disorders of pregnancy, while the relatively modest overall effect on preterm delivery may suggest that other mechanisms are unaffected. It would be interesting to explore how much this mechanism, and its modification by low-dose aspirin, contributes to preterm delivery in high income populations.

14.11. Increased weight loading reduces body weight and body fat in obese subjects – A proof of concept randomized clinical trial

Ohlsson C, Gidestrand E, Bellman J, Larsson C, Palsdottir V, Hägg D, Jansson PA, Jansson JO
EClinicalMedicine. 2020 Apr 30;22:100338.
doi: 10.1016/j.eclinm.2020.100338.

The gravitostat hypothesis was only recently proposed based on studies in rodents showing that the addition of external weights to the body limits the rate of weight gain, independent of leptin signalling. In rodents, this was achieved by inserting weighted balloons into the abdominal cavity (1).

This randomised controlled trial now tested the same hypothesis in human obese adults ($n=72$), mean age ~ 50 years, who were assigned to wear a heavy (11% of body weight) or light (1% of body weight) weighted vest for 8 hours each day for 3 weeks. Those who wore the heavy vest lost more body weight than in the light vest group (mean relative difference: -1.4%, 95% CI: -2.0 to -0.8; $P=1.5\times10-5$). Furthermore, changes were seen in fat mass (-4.0%, -6.5 to -1.5; $P=1.9\times10-3$) but not in fat-free mass (0.4%, -1.47 to 2.34; $P=0.65$).

From a clinical perspective, this seems to be a remarkably simple intervention to recommend. All those in the heavy vest group managed to wear this for at least 6 h per day, except for one person who managed on average 5.99 h/day) and there was no difference between groups in the % time spent standing. Non-serious musculoskeletal adverse events were more common in the heavy vest group (20%; 1 arthralgia, 2 myalgia, 2 pain in lower leg, 2 swelling of ankle and/or foot) than in the light vest group (2.7%; 1 myalgia), but it is possible that these are only short-term effects.

From a biological perspective, this concept is fascinating. It suggests that the homeostatic regulation of body weight involves not just the established system of hormonal feedback from adipose accumulation (e.g. leptin), but also a mechanical sensing of body weight that does not discriminate between endogenous and external loads. How this operates is yet unclear. The previous rodent studies documented no change in energy expenditure but reduced food intakes (1); no such difference was reported here in humans, although the measures of self-reported food intake are much more noisy than animal experimental conditions.

Reference

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14.12. Brain-Sparing sympathofacilitators mitigate obesity without adverse cardiovascular effects

Mahú I, Barateiro A, Rial-Pensado E, Martinéz-Sánchez N, Vaz SH, Cal PMSD, Jenkins B, Rodrigues T, Cordeiro C, Costa MF, Mendes R, Seixas E, Pereira MMA, Kubasova N, Gres V, Morris I, Temporão C, Olivares M, Sanz Y, Koulman A, Corzana F, Sebastião AM, López M, Bernardes GJL, Domingos AI
Cell Metab. 2020 Jun 2;31(6):1120-1135.e7.
doi: 10.1016/j.cmet.2020.04.013.

This experimental rodent study of a PEGylated amphetamine (PEGyAMPH) designed to not cross the blood brain barrier is interesting for 2 reasons. First, it shows that its peripheral anti-obesity effects alone (without the central anorexic effects of amphetamine) are sufficient to achieve weight loss by acting on the β2-adrenoceptor to increase lipolysis and thermogenesis. Second, it shows that the cardiac side effects of amphetamine are mediated by central, instead of direct peripheral, signaling.

Since the 1930's, amphetamine-class medications have been widely used to treat obesity, although cardiovascular safety concerns have led to repeated bans. 'Rainbow diet pills' combined an amphetamine

and other agents to block its peripheral stimulant effects, however, these were banned in the 1960's, and again in the 2000's shortly after their re-emergence in North America. Phentermine, an amphetamine derivative, has been used in obesity since the 1960's; its combination with fenfluramine (Phen-Fen) was withdrawn in 1997 due to cases of cardiac valve disease; however, its combination with topiramate remains FDA-approved since 2012 (although not approved in Europe).

There is a very long history of anti-obesity medications that have been withdrawn even several years after their approval, as long-term safety issues became apparent. Many agents are clearly effective to produce weight loss, but long-term safety concerns seem to arise repeatedly, not only for amphetamines. Lorcaserin, a centrally acting 5-HT2C receptor agonist approved by the FDA in 2012 (but not in Europe), was the latest casualty, being withdrawn in the US in February 2020 due to increased risks of pancreatic, colorectal and lung cancers.

14.13. Screen of traditional soup broths with reported antipyretic activity towards the discovery of potential antimalarials

Straschil U, Witmer K, Delves MJ, Marks SD, Baum J
Children of Eden Primary School
Arch Dis Child. 2019 Dec;104(12):1138-1142.
doi: 10.1136/archdischild-2019-317590.

Many effective drugs originated from traditional remedies. The 2016 Nobel Prize in Physiology or Medicine was awarded to Tu Youyou, whose work underpinned the development of the anti-malarial drug artemisinin by isolating the active ingredient of 'Qinghao', which has been used in Chinese traditional medicine for over 2000 years.

In this truly engaging approach, children attending an ethnically diverse primary school in London were asked to bring in soup samples with claimed antipyretic activity. Samples were then tested *in vitro* for their ability to arrest malaria parasite growth and transmission. The children provided 56 soup samples, from across their European, North African and the Middle Eastern cultures. Five samples showed >50% *in vitro* growth inhibition against P. falciparum asexual blood stages.

In our current era of large-scale screening using molecular 'omics approaches to new drug discovery, efforts should also keep an eye open for potential active ingredients in traditional medicines that have been used for hundreds of years.

14.14. T cell–mediated regulation of the microbiota protects against obesity

Petersen C, Bell R, Klag KA, Lee S-H, Soto R, Ghazaryan A, Buhrke K, Ekiz HA, Ost KS, Boudina S
Science 2019;365:eaat9351.

As in humans, weight gain in mice leads to fatty liver disease, inflammatory adipose tissue, and insulin resistance. The depletion of the microbiota through antibiotic treatment rescued this weight gain. The cohousing of T-Myd88–/– mice transferred the weight gain to wild-type mice. The major feature of the microbiota formed within T-Myd88–/– mice was a reduction in Clostridia colonization.

The microbiota has emerged as a key regulator of metabolism within the mammalian host, and the composition of the microbiota in obese individuals is sufficient to confer metabolic defects when transferred into mice. Reductions in the gene richness of the microbiota have been reported during the metabolic syndrome including decreased butyrate and methane production, while, some microbiota functions are enhanced, such as hydrogen sulfide and mucus degradation.

Gut immune responses are critical in regulating the composition of the microbiota. Young mice whose T cells have disabled Myd88 signaling (T-Myd88–/– mice) exhibit reduced follicular T cell responses and defective IgA targeting of their gut bacteria. Mice depleted of the innate adaptor molecule T-Myd88 developed many of the metabolic disease comorbidities found in humans, including visible weight gain in middle-age between 5 and 6 months of age.

What is the role of inflammatory responses during obesity? During weight, within the adipose tissue gain macrophage infiltrate and there is a reduction in regulatory T cells. Such suboptimal immune response is

associated with the metabolic syndrome and obesity. Obese adults show a decrease in immune response to immunizations, increased incidence of infection, and reduced mucosal IgG levels. The links between defective immune reactions and the metabolic syndrome are unclear. T cell–dependent events are required to prevent disease, and replacement of Clostridia rescued obesity. Inappropriate immunoglobulin A targeting of Clostridia and increased Desulfovibrio antagonized the colonization of beneficial Clostridia.

We now learn that mice defective in T follicular helper cell and gut IgA production develop the metabolic syndrome with age, they gain more weight, accumulate more fat, and show greater insulin resistance compared with controls. Their IgA inaptly targets Clostridia which enhances host lipid absorption by modulating CD36 expression.

In conclusion, gut bacteria can differentially regulate lipid metabolism. Products secreted by Desulfovibrio up-regulate CD36 expression, whereas products produced by Clostridia can down-regulate CD36 expression. Therefore, the loss of organisms that function to temper CD36 expression may lead to the inappropriate absorption of lipids, which accumulate over time, leading to obesity and the metabolic syndrome.

14.15. Fecal microbiota transplantation for the improvement of metabolism in obesity: The FMT-TRIM double-blind placebo-controlled pilot trial

Yu EW, Gao L, Stastka P, Cheney MC, Mahabamunuge J, Torres Soto M, Ford CB, Bryant JA, Henn MR, Hohmann EL
PLoS Med. 2020 Mar 9;17(3):e1003051.
doi: 10.1371/journal.pmed.1003051.

The authors describe a 12-week long randomised controlled trial of weekly oral fecal microbiota transplantation capsules, derived from healthy lean donors, given to 24 adults with obesity and mild–moderate insulin resistance. Despite evidence of successful microbiota engraftment in recipients, there was no change in fasting insulin sensitivity, HbA1c, body weight, body composition or resting energy expenditure.

The premise for this approach was the widespread associations between microbiota and metabolism in human observational studies and consistently significant effects on body weight and glycaemia from microbiota transplantation in experimental animal studies.

However, the previous experimental evidence base seems suspiciously too positive. That was the conclusion of a recent review by Walter *et al*. (1), which found an implausible 'exceedingly high (success) rate of inter-species transferable pathologies' - all but two (36/38; 95%) studies found that fecal transfer from diseased human donors could create that disease phenotype in the recipient mice. Walter *et al*. (1) made several suggestions towards a more critical and rigorous experimental approach.

Reference

1. Walter J *et al*. Establishing or Exaggerating Causality for the Gut Microbiome: Lessons from Human Microbiota-Associated Rodents. *Cell*. 2020 Jan 23;180(2):221–232. https://doi.org/10.1016/j.cell.2019.12.025.

14.16. Effect of high-dose vitamin D supplementation on volumetric bone density and bone strength

Burt LA, Billington EO, Rose MS, Raymond DA, Hanley DA, Boyd SK
JAMA. 2019 Aug 27;322(8):736–745.
doi: 10.1001/jama.2019.11889

Two legs good, four legs better? Vitamin D supplementation is widely recommended in various national guidelines at doses of 400 to 2000 iu per day in children and adults for its benefits on bone health. So you might think that more would be even better? At least 3% of US adults agree with this extrapolation and report intakes of 4000 iu per day or more.

These authors tested this question in a 3-year long, double-blind, randomized clinical trial in Calgary, Canada, on 311 healthy adults without osteoporosis, aged 55–70 years. Surprisingly, age-related reduction in bone mineral density was lowest in the recommended vitamin D dose group (400 IU/day group: −1.2% at the radius) and was progressively worse on the higher (4000 IU/day: −2.4%) and highest doses (10 000 IU/day: −3.5%).

The authors should be congratulated in admitting that the clearly significant findings of this well-designed trial were in the opposite direction of their research hypothesis. This is a reminder of the principle that the design of any scientific work must include the option to reject the hypothesis.

The findings present an important lesson against extrapolating from established to higher doses, and they also provide a timely warning against the 'pre-cautionary' use of high dose vitamin D for protection against a myriad of putative, but unproven benefits – including appropriate scientific evaluation of its use in the prevention or treatment of COVID-19 infection (1).

Reference

1. National Institute for Health and Care Excellence. COVID-19 rapid evidence summary: vitamin D for COVID-19. Published date: 29 June 2020 https://www.nice.org.uk/advice/es28/chapter/Key-messages

15. Editors' choice

Ken Ong[1], Ze'ev Hochberg[2]

[1]Medical Research Council Epidemiology Unit & Department of Paediatrics, Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Cambridge CB2 0QQ, UK, ken.ong@mrc-epid.cam.ac.uk; [2]Technion-Israel Institute of Technology, IL-31096 Haifa, Israel, rprzeev@technion.ac.il

15.1. Preconception diabetes mellitus and adverse pregnancy outcomes in over 6.4 million women: A population-based cohort study in China

Wei Y, Xu Q, Yang H, Yang Y, Wang L, Chen H, Anderson C, Liu X, Song G, Li Q, Wang Q, Shen H, Zhang Y, Yan D, Peng Z, He Y, Wang Y, Zhang Y, Zhang H, Ma X

PLoS Med. 2019 Oct 1;16(10):e1002926.

doi: 10.1371/journal.pmed.1002926.

The authors analysed a huge dataset from the Chinese national programme of preconception health checks, which include measurements of fasting plasma glucose. Overall, 13.15% ($n = 847{,}737$) had impaired fasting glucose and 1.18% ($n = 76{,}297$ women) had diabetes, of whom only 1.2% (917 women) were previously aware of having diabetes.

Women with diabetes or impaired fasting glucose had significantly higher risks of spontaneous abortion (Odds ratios: 1.11 and 1.08, respectively), pre-term birth (1.17 and 1.02), macrosomia (1.13 and 1.07), SGA (1.17 and 1.06), and perinatal infant death (1.59 and 1.08).

Despite much research focus on the prevention of gestational diabetes, there are as yet few effective interventions (1). Therefore, attention in this field is shifting to possible windows for intervention in early gestation or even before pregnancy. This study indicates that there is certainly much potential benefit for pregnancy outcomes and offspring health of correcting high glucose levels in the preconception period. A first step is to highlight the opportunity for pre-pregnancy health screening. In this regard, the Chinese National Free Pre-Pregnancy Checkups Project described here is greatly informative in identifying during preconception many women who were unaware of their diabetes status, and many more with 'pre-diabetes'. This may be a valuable model for other countries to follow.

Reference

1. H Al Wattar B, *et al.*, Mediterranean-style diet in pregnant women with metabolic risk factors (ESTEEM): A pragmatic multicentre randomised trial. *PLoS Med*. 2019 Jul 23;16(7):e1002857. doi: 10.1371/journal.pmed.1002857.

15.2. Association of maternal gastric bypass surgery with offspring birth defects

Neovius M, Pasternak B, Näslund I, Söderling J, Johansson K, Stephansson O

JAMA. 2019 Oct 15;322(15):1515–1517.

doi: 10.1001/jama.2019.12925.

The authors merged data from separate national Swedish registers for births and obesity surgery and identified 2921 women who had a Roux-en-Y gastric bypass surgery between 2007 to 2014 ('cases') and subsequent pregnancies. Controls were identified as women without bariatric surgery who were matched to cases by many factors, including pre-surgery BMI (mean 43.5), diabetes status, age, smoking and alcohol use. Median interval from surgery to conception was 1.6 years and during that time, cases lost on average 40 kg, and diabetes drug use decreased from 9.7% pre-surgery to 1.5% pre-conception.

The risk of major birth defects was substantially lower (risk ratio, 0.67) in infants of cases (3.4%, 98/2921) than in controls (4.9%, 1510/30 573), including risks of major heart defects and neural tube defects.

These findings show that obesity is teratogenic (possibly mediated by hyperglycaemia). Previous associations between maternal obesity and higher risks for birth defects are well established, but the current findings provide a higher level of causal inference and, importantly, show that reducing BMI pre-pregnancy decreases the excess risk. Furthermore, it allays concerns that any benefits of reducing BMI could be offset by harms of micronutrient deficiency following bariatric surgery, however data were not available on use of micronutrient supplements – it might be that Swedish women are more compliant with this than others.

15.3. An association between maternal weight change in the year before pregnancy and infant birth weight: ELFE, a French national birth cohort study

Lecorguillé M, Jacota M, de Lauzon-Guillain B, Forhan A, Cheminat M, Charles MA, Heude B
PLoS Med. 2019 Aug 20;16(8):e1002871.
doi: 10.1371/journal.pmed.1002871.

Women ($n = 16{,}395$; 26% overweight or obese) from the ELFE French national birth cohort were categorised into 3 groups by self-reported weight change during the year before pregnancy: weight loss >5 kg; stable weight; and weight gain >5 kg.

Among women with BMI < 25 kg/m^2 at conception, offspring birth weight was significantly higher in those reporting pre-pregnancy weight loss – this surprising association was explained by their higher rate of weight gain during pregnancy.

Among women who were overweight or obese at conception, birth weight was not associated with pre-pregnancy weight loss. Any direct effect on lower birth weight was cancelled out by their higher gestational weight gain, which was even higher if weight loss before pregnancy was reported to result from restrictive dieting.

While attention is shifting towards optimising maternal health and weight status prior to conception, the novel findings of this powerful analysis show that, importantly, efforts at weight control need to be continued during pregnancy. It is becoming well established that many homeostatic processes keep our body weights relatively stable and, in particular, act to promote weight regain after weight loss, even (unhelpfully) in overweight and obese individuals. These data show that the same applies during pregnancy. Restrictive dieting and weight loss before pregnancy may be cancelled out, or even turned to harmful effects, by weight rebound during pregnancy.

15.4. Neonatal, infant, and childhood growth following metformin versus insulin treatment for gestational diabetes: A systematic review and meta-analysis

Tarry-Adkins JL, Aiken CE, Ozanne SE
PLoS Med. 2019 Aug 6;16(8):e1002848.
doi: 10.1371/journal.pmed.1002848.

This systematic review identified 28 randomised trials ($n = 3976$ women) of metformin vs. insulin for treatment for gestational diabetes. Metformin use was associated with lower birth weights (overall mean difference -107.7 g), lower ponderal indices, and lower odds of macrosomia (odds ratio 0.59), but no difference in neonatal length or small for gestational age. Conversely, metformin use was associated with heavier infant weight (mean difference 440 g, 2 studies) and mid-childhood (5–9 years) BMI (mean difference 0.78 kg/m^2, 3 studies).

Metformin is a widely used, safe treatment option for gestational diabetes. The findings relating to birth size indicate that metformin compares well to insulin treatment, at least for these birth outcomes. However, the much fewer studies that included postnatal follow-up showed that any benefits of this reduced birth weight were reversed by a tendency to faster postnatal catch-up weight gain. The authors highlight that, in the long run, this pattern of reduced birth weight and higher postnatal catch-up may be more detrimental to offspring metabolic health than the benefits of avoiding macrosomia. Whether this is the case remains to be shown. Certainly, this review emphasises the importance of postnatal (and even longer-term) follow-up of such trials before making conclusions about the effects of maternal interventions on offspring metabolic health.

15.5. Unintentional error in formula preparation and its simulated impact on infant weight and adiposity

Altazan AD, Gilmore LA, Guo J, Rosenberg DM, Toupo D, Gowins A, Burton JH, Beyl RA, Chow CC, Hall KD, Redman LM
Pediatr Obes. 2019 Dec;14(12):e12564.
doi: 10.1111/ijpo.12564.

In an experimental setting, the authors asked 53 adults to dispense infant milk formula powder for three servings of 2, 4, 6, and 8 fl oz (57, 113, 170 and 226 grams, respectively) bottles, in random order. Remarkably, only 19% of bottles contained the recommended amount of formula powder. The large majority (78%) of bottles were overdispensed, resulting in 11% additional infant formula powder. Only 3% of bottles were underdispensed.

This study demonstrates the large potential for a fairly simple approach to avoid overfeeding and rapid infant weight gain – accurate dispensing of infant formula powder. The authors modelled that the extra unintentional feeding by 11% above energy requirements over a period of 6 months is sufficient to accelerate an infant's weight trajectory from the 50th to the 75th percentile.

There is substantial evidence and increasing awareness of the impact of infant overfeeding and excessive weight gain on higher risk for obesity in childhood and later life. An increasing number of interventions are being developed and tested to target overfeeding and rapid weight gain during this early life window. Those interventions employ a range of different strategies, from explicit education and support about these issues, different milk formula protein and energy compositions, to 'stealth' interventions that put the emphasis on other outcomes, such as parenting and sleep. It is interesting here that the errors in formula powder dispensing were far from random – 78% of bottles were overdispensed versus only 3% underdispensed. This is consistent with many parents' inherent tendencies to favour overfeeding their infants and it is an issue that needs to be addressed explicitly.

15.6. Primary prevention of cow's milk sensitization and food allergy by avoiding supplementation with cow's milk formula at birth: A randomized clinical trial

Urashima M, Mezawa H, Okuyama M, Urashima T, Hirano D, Gocho N, Tachimoto H
*JAMA Pediatr.*2019 Oct 21;173(12):1137–45.
doi: 10.1001/jamapediatrics.2019.3544.

The authors describe a randomised controlled trial to decrease risks of sensitization to cow's milk protein by avoiding supplementation with cow's milk formula at birth. The trial included 330 newborns in Japan, where the practice of supplementing breastfeeding with infant formula at birth is common. Instead, the intervention group received breastmilk plus or minus an amino acid-based elemental formula for at least the first 3 days of life.

At age 2 years (with an impressive 96.8% follow-up), the intervention group had reduced risk of sensitization to cow's milk (based on IgE levels; 16.8% vs. 32.2%; relative risk (RR), 0.52), and substantially lower prevalence of immediate (2.6% vs. 13.2%; RR, 0.20) and anaphylactic (0.7% vs. 8.6%; RR, 0.08) types of food allergy.

It is well established that breastfeeding is protective against the development of food allergies (among its many benefits). This study demonstrates that, conversely, exposure to cow's milk protein at birth causes a lasting sensitisation, which is detectable biochemically and clinically, in terms of acute food allergy. As well as having immediate lessons for routine practice, these important findings suggest potential for similar approaches to avoid autoimmune diseases, such as type 1 diabetes.

15.7. Childhood obesity intervention studies: A narrative review and guide for investigators, authors, editors, reviewers, journalists, and readers to guard against exaggerated effectiveness claims

Brown AW, Altman DG, Baranowski T, Bland JM, Dawson JA, Dhurandhar NV, Dowla S, Fontaine KR, Gelman A, Heymsfield SB, Jayawardene W, Keith SW, Kyle TK, Loken E, Oakes JM, Stevens J, Thomas DM, Allison DB
Obes Rev. 2019 Nov;20(11):1523–1541.
doi: 10.1111/obr.12923.

This is a thoughtful position statement by a number of leading obesity researchers and statisticians. They draw attention to 10 errors that are common in the scientific literature: 1. Using self-reported outcomes and 'teaching to the test' (achieving better scores simply by repeat testing); 2. Lack of control groups and ignoring regression to the mean over time; 3. Changing the goal posts (i.e. the study primary outcome); 4. Ignoring clustering when randomizing groups of children; 5. Subsetting the data, p-hacking and data dredging (to produce false positives); 6. Relying on difference from baseline (rather than between groups); 7. Equating 'no statistically significant difference' with 'equally effective'; 8. Prioritising observational analyses over intervention effects; 9. One-sided tests for statistical significance; and 10. Stating that effects are clinically significant when not statistically significant.

These highlighted errors are by no means confined (or more common) in childhood obesity studies than other fields. However, they illustrate these errors and how to avoid them by examples from the childhood obesity literature as an important step towards effective advances in reversing the obesity epidemic.

Notably, this was the last paper by Douglas Altman, who for many years was the chief statistical adviser to the British Medical Journal and, with his co-author Martin Bland, wrote the highly-regarded series of authoritative and succinct Statistical Notes in that journal. He worked hard to improve the use of statistics in medical research, and once wrote *'The majority of statistical analyses are performed by people with an inadequate understanding of statistical methods. They are then peer reviewed by people who are generally no more knowledgeable'*.

15.8. Arginine-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus: A prospective diagnostic study

Winzeler B, Cesana-Nigro N, Refardt J, Vogt DR, Imber C, Morin B, Popovic M, Steinmetz M, Sailer CO, Szinnai G, Chifu I, Fassnacht M, Christ-Crain M

Lancet. 2019 Aug 17;394(10198):587–595.
doi: 10.1016/S0140-6736(19)31255-3.

These authors previously described that the measurement of copeptin, a peptide cleaved in the posterior pituitary from the protein precursor of vasopressin and stable in circulation, is an accurate tool to diagnose diabetes insipidus (DI; vasopressin insufficiency). However, they acknowledged that their original protocol using hypertonic saline as a stimulus is challenging and requires close monitoring of hypernatraemia.

Here, they describe good diagnostic discrimination by plasma copeptin on arginine stimulation in adult patients (age 18+ years), 12 with complete DI, 9 with partial DI, 31 with primary polydipsia, and 20 healthy controls; they included similar numbers in a separate validation cohort. Optimal accuracy was reached at a cutoff of 3ñ8 pM copeptin at 60 min (sensitivity 93%; specificity 92%).

Unfortunately, for paediatric endocrinologist and our patients, they did not include children with DI and, as they found differences in plasma copeptin levels between healthy children and healthy adults, the robustness of these thresholds to diagnose children with DI needs to be assessed.

15.9. Prevalence and trends of overweight and obesity in European children from 1999 to 2016: A systematic review and meta-analysis

Garrido-Miguel M, Cavero-Redondo I, Álvarez-Bueno C, Rodríguez-Artalejo F, Moreno LA, Ruiz JR, Ahrens W, Martínez-Vizcaíno V

JAMA Pediatr. 2019 Aug 5;173(10):e192430.
doi: 10.1001/jamapediatrics.2019.2430.

The authors systematically reviewed published evidence on the prevalence of overweight and obesity in children aged 2–13 years. Based on data on 477 620 children from 28 European countries, they conclude that the prevalence is very high but appears to have stabilized in most countries.

From 1999 to 2016, the combined prevalence of overweight and obesity (defined by the International Obesity Task Forced BMI cutoffs) decreased in Iberia from 30.3% to 25.6%, increased in the Mediterranean region from

22.9% to 25.0%, and did not change substantially in Atlantic Europe 18.3% to 19.3% and Central Europe 15.8% to 15.3%.

These data are somewhat reassuring for many countries. The rising trends in the Mediterranean region and the large between-country differences are important topics for further exploration.

15.10. Parents' marital quality and children's transition to adulthood

Brauner-Otto SR, Axinn WG, Ghimire DJ
Demography 2020;57:195–220.

These authors examined the long-term consequences for children of the emotional bond between parents. In a long-term Nepalese cohort, they find that children whose parents report strong marital affection and less spousal conflict attain higher levels of education and marry later than children whose parents behave differently.

The evolutionary theory of socialization stipulates that familial psychosocial stress (e.g., marital conflict, harsh parenting, and father absence), affect reproductive strategy. Early maturation is selected under conditions of emotional risk and security uncertainty, thereby setting the stage for earlier sexual debut, more promiscuous mating, and the bearing of more offspring, along with lesser parental investment.

Unique longitudinal measures from Nepal allowed this group to link both mothers' and fathers' reports of their marital relationships with a subsequent long-term record of their children's behaviors. They focused on children's educational attainment and marriage timing because these two dimensions of the transition to adulthood have wide-ranging, long-lasting consequences. They found that children whose parents report strong marital affection and less spousal conflicts attained higher levels of education and marry later than children whose parents reported weak marital affection. Furthermore, these findings are independent of each other and of multiple factors known to influence children's educational attainment and marriage timing. These results support theories pointing toward the long-term intergenerational consequences of variations in multiple dimensions of parents' marriages. It was previously shown that maximal effect is exerted at the transition from infancy to childhood around age one year and then again at the transition from childhood to juvenility around age 6.

The authors consider three mechanisms likely to be operating in the Nepal-specific context and culture: parental investments in childrearing, general social psychological links between parents and children, and children's motivations to leave the parents' home.

15.11. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis

Chaurasia B, Tippetts TS, Mayoral Monibas R, Liu J, Li Y, Wang L, Wilkerson JL, Sweeney CR, Pereira RF, Sumida DH, Maschek JA, Cox JE, Kaddai V, Lancaster GI, Siddique MM, Poss A, Pearson M, Satapati S, Zhou H, McLaren DG, Previs SF, Chen Y, Qian Y, Petrov A, Wu M, Shen X, Yao J, Nunes CN, Howard AD, Wang L, Erion MD, Rutter J, Holland WL, Kelley DE, Summers SA
Science 2019;365:386–392.

By genetically engineering mice, the authors deleted the enzyme dihydroceramide desaturase 1 (DES1), which normally inserts a conserved double bond into the backbone of ceramides. Ablation of DES1 from whole animals or tissue-specific deletion in the liver and/or adipose tissue resolved hepatic steatosis and insulin resistance in mice caused by leptin deficiency or obesogenic diets

Sphingolipids such as ceramides and dihydroceramides are products of fat and protein metabolism that accumulate in subjects with obesity and hyperlipidemia. These lipids have been implicated in a wide range of cellular processes related to metabolism, growth, and survival. Previous research suggested associations between serum and tissue levels of ceramides and comorbidities of obesity, including insulin resistance, type 2 diabetes (T2D), and major adverse cardiac events. This article claims that ceramides are the major contributor to insulin resistance and fatty liver disease. The mechanism is related s to the enzyme dihydroceramide desaturase 1 (DES1).

Some clinics have begun using serum ceramide levels as a measure of cardiovascular disease risk. Here, they studied the role of these sphingolipids as causative agents in the development of insulin resistance and hepatic

steatosis. Clinical studies demonstrate inverse correlations between the amounts of ceramides in plasma and adiponectin in healthy individuals or those with T2D.

Inhibition of DES1 may provide a means of treating hepatic steatosis and metabolic disorders and provide evidence that therapeutically intervening in the ceramide biosynthesis pathway in mice can improve metabolic homeostasis.

15.12. A value-based healthcare approach: Health-related quality of life and psychosocial functioning in women with Turner syndrome

van den Hoven AT, Bons LR, Dykgraaf RHM, Dessens AB, Pastoor H, de Graaff LCG, Metselaar MR, Kneppers-Swets A, Kardys I, Mijnarends H, Zweerus F, Hazelzet JA, Utens EMWJ, van den Bosch AE, Roos-Hesselink JW
Clinical Endocrinology 2020;92:434–442.

The authors developed and applied questionnaires to capture various aspects of health-related quality of life (HR-QoL) and psychosocial functioning in a large cohort of adult women with Turner syndrome (TS). Women with TS reported a lower HR-QoL, perceived more stress and experienced increased fatigue compared to the general population. They found a relationship between HR-QoL and non-cardiac comorbidities (diabetes and orthopedic complaints).

In addition to the physical disorders in TS, HR?QoL these women are also affected by mild neuropsychological deficits, such as relative weaknesses in visual?spatial, executive and social cognitive domains. Additionally, other factors such as infertility, lower socioeconomic status or impaired ability to work may affect QoL in TS women.

This study aimed to investigate various aspects of HR-QoL and psychosocial functioning in 201 women with TS (age 34 $\pm$ 12 years) to establish new possible targets for therapy. Women with TS reported a lower HR-QoL, which was related to physical factors, such as diabetes and orthopedic complaints. They perceived more stress and experienced increased fatigue. Women with TS were less content with their weight, waist, build, and figure. Also, reflected in this questionnaire, is the discontent with their energy levels. Women with TS also frequently reported concerns about the future, often related to their education or work. Value-based healthcare means aiming at improving outcomes that really matter to patients. The authors suggest that the extreme fatigue may partially be explained by the mismatch that women with TS experience between what is expected of them by themselves and their environment and what they are capable of doing. This may result from the impaired concentration and attention leading to impaired executive function and subsequent fatigue.

The authors claim that perceived stress and increased fatigue should be considered targets for intervention for improvement of HR-QoL in TS women (see also paper 15.13).

15.13. Health-related quality of life in Turner syndrome and the influence of growth hormone therapy: a 20-year follow-up

Krantz E, Landin-Wilhelmsen K, Trimpou P, Bryman I, Wide U
The Journal of Clinical Endocrinology & Metabolism 2019;104:5073–5083.

On a similar theme to paper 15.12, these authors studied women with Turner syndrome (TS; $n = 200$), age range 16–78 years, between 1995 and 2018 with a focus on the impact of growth hormone (GH) therapy on health-related quality of life (HR-QoL). Despite a mean 5.7 cm increase in expected height, GH therapy was not associated with improved HR-QoL. HR-QoL in TS women was negatively related to older age at reporting, older age at diagnosis, and hearing impairment.

Children with TS have been offered GH therapy for several decades, based on the demonstration of gains in final height in the range of 4–7 cm. TS is not associated with GH deficiency, so the sole objective of GH therapy is to increase final height. However, little has been published on HR-QoL outcomes after GH therapy, particularly in TS. Studies that have made conclusions on GH therapy on HR-QoL in TS are either very small or do so in comparison to women in the general population, but do not compare the GH-treated to untreated women. There was a knowledge gap regarding the long-term effects of GH on HR-QoL in adulthood in TS.

Here, women with TS reported a similar HR-QoL to the reference population, and there was no association between height and HR-QoL in the reference population. This finding is similar to the other smaller studies that have compared GH-treated to untreated women with TS, in which no benefit of GH on HR-QoL was found in younger women.

The authors courageously conclude, *'our findings call into question whether treating short stature in childhood with such a cumbersome and expensive treatment is justified when the height gain is relatively small. There is also a risk that the treatment contributes to "health care fatigue' in childhood that may cause the young women on the cusp of transition to adult care to abstain from further monitoring, which may put them at greater risk later in life unnecessarily"*. It may be time to reconsider the TS indication for GH therapy.

15.14. Gender similarities in the brain during mathematics development

Kersey AJ, Csumitta KD, Cantlon JF
npj Science of Learning 2019;4:1–7.

To investigate the early biology of mathematics ability, these authors tested for gender differences in the neural processes of mathematics in young children using functional magnetic resonance imaging (fMRI). Across all analyses, they found similar neural functioning between genders, indicating that boys and girls engage the same neural system during mathematics development.

A single study in 1992 of intrinsic, biological gender differences in mathematics ability has been the basis of a debate about the underrepresentation of girls and women in STEM fields (science, technology, engineering, and mathematics). Some have suggested that girls and women are underrepresented in STEM careers due to biological differences.

This is the first neuroimaging study to evaluate biological gender differences in mathematics aptitude of young children. These researchers measured brain activity with fMRI in 104 young children (age 3–10 years; 55 girls) while watching an educational video covering early mathematics topics, such as counting and addition. They implemented both frequentist and Bayesian analyses that quantify gender similarities and differences in neural processes. In addition, they examined brain maturity by comparing scans of children to those in adults ($n = 63$; 25 women) who watched the same videos.

They found no difference in brain development of girls and boys. In addition, they found no difference in how boys and girls processed mathematics skills and they were equally engaged while watching the educational videos. Finally, brain maturity was equivalent in children and adults.

So, why do girls succeed less in STEM? The authors speculate that families spend more time with young boys in play that involves spatial cognition. Teachers also preferentially spend more time with boys during mathematics classes. Finally, children often pick up on cues from their parent's and teacher's expectations. This may be true, but from personal observations, we leave on the table an option that boys have a genuine greater interest than girls in mathematics. Once they attend STEM classes, the neural mechanisms for processing it seems to be gender indifferent.

15.15. Large-scale GWAS reveals insights into the genetic architecture of same-sex sexual behavior

Ganna A, Verweij KJH, Nivard MG, Maier R, Wedow R, Busch AS, Abdellaoui A, Guo S, Sathirapongsasuti JF, 23andMe Research Team, Lichtenstein P, Lundström S, Långström N, Auton A, Harris KM, Beecham GW, Martin ER, Sanders AR, Perry JRB, Neale BM, Zietsch BP
Science 2019;365:eaat7693.

These authors performed a genome-wide association study on 493 001 participants of European-ancestry from the USA, UK, and Sweden to identify genes associated with sexual orientation. They found multiple loci implicated in same-sex sexual behavior indicating that, like other behavioral traits, non-heterosexual behavior is polygenic.

As many as 4% to 10% of US individuals report ever engaging in same-sex sexual behavior. It is commonly believed that same-sex orientation and sexual behavior runs in families and has a genetic basis. Twin studies and

models to estimate inheritance indicate that same-sex sexual behavior has a significant genetic component that explains 18% of the phenotype in women and 37% in men. Other factors include shared environments of family or school, and legalization or norms regarding same-sex sexual behavior. However, previous searches for the specific genes involved had been underpowered.

This is the largest genetic study to date on this topic. They discovered 5 loci robustly associated with same-sex sexual behavior: 2 loci across both sexes, 2 only in males, and 1 only in females. However, these 5 loci together account for a small fraction ($<1\%$) of the phenotypic variation. Thus, a combined genetic score cannot be used to predict same-sex sexual behavior of an individual.

A notable finding was that these loci were also associated with more heterosexual partners (among those reporting only heterosexual partners) – suggesting the traits measured here indicated a 'promiscuous' tendency rather than sexual orientation or preference. They did not find evidence of any specific cells and tissues related to this trait. Yet, male-specific loci were associated with olfactory receptor genes, sensitivity to certain scents, and regulation of testosterone and estrogen.

15.16. Microglial UCP2 mediates inflammation and obesity induced by high-fat feeding

Kim JD, Yoon NA, Jin S, Diano S
Cell Metabolism 2019;30:952–962. e5.

These authors report that high-fat diet (HFD) in mice induces a dynamic increase in uncoupling protein 2 (Ucp2) mRNA expression in hypothalamic microglia. Ucp2 is required for HFD-induced mitochondrial changes in hypothalamic microglia and for HFD-induced inflammation, obesity, and POMC synaptic plasticity.

The brain plays a major role in the regulation of feeding and whole-body metabolism. This study focused on the brain's glia cells, rather than neurons, and shows a fundamental role for mitochondrial dynamics in microglia cells and their response to HFD.

It was known that HFD triggers microglia activation and hypothalamic inflammation as early as 3 days after HFD exposure, before changes in body weight occur. Here, they find that HFD induce a rapid and transient increase in Ucp2 mRNA expression together with changes in mitochondrial dynamics.

Ucp2 is a mitochondrial protein, which plays a critical role in mitochondrial function, including control of reactive oxygen species (ROS) generation and fuel utilization. Ucp2 has been implicated in the regulation of the activity of several hypothalamic neuronal populations involved in the control of energy and glucose homeostasis. It is highly expressed in activated microglial cells, and has also been implicated in mediating the energetic processes of microglia activation states in neuro-inflammation. Here, selective deletion of Ucp2 in microglia protected mice from HFD-induced obesity, showing decreased feeding and increased energy expenditure, associated with changes in the synaptic input organization and activation of the anorexigenic hypothalamic POMC neurons and astrogliosis. In peripheral macrophages, Ucp2 is required for mitochondrial oxidation of glutamine, while in hypothalamic NPY/AgRP neurons, Ucp2 promotes the metabolic shift from glucose oxidation to fatty acid oxidation during starvation.

In summary, this study shows that the combination of high glucose/high fat diet induces changes in microglial mitochondria dynamics and function via Ucp2. These mitochondrial changes are critical for microglia activation and neuro-inflammation that ultimately affect the susceptibility to diet-induced obesity.

15.17. Insulin-like growth factor 1 signaling in tenocytes is required for adult tendon growth

Disser NP, Sugg KB, Talarek JR, Sarver DC, Rourke BJ, Mendias CL
The FASEB Journal 2019;33:12680–12695.

These authors conditionally deleted the IGF1 receptor gene (IGF1R) in tenocytes using a tamoxifen-inducible Cre-recombinase system and then stimulated plantaris tendon growth in adult mice via mechanical loading. Mice that lacked IGF1R in their tenocytes showed reduced cell proliferation and tendon growth in response to mechanical loading. These studies indicate that IGF1 signaling is required for postnatal tendon growth.

When longitudinal bones grow, so do the adjacent tendons. We don't know much about tendons. They are a dense connective tissue that transmits force from muscle to bone during mechanical loading. Their extracellular matrix (ECM) is composed mostly of type I and type III collagen, elastin, and proteoglycans. Tendon fibroblasts ('tenocytes') are responsible for the synthesis, organization, and maintenance of the ECM. In response to high stress repetitive mechanical loading, such as during resistance exercise, tendons adapt by undergoing hypertrophy.

The authors hypothesized and showed that IGF1 signaling is required for normal tendon growth in response to mechanical loading, by regulating collagen synthesis and cell proliferation. Without IGF1 signaling, tendons were smaller and did not adapt normally. IGF1 may even become a new target for treating tendon injuries in humans.

15.18. Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa

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This genome-wide association study (GWAS) of 16,992 cases of anorexia nervosa and 55 525 controls identified 8 significant loci. The findings show that the genetic architecture of anorexia nervosa mirrors its clinical features, showing significant genetic correlations with psychiatric disorders (obsessive-compulsive disorder, depression, anxiety, and schizophrenia), physical activity, and metabolic (including glycemic), lipid and anthropometric traits, independent of the effects of common variants associated with BMI.

A previous smaller GWAS (3495 cases, 10 982 controls) estimated the common genetic variant-based heritability of anorexia nervosa to be ~20%. This new GWAS suggests that anorexia nervosa is at least partly a metabolic disorder, and not purely psychiatric as previously thought. 36 genes were predicted to be differentially expressed in tissues or blood, and 4 variants were confirmed by expression analyses, chromatin interaction studies or both. These were the locus-intersecting genes *CADM1* (Cell-adhesion molecule 1), *MGMT* (Methylguanin-DNA methyltransferase), *FOXP1* (Forkhead box P1) and *PTBP2* (Polypyrimidine tract-binding protein). The expression of *MGMT*, involved in a protein network related to brain tumors, was predicted to be downregulated in anorexia nervosa.

It was believed that metabolic abnormalities seen in individuals with anorexia nervosa are secondary to starvation and weight loss. Instead, this study shows that metabolic differences may be related to susceptibility to anorexia nervosa. The authors estimate that the metabolic factors may play nearly or as strong a role as psychiatric determinants in the development of anorexia nervosa. They conclude that anorexia nervosa should be thought of as a hybrid 'metabo-psychiatric disorder', and that it will be important to consider both metabolic and psychological factors when exploring new avenues for treating this potentially lethal illness.

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