Yearbook of Paediatric Endocrinology 2023

Editors
Ken Ong
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published by
bioscientifica

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Paediatric Endocrinology 2023

Endorsed by the European Society for Paediatric Endocrinology

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Published by Bioscientifica
Starling House, 1600 Bristol Parkway North, Bristol, BS34 8YU, UK

Typeset by OKS Prepress

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Preface

Welcome to the 2023 edition of the ESPE Yearbook. It has been a fascinating year of major advances both in the basic sciences and in clinical research, with several new genes, mechanisms and also highly promising treatments. We encourage you to explore widely the easily summarized papers and insightful comments by our expert chapter editors. And, in our ongoing quest to improve the content and format of the Yearbook, we welcome your comments and suggestions.

In this edition, we are sadly lacking our usual chapters on Antenatal and Neonatal Endocrinology and Pituitary and Neuroendocrinology, following the retirements of Khalid Hussain and Taneli Raivio, who have excellently served the Yearbook for many years. We have been fortunate to secure world-leading clinical scientists to lead these chapters – please look out for our announcements in 2024! In the meantime, for Yearbook 2023 we have endeavored to cover progress in these topics in other chapters (e.g. see reviews on congenital hyperinsulinism and maternal obesity in Chapter 13).

We conclude Yearbook 2023 with a special chapter in tribute to Professor Ze'ev Hochberg (Haifa), who sadly passed away at the beginning of 2023. Ze'ev originated and coedited the Yearbook since its first edition in 2004 until 2021. He was a close friend and colleague or collaborator of so many of us in the ESPE community of Paediatric Endocrinologists and beyond. He read, thought, discussed and published on a remarkably wide range of topics, always bringing new and original perspectives and ideas. His breadth of knowledge and interests are reflected in our selection of his papers.

Christa E. Flück and Ken K. Ong

1. Thyroid

Gabor Szinnai, Basel, Switzerland

This chapter aims at giving an overview over the most fascinating, innovative but also clinically most relevant publications in pediatric thyroidology over the last 12 months. Detailed molecular characterization of the sodium/iodide symporter, and the first thyroid organoids generated from human embryonic stem cells are highlights from basic research. A randomized controlled trial compared neurocognitive outcome in patients with congenital hypothyroidism after treatment with different starting doses. New management guidelines for pediatric thyroid cancer provide a concise summary of the current recommendations for optimal care of affected patients. Finally, a large-scale epidemiological study gives important insights into incidence trends of autoimmune thyroid diseases over the last decades and co-occurrence with other autoimmune diseases over all age groups.

Mechanism of the Year

1.1. Structural insights into the mechanism of the sodium/iodide symporter

Ravera S, Nicola JP, Salazar-De Simone G, Sigworth FJ, Karakas E, Amzel LM, Bianchet MA, Carrasco N

Nature. 2022 Dec;612(7941):795–801.

doi: 10.1038/s41586-022-05530-2. Epub 2022 Dec 14. PMID: 36517601

Brief summary: The sodium/iodide symporter (NIS) is the first and limiting step for thyroid hormone synthesis (1). NIS is located at the basolateral membrane of the thyroid follicular cells. NIS translocates iodide against its electrochemical gradient from the blood into the cytosol of the thyroid follicular cell by a co-transport with sodium. Besides iodide, the sodium/iodide symporter has also further substrates such as the environmental endocrine disruptor perchlorate, or substrates used for scintigraphy or single-photon emission computed tomography, such as pertechnetate, and perrhenate, respectively. This extensive study gives a detailed insight into the mechanism of the sodium/iodide symport by NIS.

Ravera *et al*. combined structural analyses by single-particle cryo-electron microscopy of the rat NIS protein in three different conditions: NIS without iodide binding (apo-NIS), NIS with iodide binding (NIS-I^-), and NIS with binding of perrhenate (NIS-ReO^-). They determined the binding sites of iodide and sodium within the substrate-binding pocket of the NIS-protein and investigated the functional effect of known NIS gene mutations in the binding residues. Then, they compared structural changes of the binding sites induced by iodide or perrhenate, revealing important structural dynamics between NIS-I^- and NIS-ReO^-. Finally, they describe the NIS-I^- binding mechanism in detail: binding of a first sodium, binding of a second sodium inducing an important conformational change of structure, and increasing the affinity of NIS to iodide by a factor of ten, iodine binding, occluded conformation, and finally release of the two sodium and the iodide into the cytosol of the thyroid follicular cell.

Twenty-six years after cloning and characterization of the NIS gene, this fundamental, elegant and extensive study by Ravera *et al*. describes the structural changes during sodium/iodide symport by NIS conserved in many species (2). The work represents a milestone in thyroid physiology providing the full picture of NIS function integrating and completing previous results of the last decades.

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2. Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. *Nature*. 1996 Feb 1;379(6564):458–60. doi: 10.1038/379458a0. PMID: 8559252.

1.2. DIO3 protects against thyrotoxicosis-derived cranio-encephalic and cardiac congenital abnormalities

Martinez ME, Pinz I, Preda M, Norton CR, Gridley T, Hernandez A

JCI Insight. 2022 Nov 8;7(21):e161214.
doi: 10.1172/jci.insight.161214. PMID: 36166296

Brief summary: *In utero* the embryo and the fetus are protected by different mechanisms from too high levels of maternal thyroid hormones after transplacental passage such as deiodination and sulfatation of T4 and T3 in the placenta as well as in the tissues of the unborn child (1,2). Maternal hyperthyroidism and its treatment with anti-thyroid drugs is associated with different congenital malformation. However, so far, it remains unclear, which malformations are linked to hyperthyroidism, to anti-thyroid drug toxicity, or anti-thyroid drug induced hypothyroidism *in utero*.

To shed light on the effects of hyperthyroidism *in utero*, Martinez *et al*. developed a Deiodinase 3 ($Dio3^{-/-}$) mouse strain. Embryos were exposed at the earliest stages to hyperthyroidism. Then, the authors performed a comprehensive phenotyping at different ages on all organ systems in $Dio3^{-/-}$ mice. As a consequence of *in utero* exposure to elevated thyroid hormone levels mice developed reduced viability, severe growth retardation, and cartilage loss postnatally. Mice surviving the neonatal period showed cranial and cerebral malformations, such as hydrocephalus, choanal atresia, and cleft palate and cardiac problems such as septal defects and thin ventricular walls.

In conclusion, this study provides for the first time detailed new insights into developmental effects of thyrotoxicosis *in utero* and postnatally describing mainly specific cranio-cerebral and cardiac defects.

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1.3. Incidence of and risk factors for neonatal hypothyroidism among women with Graves' disease treated with antithyroid drugs until delivery

Yoshihara A, Noh JY, Inoue K, Watanabe N, Fukushita M, Matsumoto M, Suzuki N, Suzuki A, Kinoshita A, Yoshimura R, Aida A, Imai H, Hiruma S, Sugino K, Ito K

Thyroid. 2023 Mar;33(3):373–379.
doi: 10.1089/thy.2022.0514. Epub 2023 Feb 21. PMID: 36680759

Brief summary: Neonates of mothers suffering from Graves' Disease during pregnancy are at risk for hyper- or hypothyroidism postnatally. The risk for hypothyroidism of neonates exposed to maternal anti-thyroid drugs until birth is unknown. This large retrospective study aimed at investigating 1) the incidence of hypothyroid neonates in a large cohort of mothers treated with anti-thyroid drugs until birth, and 2) identify a cutoff for maternal anti-thyroid drug doses associated with neonatal hypothyroidism.

A total of 305 pregnant women with Graves' disease were included in this retrospective cohort study. Neonates of mothers treated with methimazole were euthyroid in 62% (39/63), showed subclinical hypothyroidism in 19% (12/63) and overt hypothyroidism in 19% (12/63). Neonates of mothers treated with propylthiouracil were euthyroid in 62% (150/242), showed subclinical hypothyroidism in 24% (57/242), and were overt hypothyroid in 13% (31/242). Four patients in the propylthiouracil group showed either subclinical or overt hyperthyroidism. Anti-thyroid drug dose during the third trimester and maternal FT4 and TRAB levels were the best predictors for neonatal hypothyroidism. The cut-off for predicting neonatal hypothyroidism was 10mg for methimazole and 150mg for propylthiouracil.

In conclusion, these results allow to anticipate neonatal hypothyroidism for both treatments in newborns of mothers treated with anti-thyroid drugs until delivery. The defined cut-off doses inform adult endocrinologists to optimally titrate the anti-thyroid drug dose during the third trimester for mother and child.

Thyroid Development

1.4. Transplantable human thyroid organoids generated from embryonic stem cells to rescue hypothyroidism

Romitti M, Tourneur A, de Faria da Fonseca B, Doumont G, Gillotay P, Liao XH, Eski SE, Van Simaeys G, Chomette L, Lasolle H, Monestier O, Kasprzyk DF, Detours V, Singh SP, Goldman S, Refetoff S, Costagliola S

Nat Commun. 2022 Nov 17;13(1):7057.
doi: 10.1038/s41467-022-34776-7. PMID: 36396935

Brief summary: In recent years, generation of human organoids of different tissues from human embryonic stem cells have been realized, e.g. intestine, liver, and lung among others. In contrast, so far all attempts to generate fully mature and functional human thyroid follicular cells from stem cells was not successful. Romitti *et al*. present for the first time successful generation of transplantable and functional human thyroid organoids derived from human embryonic stem cells.

The Costagliola team has published in 2012 the generation of functional thyroid tissue derived from murine embryonic stem cells (1). Based on their experience, it took them ten years to establish a robust model to generate fully mature and functional human thyroid organoids. They report in detail on the doxycycline inducible transient overexpression of *NKX2-1* and *PAX8* in their human embryonic stem cell *NKX2-1*$^{WT/GFP}$ line. Subsequent differentiation steps as proliferation and maturation were then induced by addition of cAMP, hrTSH, and dexamethasone in the culture until stem cells became mature thyroid follicular cells. These thyroid follicular cells were analyzed extensively by immunohistochemistry, single cell RNA sequencing, and biochemistry to prove structural and functional differentiation with full synthetic capacity. Sequential single cell analysis over the whole culture period of 45 days documented in detail the changing gene expression pattern until reaching fully mature thyroid follicular cell expression. Immunohistochemistry showed mature follicular structure with expression of thyroperoxidase and thyroglobulin. Finally, the authors provided evidence for stable thyroid hormone synthesis of these human thyroid organoids after transplantation into mice that underwent radioablation of their thyroids and were hypothyroid. T3 levels in transplanted mice were completely normalized.

In conclusion, this extensive and elegant work is an important step forward in thyroidology. It opens avenues first, for detailed studies of human thyroid development not accessible by other models, and second, options for possible regenerative medicine in patients with thyroid diseases in the future.

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Antonica F, Kasprzyk DF, Opitz R, Iacovino M, Liao XH, Dumitrescu AM, Refetoff S, Peremans K, Manto M, Kyba M, Costagliola S. Generation of functional thyroid from embryonic stem cells. *Nature*. 2012 Nov 1;491(7422):66–71. doi: 10.1038/nature11525. Epub 2012 Oct 10. PMID: 23051751.

Follow-up Paper from the 2022 Yearbook

1.5. Evaluation of the molecular landscape of pediatric thyroid nodules and use of a multigene genomic classifier in children

Gallant JN, Chen SC, Ortega CA, Rohde SL, Belcher RH, Netterville JL, Baregamian N, Wang H, Liang J, Ye F, Nikiforov YE, Nikiforova MN, Weiss VL

JAMA Oncol. 2022 Sep 1;8(9):1323–1327.
doi: 10.1001/jamaoncol.2022.1655. PMID: 35679040

Brief summary: In 2021 and 2022, two important publications on pediatric thyroid carcinomas revealed a distinct molecular landscape compared to adult thyroid carcinomas (1,2). Pediatric differentiated thyroid carcinoma was mainly caused by fusion oncogenes, especially in children younger than 10 years (93%), compared to children aged 10–15 years (28%) and 15–20 years old patients (14%). In contrast, PTC due to BRAF mutations showed increasing frequency with age (7%, 30%, and 65%, respectively in children <10 years, 10–15 years, and 15–20 years. The major clinical consequence of these results was that pediatric thyroid carcinomas caused by fusion oncogenes could successfully be treated with fusion targeted therapies in patients after molecular diagnosis (1). These results were confirmed in a second large study (2).

The presented retrospective monocenter case series by Gallant *et al*. investigated $n=95$ patients with a median age of 16.3 years (range: 4.8–21.1 years) who underwent thyroidectomy for thyroid nodule. Surgical samples were analyzed by a commercially available DNA/RNA next generation sequencing genomic classification test used for adult thyroid tumors. The molecular analyses confirmed the results of the two papers presented in the 2022 Yearbook that fusion oncogenes and *DICER1* variants were more frequent in the pediatric cohort than in published adult series and showed that genomic classifiers are useful for molecular diagnosis.

In summary, these results first confirm recent data on different molecular landscape of pediatric thyroid carcinomas, and second show that genomic classifiers established for adult thyroid tumors are amendable in pediatric thyroid tumor patients for better molecular diagnosis. Better molecular diagnosis is the key for targeted therapies in patients with unfavourable outcome.

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Lee YA, Lee H, Im SW, Song YS, Oh DY, Kang HJ, Won JK, Jung KC, Kwon D, Chung EJ, Hah JH, Paeng JC, Kim JH, Choi J, Kim OH, Oh JM, Ahn BC, Wirth LJ, Shin CH, Kim JI, Park YJ. NTRK and RET fusion-directed therapy in pediatric thyroid cancer yields a tumor response and radioiodine uptake. *J Clin Invest*. 2021 Sep 15;131(18):e144847. doi: 10.1172/JCI144847. PMID: 34237031.
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Franco AT, Ricarte-Filho JC, Isaza A, Jones Z, Jain N, Mostoufi-Moab S, Surrey L, Laetsch TW, Li MM, DeHart JC, Reichenberger E, Taylor D, Kazahaya K, Adzick NS, Bauer AJ. Fusion oncogenes are associated with increased metastatic capacity and persistent disease in pediatric thyroid cancers. *J Clin Oncol*. 2022 Apr 1;40(10):1081–1090. doi: 10.1200/JCO.21.01861. Epub 2022 Jan 11. PMID: 35015563.

Congenital Hypothyroidism

1.6. Effect of initial levothyroxine dose on neurodevelopmental and growth outcomes in children with congenital hypothyroidism

Esposito A, Vigone MC, Polizzi M, Wasniewska MG, Cassio A, Mussa A, Gastaldi R, Di Mase R, Vincenzi G, Pozzi C, Peroni E, Bravaccio C, Capalbo D, Bruzzese D, Salerno M

Front Endocrinol (Lausanne). 2022 Sep 5;13:923448.
doi: 10.3389/fendo.2022.923448. eCollection 2022. PMID: 36133316

Brief summary: Current guidelines for congenital hypothyroidism recommend a starting dose of 10–15 µg/d of levothyroxine for optimal treatment (1). Over the last years, some studies suggested that overtreatment of patients during infancy by high levothyroxine doses might have negative effects on neurocognitive and behavioral development (2). The presented multicenter prospective randomized trial aimed at comparing the effect of higher (12.5–15.0 µg/d levothyroxine starting dose) *versus* lower (10.0–12.5 µg/d levothyroxine starting dose) on growth and neurocognitive outcome.

The two treatment groups (higher dose $n=24$ patients, lower dose $n=21$ patients) were balanced for sex, gestational age, age at diagnosis and treatment start, biochemical severity of congenital hypothyroidism, and thyroid morphology (dysgenesis *versus* gland *in situ*). The main results are the following: First, the authors found no difference concerning growth parameters (weight, height) at 24 and 48 months. Second, they observed Griffiths Mental Development Scales and Subscales at 24 months, and in Wechsler Preschool and Primary scale of Intelligence at 48 months of age for both groups in the normal range without significant

differences between the higher *versus* lower levothyroxine treatment group. Third, despite biochemically optimal treatment over the 48 months of the study 6/45 patients showed IQ below normal range, associated with delayed bone age at diagnosis and lower socioeconomic status.

The so far only randomized trial for different starting doses (37.5 μg/d, 50 μg/d, and 62.5 μg for three days then lowered to 37.5 μg/d) in patients with congenital hypothyroidism was published in 2002 and 2005 by Selva *et al*. Those authors found differences in TSH and FT4 normalization in the first 4 weeks as well as in full scale IQ at the age of four years (3,4). This randomized clinical trial by Esposito *et al*. provides for the first-time important prospective data on neurocognitive outcome comparing two starting doses within the recommended starting dose range of 10–15 μg/d. The results show that the higher range of the recommended starting dose (12.5 μg–15.0 μg) did not result in better neurocognitive outcome. If confirmed in a larger cohort, and in the context of potential overtreatment with the higher dose group (12.5 μg–15.0 μg) these results might lead to adaptation of the current dosing recommendations to use rather the 10.0 μg–12.5 μg starting dose than 12.5 μg–15.0 μg starting dose.

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5. Thyroid hormone signaling specifies cone subtypes in human retinal organoids.
Eldred KC, Hadyniak SE, Hussey KA, Brenerman B, Zhang PW, Chamling X, Sluch VM, Welsbie DS, Hattar S, Taylor J, Wahlin K, Zack DJ, Johnston RJ Jr. Thyroid hormone signaling specifies cone subtypes in human retinal organoids. *Science*. 2018 Oct 12;362(6411):eaau6348. doi: 10.1126/science.aau6348. PMID: 30309916.

1.7. Comorbidity in congenital hypothyroidism – A nationwide population-based cohort study

Danner E, Jääskeläinen J, Niuro L, Huopio H, Niinikoski H, Viikari L, Kero J, Sund R

J Clin Endocrinol Metab. 2023 Jun 6:dgad334.

doi: 10.1210/clinem/dgad334. Online ahead of print. PMID: 37279943

Brief summary: Patients with congenital hypothyroidism have a higher rate of comorbidities in adult age (1). This nationwide population-based register study aimed at investigating the incidence of comorbidities present at birth or acquired during childhood in patients with congenital hypothyroidism and prescription of specific drugs (antidepressants, antipsychotics, medication for ADHS).

The study analyzed data from $n = 438$ full-term patients with congenital hypothyroidism with $n = 835$ matched controls with a median follow-up time of 11.6 years (range 0–23 years). The incidences of the following neonatal comorbidities were significantly higher in patients with congenital hypothyroidism than in controls: jaundice (11.2% and 2.0%), hypoglycemia (8.9% and 2.8%), metabolic acidosis (3.2% and 1.1%), and respiratory distress (3.9% and 1.3%). The incidence of congenital malformations was twice as high in the congenital hypothyroidism group compared to controls (15.1% and 7.4%). At the age of 15 years, cumulative incidence of hearing loss (5.0% and 1%), disorders of neurocognitive development (14.1% and 5.6%), and intellectual impairment (2.9% and 0.5%) were significantly increased in patients *versus* controls. Use of antidepressants, antipsychotics, medication for ADHS were statistically not different.

Based on national registry data, this study confirms earlier reports of increased neonatal morbidity and incidence of congenital malformations in congenital hypothyroidism. The study further provides important data on significantly increased cumulative incidences of hearing loss, neurodevelopmental problems, and intellectual disabilities in patients until the age of 20 years. This work demonstrates the importance of regular screening for hearing loss and neurocognitive deficits in patients with congenital hypothyroidism as recommended in the guidelines.

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Genetics

1.8. The severity of congenital hypothyroidism with gland-in-situ predicts molecular yield by targeted NGS

Levaillant L, Bouhours-Nouet N, Illouz F, Jager JA, Bachelot A, Barat P, Baron S, Bensignor C, De La Perriere AB, Djellas YB, Caillot M, Caldagues E, Campas MN, Caquard M, Cartault A, Cheignon J, Decrequy A, Delemer B, Dieckmann K, Donzeau A, Doye E, Fradin M, Gaudillière M, Gatelais F, Gorce M, Hazart I, Houcinat N, Houdon L, Ister-Salome M, Jozwiak L, Jeannoel P, Labarthe F, Lacombe D, Lambert AS, Lefevre C, Leheup B, Leroy C, Maisonneuve B, Marchand I, Marquant E, Muszlak M, Pantalone L, Pochelu S, Quelin C, Radet C, Renoult-Pierre P, Reynaud R, Rouleau S, Teinturier C, Thevenon J, Turlotte C, Valle A, Vierge M, Villanueva C, Ziegler A, Dieu X, Bouzamondo N, Rodien P, Prunier-Mirebeau D, Coutant R

J Clin Endocrinol Metab. 2023 Mar 8:dgad119.
doi: 10.1210/clinem/dgad119. Online ahead of print. PMID: 36884306

Brief summary: Over the recent years several publications reported on next generation sequencing (NGS) in cohorts of patients with congenital hypothyroidism (1). Based on these data, diagnostic yield was higher in patients with gland-*in-situ*, than with thyroid dysgenesis. Further studies performed NGS only in cohorts of patients with gland-*in-situ*, excluding thyroid dysgenesis forms such as athyreosis, ectopy, or hypoplasia (2). The publication of Levaillant *et al*. is so far the largest genetic study of patients with congenital hypothyroidism with gland-*in-situ* comparing for the first time diagnostic yield with disease severity of congenital hypothyroidism.

This genetic monocenter study presents systematic NGS results in $n=103$ patients with congenital hypothyroidism with gland-*in-situ*. Detailed clinical and laboratory phenotyping of all patients was performed. The used NGS panel covered $n=48$ genes either known to be associated with congenital hypothyroidism or known to be involved in thyroid physiology. In total in 42/103 (42%) patients a genetic cause of congenital hypothyroidism was identified. This diagnostic yield in the complete cohort is comparable to earlier results of previous studies. However, for the first time, this study analyzed the relationship between biochemical severity of congenital hypothyroidism based on TSH values at screening and at diagnosis and FT4 at diagnosis with diagnostic yield and found a higher diagnostic yield in patients with more severe hypothyroidism: the diagnostic yield in patients with TSH at screening of $>$ 80 mU/L, with TSH at diagnosis of $>$ 100 mU/L, or FT4 at diagnosis of <5 pmol/L was 73%, 60%, and 69% respectively compared to the diagnostic yield in patients with TSH and FT4 values below these cut-offs (25%, 30%, 29%).

In summary, this innovative study not only confirms earlier results of diagnostic yield in the so far largest cohort of patients with congenital hypothyroidism with gland-*in-situ*, but shows that a genetic diagnosis is far more probable in severe cases of congenital hypothyroidism than in milder forms. This observation is of importance in the context of increasing incidence of congenital hypothyroidism, especially of milder degree over the last decades, suggesting other than genetic causes for the increased incidence, such as e.g. lowering of screening cut-offs, mild iodine deficiency, or epigenetic effects.

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1.9. IGSF1 mutations are the most frequent genetic aetiology of thyrotropin deficiency

Fourneaux R, Reynaud R, Mougel G, Castets S, Bretones P, Dauriat B, Edouard T, Raverot G, Barlier A, Brue T, Castinetti F, Saveanu A

Eur J Endocrinol. 2022 Nov 3;187(6):787–795.
doi: 10.1530/EJE-22-0520. Print 2022 Dec 1. PMID: 36201163

Brief summary: Congenital central hypothyroidism is caused by thyrotropin deficiency, either isolated or in combination with other pituitary deficiencies. So far, mutations in five genes have been identified in patients with isolated thyrotropin deficiency: thyroid stimulating hormone subunit β (*TSHβ*), thyrotropin-releasing hormone receptor (*TRHR*), immunoglobulin superfamily member 1 (*IGSF1*), transducin-like protein 1 (*TBLX1*), and insulin receptor substrate 4 (*IRS4*). The phenotype of patients with mutations in IGSF1 is more complex, as transient growth hormone deficiency can occur during childhood. This study aimed at systematically investigate the genetic cause of non-syndromic isolated thyrotropin deficiency (ITSHD) or thyrotropin deficiency combined with growth hormone deficiency (TSHD-GHD) in a large cohort.

In the context of the GENHYPOPIT network, the authors identified $n=22$ index cases with ITSHD and $n=42$ index cases with TSHD-GHD. All $n=64$ patients were analyzed by a next generation sequencing panel for mutations in genes associated with ITSHD as well as all known genes associated with combined pituitary deficiencies. In $n=22$ ITSHD cases mutations in *IGSF1*, and *TSHβ* were found in $n=6$ patients, and $n=2$ patients, respectively. In $n=42$ patients with TSHD-GHD, $n=2$ patients suffered from *IGSF1* mutations, while $n=7$ patients showed mutations in *POUF, PROP1, GH1, THRH*. A genetic cause of TSHD was identified in 36.3% of ITSHD patients and in 21.4% of TSHD-GHD patients.

Data on genetic causes of central hypothyroidism are scarce. This is the so far largest cohort study of ITSHD and TSHD-GHD. The authors confirm earlier results from a smaller cohort that *IGSF1* mutations are the most frequent cause of ITSHD. *IGSF1* mutations need also be considered in TSHD-GHD patients because of the specific phenotype with transient GHD in childhood.

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Naafs JC, Verkerk PH, Fliers E, van Trotsenburg ASP, Zwaveling-Soonawala N. Clinical and genetic characteristics of Dutch children with central congenital hypothyroidism, early detected by neonatal screening.. *Eur J Endocrinol*. 2020 Dec;183(6):627–636. doi: 10.1530/EJE-20-0833. PMID: 33107432.

1.10. The effects of common genetic variation in 96 genes involved in thyroid hormone regulation on TSH and FT4 concentrations

Sterenborg RBTM, Galesloot TE, Teumer A, Netea-Maier RT, Speed D, Meima ME, Visser WE, Smit JWA, Peeters RP, Medici M

J Clin Endocrinol Metab. 2022 May 17;107(6):e2276–e2283.
doi: 10.1210/clinem/dgac136. PMID: 3526217

Brief summary: The so far largest GWAS study on thyroid function in 72'167 individuals testing 8 million genetic variants identified 92 common genetic variants associated with variation of TSH and FT4 in the reference range. These variants explained 21% of the variance in normal thyroid function, however many

identified variants were localized in genes without obvious link to thyroid function (1). This GWAS paper was commented in the 2019 Yearbook. The same research team aimed now at defining the role of variants in genes involved in the hypothalamo-pituitary thyroid axis.

For this, the authors analyzed variants in 96 genes known to be involved in thyroid development (transcription factors such as *FOXE1*, *PAX8*, *NKX2-1*), regulation of thyroid function (e.g. *TRH*, *TRHR*, *TSHR*), thyroid hormone synthesis (e.g. *TPO*, *TG, SLC27A7*), and thyroid hormone metabolism and action (e.g. *DIO2*, *ABCB1, SLC16A2*) in a large cohort from the ThyroidOmics Consortium with 54'288 and 49'269 participants with TSH and FT4 data. With this extensive study, the authors identified 23 and 25 new genetic variants, associated with TSH levels, and FT4 levels in the normal range, respectively. Variants of genes of the hypothalamo-pituitary-thyroid axis were predominantly associated with TSH levels, while variants of genes responsible for thyroid hormone metabolism and action were mainly associated with FT4 levels. However, unexpectedly, these large number of genetic variants in thyroid hormone axis genes explained only 1.9% and 2.6% of variance of TSH, and FT4 levels respectively in contrast to GWAS results of the whole genome.

In conclusion, these important results did not confirm the hypothesis of the authors that genetic variants in genes involved in thyroid axis had an important contribution to variance of TSH and FT4 in the normal population. First, based on the previous GWAS data, these results suggest the presence of yet unknown pathways being involved in thyroid hormone level regulation. Second, the authors recommend analyzing not only frequent but also rare genetic variants in future large genetic studies.

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Autoimmune Thyroid Disease

1.11. Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort study of 22 million individuals in the UK

Conrad N, Misra S, Verbakel JY, Verbeke G, Molenberghs G, Taylor PN, Mason J, Sattar N, McMurray JJV, McInnes IB, Khunti K, Cambridge G

Lancet. 2023 Jun 3;401(10391):1878–1890.
doi: 10.1016/S0140-6736(23)00457-9. Epub 2023 May 5. PMID: 37156255

Brief summary: Over the last decades changing incidences for autoimmune diseases have been observed. However, no data are available so far on long-term trends, incidence over the whole age spectrum (child, adults, geriatric patients), and incidence of co-occurrence of the different autoimmune diseases. The presented study provides an extensive population-based cohort study performed in the UK.

The authors used electronic health care records of 22 million individuals of all ages and sexes over a twenty-year period (2000–2019), and ethnicities representative for the population in the UK. The main results are the following: First, incidence rate of any autoimmune disease increased significantly. Second, opposite trends were observed for autoimmune thyroid diseases. While Graves' disease was among the three diseases with highest increase (celiac disease, Sjögren's syndrome and Graves' disease), incidence of Hashimoto thyroiditis decreased

most significantly (together with pernicious anemia). Finally, a detailed co-occurrence table is presented including all twelve organ specific autoimmune diseases and seven connective tissue diseases. Both Grave's disease and Hashimoto thyroiditis can occur in the context of all other autoimmune diseases, the most frequent diseases co-occurring with Graves' disease were Myasthenia gravis, type 1 diabetes, and Addison's disease, and type 1 diabetes, Addison's disease and vitiligo for Hashimoto thyroiditis.

This exhaustive population-based cohort study provides important insights on incidence trends, age-specific incidence, and co-occurrence of 19 autoimmune diseases in general and more specifically also for the pediatric population.

1.12. Impact of definitive surgery for Graves' disease on adolescent disease-specific quality of life and psychosocial functioning

Halada S, Baran JA, Isaza A, Patel T, Sisko L, Kazahaya K, Adzick NS, Katowitz WR, Magee L, Bauer AJ

Thyroid. 2022 Dec;32(12):1519–1528.
doi: 10.1089/thy.2022.0334. Epub 2022 Nov 30. PMID: 36254382

Brief summary: Treatment of Graves' disease comprises anti-thyroid drugs, radioactive iodine ablation or total thyroidectomy (1,2). While definitive treatment of Graves' disease is widely used in adults, anti-thyroid drug treatment is often used in the pediatric age group over years (1,2). The presented prospective monocenter study provides detailed information on quality of life of adolescents undergoing total thyroidectomy.

Two recent studies on quality of life in adults showed significant improvement in quality of life after total thyroidectomy for hyperthyroidism (3,4). Halada *et al*. present for the first time prospective data on quality of life in children and adolescents between 12 and 19 years, thyroidectomized for Graves' disease in a tertiary care center by high volume thyroid surgeons. Eleven of 20 patients had poorly controlled Graves' disease preoperatively. Patients and parents completed the surveys. The authors used the following five questionnaires for assessing quality of life of patients with Graves' disease before and after total thyroidectomy: Pediatric Quality of Life Scales (PedsQL), the Quality of Life Questionnaire for Patients with Thyroid Disease (ThyPRO), the Perceived Stigmatization Questionnaire (PSQ), the Body Esteem Scale for Adolescents and Adults (BESAA), and the European Group on Graves' Orbitopathy GO Quality of Life Questionnaire (EUGOGO-QOL). Significant improvements after total thyroidectomy were observed for the following parameters: goitre resolution, hyperthyroid symptoms, tiredness, cognitive impairment, anxiety, physical and school related functioning. Further, median time to full recovery was reported by parents after two months.

Despite the limitation of the small sample size, this first prospective pediatric study provides important results and suggests that not only biochemical stability of the disease but also measures of quality of life in this age group should be considered for optimal treatment. Further, discussion on definitive treatment by total thyroidectomy of Graves' disease should have a place in the ongoing care of children and adolescents with Graves' disease, especially in those with poor control.

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Mooij CF, Cheetham TD, Verburg FA, Eckstein A, Pearce SH, Léger J, van Trotsenburg ASP. 2022 European Thyroid Association Guideline for the management of pediatric Graves' disease. *Eur Thyroid J*. 2022 Jan 1;11(1):e210073. doi: 10.1530/ETJ-21-0073. PMID: 34981748.
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Gunn AH, Frisco N, Thomas SM, Stang MT, Scheri RP, Kazaure HS. Patient-reported outcomes following total thyroidectomy for Graves' disease. *Thyroid*. 2022 Jan;32(1):54–64. doi: 10.1089/thy.2021.0285. Epub 2021 Dec 3. PMID: 34663089.
4. Impaired Quality of Life After Radioiodine Therapy Compared to Antithyroid Drugs or Surgical Treatment for Graves' Hyperthyroidism: A Long-Term Follow-Up with the Thyroid-Related Patient-Reported Outcome Questionnaire and 36-Item Short Form Health Status Survey.

Törring O, Watt T, Sjölin G, Byström K, Abraham-Nordling M, Calissendorff J, Cramon PK, Filipsson Nyström H, Hallengren B, Holmberg M, Khamisi S, Lantz M, Wallin G. Impaired quality of life after radioiodine therapy compared to antithyroid drugs or surgical treatment for Graves' hyperthyroidism: A long-term follow-up with the thyroid-related patient-reported outcome Questionnaire and 36-item short form health status survey. *Thyroid*. 2019 Mar;29(3):322–331. doi: 10.1089/thy.2018.0315. PMID: 30667296.

Pediatric Thyroid Cancer

1.13. 2022 European Thyroid Association Guidelines for the management of pediatric thyroid nodules and differentiated thyroid carcinoma

Lebbink CA, Links TP, Czarniecka A, Dias RP, Elisei R, Izatt L, Krude H, Lorenz K, Luster M, Newbold K, Piccardo A, Sobrinho-Simões M, Takano T, Paul van Trotsenburg AS, Verburg FA, van Santen HM

Eur Thyroid J. 2022 Nov 29;11(6):e220146.

doi: 10.1530/ETJ-22-0146. Print 2022 Dec 1. PMID: 36228315

Brief summary: American Thyroid Association Guidelines for management of children with thyroid nodules and differentiated thyroid cancer were published in 2015 (1). Now Lebbink *et al*. published management guidelines from the European Thyroid Association.

Pediatric differentiated thyroid carcinoma is rare. For many aspects of the disease (diagnosis, treatment, follow-up) clear evidence from randomized controlled trials are therefore missing. The presented management guidelines reviewed the current literature and present 29 recommendations ranging from organization of care of a multidisciplinary thyroid team, diagnostic evaluation of thyroid nodules, detailed recommendation for surgical management of differentiated thyroid carcinoma, indication for I-131 therapy, molecular testing, and targeted therapy, follow-up, and late effects of treatment.

Especially the new, but only in case reports described options of targeted therapies in patients with refractory disease should clearly only be performed in clinical studies (2).

In summary, this paper represents an extensive review and interpretation of the actual knowledge on pediatric thyroid nodules and pediatric differentiated carcinoma. Together with the 2015 published ATA Guidelines it provides important guidance for optimal treatment of affected patients.

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2. NTRK and RET fusion-directed therapy in pediatric thyroid cancer yields a tumor response and radioiodine uptake.
   Lee YA, Lee H, Im SW, Song YS, Oh DY, Kang HJ, Won JK, Jung KC, Kwon D, Chung EJ, Hah JH, Paeng JC, Kim JH, Choi J, Kim OH, Oh JM, Ahn BC, Wirth LJ, Shin CH, Kim JI, Park YJ. NTRK and RET fusion-directed therapy in pediatric thyroid cancer yields a tumor response and radioiodine uptake. *J Clin Invest*. 2021 Sep 15;131(18):e144847. doi: 10.1172/JCI144847. PMID: 34237031.

1.14. Longitudinal analysis of cancer risk in children and adults with germline PTEN variants

Yehia L, Plitt G, Tushar AM, Joo J, Burke CA, Campbell SC, Heiden K, Jin J, Macaron C, Michener CM, Pederson HJ, Radhakrishnan K, Shin J, Tamburro J, Patil S, Eng C

JAMA Netw Open. 2023 Apr 3;6(4):e239705.

doi: 10.1001/jamanetworkopen.2023.9705. PMID: 37093598

Brief summary: *PTEN* hamartoma tumor syndrome is one of five well known genetic syndromes associated with differentiated thyroid carcinoma (1,2). *PTEN* hamartoma tumor syndrome comprises four different entities: Cowdwn syndrome, Bannayan-Riley-Ruvalcaba syndrome, *PTEN*-related Proteus syndrome, and Proteus-like syndrome caused by mutations in the *PTEN* (phosphatase and tensin homologue) tumor

suppressor gene [3]. This prospective longitudinal multicenter cohort study aimed at defining the lifetime cancer risk of children and adults affected with *PTEN* germline mutations.

The study on 701 pediatric and adult patients revealed the following important results: 1) The median age at cancer diagnosis was 47 years with breast cancer, thyroid cancer and endometrial cancer being the three most frequent cancers. 2) In children and young adults <29 years, thyroid cancer was by far the most frequent tumor observed, followed by breast cancer, and endometrial cancer. 3) Patients with neurodevelopmental delay associated with *PTEN* hamartoma tumor syndrome developed any cancer at an earlier age (70% before the age of 29 years) than patients without neurodevelopmental delay.

In conclusion, these results show an elevated lifetime cancer risk for patients with *PTEN* hamartoma tumor syndrome, and for the first time also for the pediatric age group. Therefore, cancer surveillance should be started at an early age in patients with a known *PTEN* variant, especially in those with neurodevelopmental delay.

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Endocrine Disruptors

1.15. Thyroid-on-a-chip: An organoid platform for in vitro assessment of endocrine disruption

Carvalho DJ, Kip AM, Romitti M, Nazzari M, Tegel A, Stich M, Krause C, Caiment F, Costagliola S, Moroni L, Giselbrecht S

Adv Healthc Mater. 2023 Mar;12(8):e2201555.
doi: 10.1002/adhm.202201555. Epub 2023 Jan 10. PMID: 36546709

Brief summary: Exposure to endocrine disrupting chemicals may have adverse effects on humans. Tissue specific organoids are a helpful model to study organ specific impact of compounds alone or in combination, at different doses and during different stages of tissue development (1). While such organoid models have been developed for different tissues, such models were lacking to study differentiated thyroid follicles and thyroid tissue during development and differentiation.

Carvalho *et al*. present an elegant new model of mouse embryonic stem cell derived thyroid organoids on a chip device. This microfluidic culture allows different modes of flows (single and dual flow) with the technical possibility of imaging and continuous oxygen measurements. Optimized culture conditions result in a high percentage of T4-expressing follicles. Exposure of thyroid follicles to an endocrine disruptor (benzo(k)fluroanthene) for 24 hours induced a significant change in gene expression of genes involved in thyroid hormone synthesis.

In summary, this elegant standardized first thyroid organoid culture device on a chip provides a robust new model for serial investigation of endocrine disrupting chemicals on the thyroid tissue.

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2. Establishment of human fetal hepatocyte organoids and CRISPR-Cas9-based gene knockin and knockout in organoid cultures from human liver.
   Hendriks D, Artegiani B, Hu H, Chuva de Sousa Lopes S, Clevers H. Establishment of human fetal hepatocyte organoids and CRISPR-Cas9-based gene knockin and knockout in organoid cultures from human liver. *Nat Protoc*. 2021 Jan;16(1):182–217. doi: 10.1038/s41596-020-00411-2. Epub 2020 Nov 27. PMID: 33247284.

2. Growth and Growth Factors

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Most papers selected for this chapter tackle issues with implications for clinical practice. Phase 2 and 3 long-acting GH clinical trials in GHD, SGA and even ISS children have been published in the last year. Use of the IGF-1/IGFBP-3 molar ratio has been re-proposed, with more convincing evidence, for the diagnosis of GH deficiency. An umpteenth reassuring pharma sponsored observational study on rhGH safety with short patient follow-up has been published. The safety and efficacy of long-term rhGH therapy in large cohorts of short children born SGA followed up to the achievement of adult height and even at 12 years after the completion of treatment have been extensively investigated in different articles. The importance of a comprehensive genetic approach to better characterize short SGA children and possibly predict the responders to rhGH therapy has been highlighted in an analytic observational study. The close link between early postnatal growth and adult height has been confirmed in a large retrospective longitudinal study. Two new classes of factors, pappalysins and stanniocalcins, have been identified as modulators of IGF bioavailability at different stages of development. Unexpectedly, GH has been reported to exert a direct effect on neuroinflammation in an animal model. Finally, the yet unanswered old question if faster height growth rate may be associated and even contribute to the development of type 1 diabetes in childhood has probably obtained an answer eventually.

Important for Clinical Practice

2.1. Serum IGF-1 to IGFBP-3 molar ratio: a promising diagnostic tool for growth hormone deficiency in children

Haj-Ahmad LM, Mahmoud MM, Sweis NWG, Bsisu I, Alghrabli AM, Ibrahim AM, Zayed AA
J Clin Endocrinol Metab. 2023 Mar 10;108(4):986–994.
doi: 10.1210/clinem/dgac609. PMID: 36251796

Brief summary: This cross-sectional study provided evidence regarding the usefulness of serum IGF-1/IGFBP-3 ratio as a marker for the diagnosis of GHD in children.

Diagnosis of growth hormone deficiency (GHD) is still challenging, due to the insufficient specificity of GH stimulation tests. Serum IGF-1, IGFBP-3, and their combination have been proposed as an alternative to GH stimulation tests. However, despite relatively high specificity, their validity in the diagnosis of GHD is limited by low sensitivity (1–3). The serum IGF-1 to IGFBP-3 ratio has been suggested as a surrogate marker of biologically active IGF-1 and a few studies investigated its potential in the diagnosis of GHD in children (4, 5).

This study aimed at investigating the utility of serum IGF-1 to IGFBP-3 ratio in the diagnosis of GHD in short-statured children and adolescents referred to a university hospital in Jordan.

A cohort of 235 children with short stature was enrolled in this cross-sectional observational study. Participants were classified into GHD ($n=64$) and non-GHD ($n=171$) groups. GHD was defined as a slow growth rate (growth velocity $<$ 25th percentile during 1 year) and reduced growth hormone response to 2 GH stimulation tests ($<$6.25 ng/mL). The sensitivity and specificity of serum IGF-1, IGFBP-3, and IGF-1/IGFBP-3 molar ratio were determined.

Fifty-six (87.5%) of 64 GHD participants had a low IGF-1 to IGFBP-3 ratio as compared to 29 (17.0%) of 171 non-GHD participants. The resulting sensitivity was 87.5%, whilst specificity was 83%. As expected, the combination of low IGF-1, IGFBP-3, and IGF-1 to IGFBP-3 ratio elevated the specificity to 97.7% but lowered the sensitivity to 29.7%. On the other hand, the combination of normal serum IGF-1, IGFBP-3, and IGF-1/IGFBP-3 ratio demonstrated the greatest specificity for a non-GHD cause of short stature (100.0%).

In conclusion, this study showed that serum IGF-1/IGFBP-3 ratio may be useful marker for the diagnosis of GHD in children.

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2.2. Safety and efficacy of pediatric growth hormone therapy: results from the full KIGS cohort

Maghnie M, Ranke MB, Geffner ME, Vlachopapadopoulou E, Ibáñez L, Carlsson M, Cutfield W, Rooman R, Gomez R, Wajnrajch MP, Linglart A, Stawerska R, Clayton PE, Darendeliler F, Hokken-Koelega ACS, Horikawa R, Tanaka T, Dörr HG, Albertsson-Wikland K, Polak M, Grimberg A

J Clin Endocrinol Metab. 2022 Nov 25;107(12):3287–3301.
doi: 10.1210/clinem/dgac517. PMID: 36102184; PMCID: PMC9693805

Brief summary: This study reports data from the full Kabi/Pfizer International Growth Database (KIGS), a large international database including 83803 children treated with rhGH for GHD and non-GHD conditions. The data confirm that rhGH is effective in increasing short-term height gain and adult height in various conditions and validate rhGH safety, with no new serious adverse events reported.

KIGS (Kabi/Pfizer International Growth Database), first established in Sweden in 1987, has evolved into one of the largest and longest databases for rhGH treatment, with more than 80 000 rhGH-treated children from more than 50 countries, allowing assessment of long-term efficacy and safety of rhGH treatment in real-world clinical setting.

Safety concerns regarding rhGH therapy have been raised by postmarketing surveillance databases and registries data analyses (1–5). The major concerns include recurrent or new malignancies in children with preexisting malignancies, development of intracranial hypertension, unmasking of type 2 diabetes mellitus, induction of stroke, and a possible association with increased overall mortality.

Data analysis from KIGS revealed that adverse events (AEs), serious adverse events (SAEs) and deaths occur at low frequencies, and the majority were defined by investigators as not related to rhGH therapy. Benign intracranial hypertension and epiphysiolysis (slipped upper femoral epiphysis), were infrequent in KIGS.

The Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) study reported increased risks of mortality and hemorrhagic stroke after a mean follow-up of 17 years in young adults treated with rhGH in childhood. The data in KIGS seem to be reassuring in the short term, as intracranial hemorrhage events occurred at low frequencies in the full KIGS cohort and death in only 0.4% of patients during the study. However, KIGS median follow-up was 3.1 years (range, 0.5–8.2 years) considerably shorter than SAGhE follow-up, averaging 17.1 years per patient. This KIGS report shows low frequencies of both type 1 and type 2 diabetes. However, previous large observational studies reported an increased incidence of type 2 diabetes,

likely driven by genetic predisposition and overweight. The overall available evidence suggests the need of monitoring glucose homeostasis in patients treated with rhGH.

The SAGhE study reported a significantly increased risk of all-site cancer for patients with a history of childhood rhGH treatment and initial cancer diagnosis. Increased risks for bone and bladder cancer in patients without prior cancer were also reported, suggesting a potential impact of rhGH therapy on these cancer types. In the present study, neoplasms were reported in 1% of patients, bone tumor events were rare, and craniopharyngioma recurrence was described in 11.9% of patients, in line with previous reports.

These KIGS data confirmed the overall efficacy of rhGH treatment in all diagnostic groups at least in the first year of treatment.

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2.3. Timing of puberty, pubertal growth, and adult height in short children born small for gestational age treated with growth hormone

Upners EN, Raket LL, Petersen JH, Thankamony A, Roche E, Shaikh G, Kirk J, Hoey H, Ivarsson SA, Söder O, Juul A, Jensen RB
J Clin Endocrinol Metab. 2022 Jul 14;107(8):2286-2295.
doi: 10.1210/clinem/dgac282. PMID: 35521800

Brief summary: In this study the authors reported the adult height and timing of puberty of a cohort of Danish children born small for gestational age (SGA) and treated with rhGH in comparison with national growth standards. rhGH treatment significantly increased height SDS in adulthood as compared to the height SDS at treatment start. Age at peak height velocity did not differ compared with the reference cohort, although peak height velocity was reduced in SGA subjects. SGA boys had an earlier onset of puberty compared with the reference cohort.

Approximately 10% of children born small for gestational age (SGA) fail to catch-up their growth during postnatal life, ending up with a shorter adult height (1, 2). Short stature is an approved indication for rhGH treatment in Europe since 2003. Previous studies have reported the overall efficacy of rhGH therapy in increasing adult height, though a considerable variability in the individual responses to therapy exists (3, 4). In this prospective longitudinal multicenter study, 102 short children born SGA treated with rhGH were evaluated for final height ($n=47$), peak height velocity, age at peak height velocity and puberty onset.

Height gain SDS from start of treatment up to adult height was on average 0.9 SDS in girls and 1.6 SDS in boys with wide individual variability. 57% of the patients reached an adult height within the normal range (above –2 SDS) and 53% achieved an adult height within their genetic target. A prediction model was applied to the dataset and adult height (SDS) was compared with the predicted adult height (SDS). In girls gain in height compared with the prediction was 0.71 SDS, which is equal to 4.5 cm, and in boys 0.61 SDS, which is equal to 4.0 cm. In both genders, peak height velocity was lower than the reference cohort, with no difference in age at peak height velocity. Age at puberty onset was earlier in SGA boys compared with the reference cohort but not in girls.

These data confirm the moderate efficacy of rhGH treatment in short SGA children and the large individual variability in the response to treatment, due to the heterogeneity of the conditions underlying the ‘diagnosis’ of SGA.

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2.4. Childhood growth hormone treatment and metabolic and cardiovascular risk in adults born small for gestational age after growth hormone cessation in the Netherlands: a 12-year follow-up study

Goedegebuure WJ, van der Steen M, Smeets CCJ, Hokken-Koelega ACS

Lancet Child Adolesc Health. 2022 Nov;6(11):777–787.
doi: 10.1016/S2352-4642(22)00240-1. Epub 2022 Sep 16. PMID: 36122581

Brief summary: In this longitudinal study, the authors investigated the metabolic and cardiovascular health profile of 167 adults born SGA and previously treated with rhGH during a period of 12 years after therapy discontinuation. No relevant differences were found in treated SGA subjects compared with untreated SGA adults or adults born appropriate for gestational age (AGA), thus suggesting long-term metabolic and cardiovascular safety of rhGH treatment in short SGA children.

Subjects born small for gestational age (SGA) have an increased risk of cardiometabolic disorders in adulthood. Treatment with rhGH causes changes in body composition and insulin sensitivity that could have long-term effects (1–4). Postmarketing surveillance studies, including the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) study, reported an increased risk of cardiovascular mortality in adults born SGA who were treated with rhGH during childhood (5).

In this study the metabolic and cardiovascular profile of 167 adults born SGA and previously treated with rhGH was compared with that of 219 untreated adults: 127 born SGA with either persistent short stature or spontaneous catch-up growth, and 92 born AGA. Subjects were followed-up for 12 years (from GH cessation), and data were collected at the age of 30 years. Insulin sensitivity increased significantly in adults previously treated with rhGH during the 12 years follow-up and a normal β-cell function was maintained. Fat mass, trunk fat and limb fat increased in SGA adults treated with rhGH after cessation of therapy, whereas lean body mass significantly decreased. However, these parameters did not differ from those of untreated SGA adults. All groups born SGA had significantly lower HDL cholesterol than adults born AGA. Systolic and diastolic blood pressure, visceral adipose tissue, subcutaneous adipose tissue, and liver fat fraction corrected for age, sex, and adult height SD score, were similar in all SGA and AGA groups. The prevalence of metabolic syndrome was also comparable in the different groups. In conclusion, the results of this study clearly show that rhGH therapy in SGA children is not associated with a worse cardiometabolic profile even after 12 years from cessation of treatment.

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2.5. Tracking and cumulative lifetime exposure to IGF-I in 6459 healthy individuals and in SGA children treated with GH

Kjaer ASL, Jensen RB, Petersen JH, Linneberg A, Kårhus LL, Henriksen LS, Johannsen TH, Main KM, Hoffman AR, Juul A
J Clin Endocrinol Metab. 2023 Feb 15;108(3):642–652.
doi: 10.1210/clinem/dgac605. PMID: 36250350

Brief summary: The objective of this study was to examine whether IGF-I and IGF-binding protein-3 (IGFBP-3) levels track through childhood into adulthood and to estimate the cumulative lifetime exposure to IGF-I in healthy individuals and in individuals with a defined period of increased IGF-I levels because of GH therapy.

Measurement of serum IGF-I is a cornerstone in rhGH treatment monitoring. Keeping serum IGF-I concentrations within the normal reference range is recommended as a safety measure, given the epidemiologic associations between high serum IGF-I and cancer risk (1-2).

Samples from 6459 healthy participants (cross-sectional = 5326; longitudinal = 1133) aged 0–76 years (9963 serum samples) and 9 patients born small-for-gestational-age (SGA) with 238 serum samples during rhGH treatment were included.

IGF-I and IGFBP-3 levels showed age-dependent changes with a rapid decrease after birth, increasing levels during childhood, and peak levels around puberty followed by a post-pubertal decrease and a continuous subtle decline throughout adulthood.

Individuals were stratified into tertiles based on their mean IGF-I or IGFBP-3 SD score (SDS). Throughout the period of life considered, the majority of the participants stayed within the same tertile, allowing the authors to draw the conclusion that a single measurement of IGF-I (SDS) is a reliable approximation of cumulative lifetime exposure.

As an estimate of cumulative lifetime exposure to IGF-I, the area under the curve (AUC) for 0 to 76 years was calculated. For SGA patients, the actual AUC during the years of rhGH treatment and a predicted AUC (under the hypothesized scenario that rhGH treatment was not used) were calculated.

The estimated cumulative lifetime exposure to IGF-I without GH treatment was below the average in SGA patients, whereas GH treatment increased IGF-I by 0.6 SDS, shifting the patients from having IGF-I levels in the lowest tertile to the middle tertile. However, the cumulative lifetime IGF-I exposure in GH-treated SGA patients remained below the average of the reference population.

In conclusion, IGF-I and IGFBP-3 levels track throughout life, suggesting that a single IGF-I measurement is reliable to assess long-term exposure and rhGH therapy in childhood does not increase the cumulative lifetime exposure to IGF-I beyond levels of healthy individuals. This latter seems to be reassuring in relation to the safety of rhGH treatment, especially in non-GHD children, who more easily reach supraphysiologic IGF-I concentrations during rhGH treatment. However, the reported data clearly show that rhGH treated subjects experience a transient period of exposure to IGF-I levels significantly higher than the genetically determined concentrations thus leaving unanswered the question about a possible detrimental effect of this transitory overexposure.

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2.6. Growth patterns of children with short stature in adulthood according to auxological status and maturity at birth

Pfäffle R, Knüpfer M, Göbert M, Vogel M, Gausche R, Beger C, Keller E, Körner A, Thome U, Kiess W
J Clin Endocrinol Metab. 2022 Nov 25;107(12):3320–3327.
doi: 10.1210/clinem/dgac510. PMID: 36099499

Brief summary: This retrospective longitudinal study included 5698 patients with multiple height measurements since birth to the near-adult age. The aim was to define different growth patterns of children from birth to 18 years corrected for gestational age, sex and auxological status at birth in order to early identify children at risk for adult short stature. Adult height was significantly reduced in preterm and/or in small-for-gestational-age (SGA) children, with no sex differences. A decreased height-rate during the first year of life in SGA children is a predictor of final short stature.

The population of this study included 5698 children with growth data at birth, at near final height (NFH) and at least 2 further time points during childhood. Children were stratified according to maturity at birth and sex: 526 children preterm children were compared to 5172 children born full-term. The SGA cohort included 1204 children. Children treated with Growth Hormone (GH) were excluded. The determination of the NFH was carried out for girls at an age of 16.2 ± 1.64 and for boys at an age of 16.8 ± 1.45 years.

A total of 741 of 5698 (13%) children presented a NFH $<$ 3rd percentile, amounting to 20.5% (108/526) in the cohort of preterm children and 12% (633/5172) in the cohort of full-term children. The percentage of short children was also higher in children born SGA than those born AGA or LGA (SGA, 33.9%; AGA 11%; LGA, 3.5%; $P < 0.001$). Preterm children had a significantly lower mean NFH than term children (preterm, -0.61 SDS; term, -0.18 SDS). SGA children also had a lower mean NFH than AGA children (SGA, -1.06 SDS; AGA, -0.15 SDS). Of 1204 SGA children, 672 (56%) showed successful catch-up growth (CUG) achieving NFH $\geq$10th percentile (SGA-CU), and 532 children (44%) did not (SGA-S). The difference in their mean NFH SDS can only partly be explained by the differences in mean mid-parental height SDS (SGA-CU, -0.3; SGA-S, -1.19). The growth-velocity during the first year of life in children born SGA was strongly associated with the final growth outcome. Therefore, careful monitoring of growth during the first 12 months of postnatal life in SGA children could identify those to be destined for remaining permanently short who could benefit from an early start of growth promoting therapies.

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Long-Acting Growth Hormone (LAGH)

2.7. Weekly somapacitan is effective and well tolerated in children with GH deficiency: The randomized phase 3 REAL4 trial

Miller BS, Blair JC, Rasmussen MH, Maniatis A, Kildemoes RJ, Mori J, Polak M, Bang RB, Böttcher V, Stagi S, Horikawa R
J Clin Endocrinol Metab. 2022 Nov 25;107(12):3378–3388.
doi: 10.1210/clinem/dgac513. PMID: 36062966; PMCID: PMC9693810

Brief summary: This multicenter, randomized, controlled, phase 3 study compared the effects of long-acting GH (Somapacitan; 0.16 mg/kg/wk) with daily GH (Norditropin; 0.034 mg/kg/d), in GHD children. The trial was conducted over 52 weeks, followed by an ongoing 3-year single-group extension period. Similar efficacy and safety for somapacitan compared to daily GH was demonstrated over 52 weeks of treatment.

Long-acting growth hormone (LAGH) formulations have been under intense investigation in the last decades for improving adherence to chronic GH therapy in both children and adults with GHD.

Somapacitan, a once-weekly reversible albumin-binding GH derivative, has been approved for the treatment of adults in Europe, United States and Japan and trials in GHD children are ongoing (1).

A previous phase 2 dose-finding and safety trial in prepubertal children with GHD showed that 0.16 mg/kg/wk Somapacitan had the same efficacy and safety profile of daily GH treatment (0.034 mg/kg/d) over 3 years of treatment (2).

In this phase 3 REAL4 trial, the authors aimed at evaluating the efficacy, safety, and tolerability of once-weekly Somapacitan 0.16 mg/kg/wk compared with daily GH in prepubertal, treatment-naïve children with GHD. Efficacy estimates were height velocity (HV; cm/y) at week 52 and changes from baseline to end in height SDS (HSDS), HVSDS, IGF-I SDS, and bone age. Incidence of adverse events (AEs), occurrence of anti-somapacitan and anti-GH antibodies, changes in laboratory parameters, including lipid profile, glucose, insulin, and glycated hemoglobin level, were assessed as safety endpoints.

Efficacy of Somapacitan was confirmed by mean HV after 52 weeks of treatment (11.2 cm/y for Somapacitan and 11.7 cm/y for daily GH). HVSDS and HSDS increased from baseline to week 52 for both Somapacitan and daily GH, with non-statistically significant differences between treatment groups.

Mean IGF-I SDS values at week 52 and change in mean IGF-I SDS from baseline to week 52 were similar between treatment groups. IGF-I SDS increased after Somapacitan injection to an estimated mean peak of $+1.7$ SDS after an average time of 58 hours. Thereafter, the profile declined to a mean pre-dose IGF-I of -0.8 SDS.

In conclusion, noninferiority in HV for somapacitan compared with daily GH was demonstrated with similar safety and mean IGF-I SDS in treatment-naïve children with GHD.

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2.8. Efficacy and safety of weekly somatrogon vs daily somatropin in children with growth hormone deficiency: A phase 3 study

Deal CL, Steelman J, Vlachopapadopoulou E, Stawerska R, Silverman LA, Phillip M, Kim HS, Ko C, Malievskiy O, Cara JF, Roland CL, Taylor CT, Valluri SR, Wajnrajch MP, Pastrak A, Miller BS

J Clin Endocrinol Metab. 2022 Jun 16;107(7):e2717–e2728.
doi: 10.1210/clinem/dgac220. PMID: 35405011; PMCID: PMC9202717

Brief summary: This 12-months randomized, controlled, phase 3 study compared the efficacy and safety of once-weekly Somatrogon 0.66 mg/kg/week with once-daily somatropin in prepubertal children with GHD. The efficacy of once-weekly Somatrogon was noninferior to once-daily somatropin, with similar safety and tolerability profiles.

Somatrogon (MOD-4023) is a long-acting rhGH recently approved by the European Medicines Agency for the treatment of children with GHD. Somatrogon contains the amino acid sequence of human GH and 3 copies of the C-terminal peptide (CTP) of human chorionic gonadotropin, which prolongs its half-life. A previous randomized dose-finding phase 2 study was conducted in children with GHD to compare the safety, tolerability, and efficacy of 3 different doses of Somatrogon (0.25, 0.48, and 0.66 mg/kg/week) administered once weekly vs rhGH administered once daily. The 0.66 mg/kg/week dose proved to have comparable efficacy and safety profile during 12-months follow-up as daily-GH (1).

In this phase 3 study, a total of 228 treatment-naiäve prepubertal children with GHD were randomized 1:1 to receive once-weekly Somatrogon (0.66 mg/kg/week) or once-daily somatropin (0.24 mg/kg/week) for 12 months. Height velocity (HV) at month 12 was the primary endpoint, whereas secondary efficacy measures were HV at 6 months and change in height standard deviation score (SDS) at months 6 and 12.

HV at month 12 was 10.1 cm/year for Somatrogon-treated subjects and 9.8 cm/year for somatropin-treated subjects, demonstrating noninferiority of once-weekly Somatrogon vs daily somatropin. HV at month 6 and

change in height SDS at months 6 and 12 were similar between the two treatment groups. Change in bone maturation at month 12 was also similar between treatment groups. The mean value for IGF-1 SDS was −1.9 at baseline and reached 0.6 SDS at 12 months in the Somatrogon group. In the somatropin group mean IGF-1 SDS was −1.7 at baseline and remained near 0 at all post-baseline visits.

A similar percentage of subjects experienced mild to moderate adverse events (AEs) in both groups (Somatrogon: 78.9%, somatropin: 79.1%). The incidence of severe AEs was 8.3% and 5.2% in the respective groups. AEs with ≥ 5% higher incidence in the Somatrogon group than in the somatropin group were injection site erythema, injection site pain, and injection site pruritus.

In conclusion, Somatrogon administered once weekly was noninferior to somatropin administered once daily for the treatment of prepubertal children with GHD, with similar safety and tolerability profiles.

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2.9. Safety and efficacy of lonapegsomatropin in children with growth hormone deficiency: enliGHten trial 2-year results

Maniatis AK, Casella SJ, Nadgir UM, Hofman PL, Saenger P, Chertock ED, Aghajanova EM, Korpal-Szczyrska M, Vlachopapadopoulou E, Malievskiy O, Chaychenko T, Cappa M, Song W, Mao M, Mygind PH, Smith AR, Chessler SD, Komirenko AS, Beckert M, Shu AD, Thornton PS

J Clin Endocrinol Metab. 2022 Jun 16;107(7):e2680–e2689.

doi: 10.1210/clinem/dgac217. PMID: 35428884; PMCID: PMC9202697

Brief summary: This open-label extension trial enrolled all subjects completing the two previous phase 3 Lonapegsomatropin trials, heiGHt and fliGHt. The results confirm the efficacy of this LAGH formulation in improving height SDS, without major adverse events.

Lonapegsomatropin is a long-acting GH consisting of 3 components: unmodified human GH (hGH), an inert glycol carrier, and a TransCon linker that transiently binds the other 2 components. The glycol carrier extends hGH circulation time in the body by shielding GH receptor binding and renal excretion. The TransCon linker releases hGH by autocleavage in a controlled manner.

This study reports the results from EnliGHten, an open-label extension trial that aimed at assessing the long-term safety of weekly Lonapegsomatropin (0.24 mg/kg/week) in children with GHD previously treated in the phase 3 Lonapegsomatropin trials heiGHt or fliGHt (1, 2). Almost all subjects who completed the heiGHt (158/159) and fliGHt (140/144) trials continued into the EnliGHten long-term extension trial for up to 2 years. Efficacy assessments included the endpoints of height SDS and annualized height velocity (AHV).

In subjects from heiGHt trial, height SDS improved from −2.9 at heiGHt trial baseline to −1.4 at week 104 (=2 yrs). The mean AHV was 10.9 cm/year at week 52 and 8.5 cm/year at week 104.

Subjects who were on daily somatropin during the heiGHt trial and switched to Lonapegsomatropin also improved their height SDS from −3.0 at heiGHt trial baseline to −1.5 at week 104; their mean AHV was 10.2 cm/year at week 52 and 8.9 cm/year at week 104.

In subjects from fliGHt trial, height SDS improved from −1.4 at baseline to −0.7 and mean AHV was 8.4 cm/year at week 78. There was no advancement in bone age in children receiving Lonapegsomatropin, indicating that the clinical effects did not occur at the expense of skeletal maturation.

Mean IGF-1 SDS remained stable for heiGHt subjects already on Lonapegsomatropin, whereas in those who were initially on daily GH an initial increase in mean IGF-1 SDS with subsequent stabilization was observed. For fliGHt subjects, mean IGF-1 SDS increased from 0.8 at fliGHt baseline to 1.6 at week 26 and 1.8 at week 78.

As observed in the heiGHt and fliGHt trials, AEs were generally mild and consistent with those reported in other clinical trials evaluating daily GH children with GHD. Blood parameters remained stable and no serious AEs related to Lonapegsomatropin were reported.

In conclusion, weekly Lonapegsomatropin produced a continued improvement of height SDS through 2 years of therapy without advancing bone age. The safety and tolerability profile of Lonapegsomatropin remained comparable to daily GH.

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2.10. Long-acting PEGylated growth hormone in children with idiopathic short stature

Luo X, Zhao S, Yang Y, Dong G, Chen L, Li P, Luo F, Gong C, Xu Z, Xu X, Gong H, Du H, Hou L, Zhong Y, Shi Q, Chen X, Chen X, Xu L, Cheng R, Su C, Ma Y, Xu L, Zhang L, Lu H

Eur J Endocrinol. 2022 Oct 13;187(5):709–718.
doi: 10.1530/EJE-22-0449. PMID: 36130048

Brief summary: This randomized, multicenter, controlled, phase II study compared the effects of high-dose (HD) once-weekly PEGylated-recombinant human growth hormone (PEG-rhGH) to low-dose (LD) and to an untreated control group of children with idiopathic short stature (ISS) over a period of 52 weeks. PEG-rhGH was effective in increasing height gain in a dose dependent manner with both doses being well tolerated during the observation period.

PEG-rhGH is a once-weekly long-acting rhGH obtained by adding a hydrophilic polyethylene glycol residue to rhGH, in order to extend half-life and reduce antigenicity and immunogenicity (1). Previous phase I–III studies established the safety and efficacy of weekly PEG-rhGH in children with GHD, supporting its approval in China (2).

The authors reported the results of the Phase 2 randomized, multicenter, controlled study in ISS children comparing two different dose-regimens of PEG-rhGH with a control group.

Study population included 360 children with idiopathic short stature randomized 1:1:1 to weekly s.c. injections of PEG-rhGH 0.1 (LD) or 0.2 mg/kg/week (HD) or untreated controls for 52 weeks.

The study was completed by 118/120 patients in LD arm, 116/120 patients in HD arm and 117/120 controls.

At week 52, the height improvement (HT-SDS) was 0.56 ± 0.26, 0.98 ± 0.35, and 0.20 ± 0.26 in the LD, HD, and control groups, respectively ($P < 0.0001$). Statistically significant increase in height velocity, IGF-1, IGF-1/ IGFBP-3 ratio, and IGF-1 SDS at week 52 from baseline were observed in both treatment groups ($P < 0.0001$) with a dose-dependent response for all auxological variables. Adverse events occurring during treatment were reported in 86.5%, 84.6%, and 91.3% of children in the HD, LD, and control groups, respectively. A total of 27 (8.7%) children experienced mild to moderate drug-related adverse effects that did not affect treatment efficacy and compliance. There was no significant change in the other safety parameters, such as complete blood count and glucose metabolism during treatment. To our knowledge, this is the first study reporting efficacy and safety of a long acting formulation of GH in ISS children. Preliminary results look encouraging but a phase 3 trial and a longer observation time are needed to confirm these results.

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2.11. Somapacitan in children born small for gestational age: a multi-centre, open-label, controlled phase 2 study

Juul A, Backeljauw P, Højby M, Kawai M, Kildemoes RJ, Linglart A, Zuckerman-Levin N, Horikawa R

Eur J Endocrinol. 2023 Jan 10;188(1):lvac008.
doi: 10.1093/ejendo/lvac008. Erratum in: *Eur J Endocrinol*. 2023 Jan 10;188(1): PMID: 36651161

Brief summary: This is the first multi-national phase 2 dose finding trial aimed at investigating efficacy and safety of the long acting GH (Somapacitan) with daily GH in short children born SGA. 62 GH treatment-naïve short children born SGA were enrolled in the study. Efficacy was valuated with estimated mean annualized height velocity (HV) after 26 weeks of follow-up. Somapacitan showed similar efficacy of daily rhGH. The study will be extended for further 4 years in order to investigate the long-term efficacy and safety profile.

Somapacitan is a long acting human GH (LAGH) approved for treatment of adults with growth hormone deficiency (GHD). It has a longer half-life than rhGH due to its bond to a small non-covalent albumin-binding moiety that facilitates connection with endogenous albumin thus slowing down drug elimination (1–3). SGA children treated with rhGH have a sub-optimal growth response often due to low adherence to treatment, being daily rhGH therapy burdensome for patients and caregivers (3,4).

REAL5 was a dose-finding, randomised, multi-national, open-label, 5-arm design, controlled phase 2 study investigating the efficacy and tolerability of weekly Somapacitan compared with daily rhGH therapy in a cohort of GH treatment-naïve prepubertal short children born SGA. Sixty-two patients were randomized 1:1:1:1 to receive Somapacitan 0.16 mg/kg/week, Somapacitan 0.20 mg/Kg/week, Somapacitan 0.24 mg/kg/week, rhGH 0.035 mg/kg/day and rhGH 0.067 mg/kg/day for 26 weeks. In total, 61/62 children completed the study. After 26 weeks, HV increased in a dose-dependent manner and resulted 8.9, 11.0, 11.3, 10.3, 11.9 cm/yr respectively in the aforementioned groups. Somapacitan showed similar tolerability and safety of daily rhGH. No serious adverse effects (AE) were reported in the treatment groups and no AEs led to treatment discontinuation.

These results suggest that Somapacitan may be considered in the treatment of short children born SGA as an alternative to rhGH to reduce the burdensome of chronic therapy thus improving the adherence and, consequently, the growth outcome of treatment.

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New Perspectives

2.12. Pappalysins and stanniocalcins and their relationship with the peripheral IGF axis in newborns and during development

Martín-Rivada Á, Guerra-Cantera S, Campillo-Calatayud A, Andrés-Esteban EM, Sánchez Holgado M, Martos-Moreno GÁ, Pozo J, Güemes M, Soriano-Guillén L, Pellicer A, Oxvig C, Frystyk J, Chowen JA, Barrios V, Argente J

J Clin Endocrinol Metab. 2022 Sep 28;107(10):2912–2924.
doi: 10.1210/clinem/dgac453. PMID: 35902207

Brief summary: Growth Hormone (GH)-Insulin-Growth-Factor-1 (IGF-1) axis plays the major role in promoting growth, but novel factors seem to modulate GH-IGF1 axis functioning. The majority of circulating IGF-1 and IGF-2 are bound to IGF-binding proteins (IGF-BPs) which prolong their half-life and regulate their tissue distribution (1,2). Pappalysins (PAPP-A, PAPP-A2) increase IGF-1 bioavailability through cleavage of IGFBPs and are inhibited by stanniocalcins (STC1, STC2) (3,4). This translational study provides normative data about pappalysins, stanniocalcins and IGF-BPs in relation to Tanner pubertal stages and gestational age of newborns.

This descriptive cross-sectional study aims to delineate the trend of values of PAPPAs and STCs in different periods of life, from birth to adulthood. The study population included 190 appropriate-for-gestational-age (AGA) newborns, divided into 150 full term newborns and 40 preterm newborns and 1071 healthy individuals aged 1-30 years with normal height, weight, and BMI, subdivided ac-cording to their pubertal Tanner stage. Newborn samples were compared to 317 healthy children samples with Tanner pubertal stage I.

Blood levels of IGFBP-2, IGFBP-4, IGFBP-5, PAPP-As, STCs, the free IGF1/total IGF1 ratio were higher at birth than in childhood. In postnatal life, PAPP-A2 concentrations decreased progressively in concomitance with the free/total IGF-I ratio (positive correlation, $P < .001$) supporting the hypothesis that PAPP-A2 is one of the main regulators of IGF-I bioavailability (5). PAPP-A2 negatively correlated with the intact/total IGFBP-3 ratio ($P < .001$) and PAPP-A concentrations negatively correlated with intact/total IGFBP-4 ratio ($P < .001$), with PAPP-A concentrations being lower in females. Overall, STCs and PAPPAs levels showed a progressive decrease from prenatal to postnatal life except from a peak of STC2 in Tanner V females that could contribute to the deceleration in growth observed in this stage, as STC2 appears to be a more potent brake for linear bone growth than STC1 (6). In conclusion, this study provides for the first time reference data for PAPP-A, PAPP-A2, STC1 and STC2 relating these factors with the GH-IGf1 axis parameters, including IGF bioavailability at the different stages of development.

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2.13. Central growth hormone action regulates neuroglial and proinflammatory markers in the hypothalamus of male mice

Wasinski F, Tavares MR, Gusmao DO, List EO, Kopchick JJ, Alves GA, Frazao R, Donato J Jr

Neurosci Lett. 2023 May 29;806:137236.

doi: 10.1016/j.neulet.2023.137236. Epub 2023 Apr 6. PMID: 37030549; PMCID: PMC10133206

Brief summary: This translational study evaluated mRNA expression of hypothalamic neuroglial markers in two transgenic mice with either enhanced or reduced consensual GH-IGF-1 signaling and in Hepatocyte-specific GH receptor (GHR) knockout male mice, with high GH circulating-levels and low IGF-1 concentrations. The study demonstrated a direct action of GH on neuroinflammation, independently of IGF-1 levels.

Although the brain is not considered a classical target tissue of GH, recent studies conducted in animal models and humans highlighted the potential role of GH in brain functions, such as spatial learning, memory and stress resilience in particular in the hippocampal region (1,2,3). However, these effects may be indirect and mediated by insulin and IGF1 [4,5]. Neuroglial cells represent the most numerous cell family in the central nervous system and contribute to brain homeostasis, supporting synaptic contacts and neuron signalling. This study aimed to determine the action of GH in the regulation of neuroglial and proinflammatory markers in the

hypothalamus of male mice. GH-IGF1 deficient mice with a null mutation in the GH-releasing hormone receptor gene, showed decreased mRNA expression of Nes (Nestin), Gfap, Iba1, Adgre1 (F4/80), and Tnf (TNFα) compared to wild-type animals. Conversely, mice with transgenic overexpression of bovine GH with high serum GH and IGF-1 levels had increased hypothalamic expression of Nes, Gfap, Adgre1, Iba1, and Rax. In order to investigate the direct effect of GH on neuroglia independently from IGF-1, the authors utilized mice with knockout of hepatocyte GH receptor (GHR), having high serum GH levels, but reduced IGF-1 concentrations. Hepatic GHR-ablated mice showed increased mRNA expression of Gfap, Iba1, Tnf, and Sox10, thus indicating that GH has a direct effect on the hypothalamic expression of glial markers associated with neuroinflammation. Conversely, brain-specific GH-Receptor (GHR) knockout mice showed reduced expression of several neuroinflammatory markers. Taken together these experimental findings suggest that GH exerts a direct effect on neuroglial markers of inflammation in male mice.

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2.14. Pathogenic copy number and sequence variants in children born SGA with short stature without imprinting disorders

Hara-Isono K, Nakamura A, Fuke T, Inoue T, Kawashima S, Matsubara K, Sano S, Yamazawa K, Fukami M, Ogata T, Kagami M

J Clin Endocrinol Metab. 2022 Jul 14;107(8):e3121–e3133.

doi: 10.1210/clinem/dgac319. PMID: 35583390

Brief summary: This observational study aimed at clarifying the contribution of pathogenic copy number variants (PCNVs) and candidate pathogenic variants in 86 children born small-for-gestational-age with short stature (SGA-SS).

Approximately 10% of children born SGA do not show catch-up and remain permanently short (SGA-SS) (1,2). There is increasing evidence suggesting that genetic abnormalities underlie a high proportion of SGA-SS children. In this study, 86 children born SGA and with short stature underwent a comprehensive molecular analysis consisting of methylation analysis, copy number variant evaluation, and multigene sequencing. Children with imprinting disorders, including Silver-Russel-syndrome, were excluded. The definition criteria of SGA-SS were: (A) a child with both BW and BL below the 10th percentile for the gestational age and sex and (B) a stature at 24 months of age below −2 SDS (3,4). The cohort included 20 preterm children. About 60% of patients showed no clinical features other than SGA-SS.

Pathogenic copy number variants (PCNVs) were identified in 8 patients (9.3%) in a heterozygous state. A candidate pathogenic genetic variant was identified in 11 patients (12.8%), in a heterozygous state, including one nonsense and 10 missense variants. According to the American College of Medical Genetics standards and guidelines, 5 variants were classified as pathogenic and 6 variants of unknown significance (VUS). The 5 pathogenic variants included 4 previously reported variants of Myhre syndrome (SMAD4), Noonan syndrome (PTPN11), Noonan syndrome–like disorder (CBL), Feingold syndrome (MYCN) and 1 causative variant for short stature (NPR2). Among 6 VUS, 2 were genes (GHR and IGF1R) involved in the GH/IGF-I axis, and 4 were genes (COL2A1, SHOX, ACAN, and FGFR3) involved in growth plate physiology. Interestingly, all cases were not clinically diagnosed before molecular analyses because their phenotype was not suggestive of a specific genetic disease. These results highlight the importance of performing a comprehensive genetic assessment in children born SGA with unexplained short stature.

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2.15. Childhood height growth rate association with the risk of islet autoimmunity and development of type 1 diabetes

Li Z, Veijola R, Koski E, Anand V, Martin F, Waugh K, Hyöty H, Winkler C, Killian MB, Lundgren M, Ng K, Maziarz M, Toppari J
J Clin Endocrinol Metab. 2022 May 17;107(6):1520–1528.
doi: 10.1210/clinem/dgac121. PMID: 35244713; PMCID: PMC9113806

Brief summary: In this study, 10 145 children of 1–8 years of age, selected from a prospective systematic cohort study and stratified according to HLA-risk categories for type-1-diabetes (T1D), underwent a combined evaluation of pancreatic autoimmunity, glucose metabolism and anthropometry at different timeframes. Diagnosis of T1D occurred in 131/10,145 children (1.3%). Faster height growth, both before and after age 3 years, was significantly associated with the appearance of islet autoimmunity and the progression to T1D among subjects who seroconverted. The inclusion of growth dynamics in the risk score models may improve the accuracy of predicting time to islet autoimmunity and overt disease.

Growth is mainly driven by different factors according to a distinct time period, in particular nutrition is the main driver during infancy, growth hormone in childhood, and sex hormones in adolescence (1). Since each factor could contribute differently to the onset of T1D, the peculiar approach of this study was to focus only to the childhood period and to explore the association between the growth rate and the risk of type-1-diabetes-autoimmunity in children at high genetic risk for T1D.

Longitudinal data were collected in 10,145 children from 1 to 8 years of age, enrolled in a prospective cohort study, the 'Type 1 Diabetes Intelligence study (T1DI) cohort', from Finland (61.3%), Sweden (12.2%), Germany (13.1%), and the United States (13.5%), with an average follow-up period of 6.94 years (2). The study evaluated the association between 4 main predictors (height, weight, and rates of change in height and weight) and 2 main outcomes (seroconversion and T1D-onset) in 3 analysis timeframes (from 1 year of age to seroconversion; from one year of age to the onset of T1D; and from seroconversion to T1D), stratifying children into 4 different HLA-risk categories. Rapid increase in height was associated with increased risk of seroconversion to autoantibodies (hazard ratio [HR] $=1.26$ for 1–3 years of age and HR $=1.48$ for >3 years of age) and positively associated with the development of T1D (HR $=1.80$).

These results provide the basis to investigate the role of GH-IGF-1 axis in the onset of T1D during childhood.

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3. Bone, Growth Plate and Mineral Metabolism

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Premium research efforts, encompassing laboratory, translational, clinical studies and clinical trials, continually augment our understanding of skeletal biology, along with disorders associated with growth plate, bone, and mineral metabolism. This progression is currently offering innovative treatments for rare skeletal disorders. In this chapter, we underscore several promising clinical trials, notably: a phase 2 study investigating denosumab treatment for fibrous dysplasia, a phase 1 study of a slow-release formulation of C-natriuretic peptide (CNP) in achondroplasia, and a successful anti-FGF23 treatment for autosomal recessive hypophosphatemic rickets.

Among the important clinical advances, we highlight an updated and expanded nosology of genetic skeletal disorders, and a large retrospective cohort study of neonatal and early infancy features of patients with inactivating PTH/PTHrP signalling disorders/pseudohypoparathyroidism. We also highlight two excellent review articles, one on high bone mass disorders and the other on disorders of biomineralization.

Translational highlights include a novel overgrowth syndrome due to mutations in *SPIN4* and description of its molecular underpinning, as well as an animal model of childhood-onset osteoporosis due to *PLS3* mutations.

Several seminal findings in diverse areas of skeletal biology are highlighted and include articles showing: evidence for lymph vessels in bone and their importance in bone tissue repair, and proof of liver-bone crosstalk involving small extracellular vesicles. Another interesting article reports the tracing of resting zone chondrocytes during food restriction and subsequent catch-up growth, describing a potential mechanism by which growth potential is conserved during growth-inhibiting conditions. In addition, the largest genome-wide association study finds that as much as 21% of the human genome may be involved in variation in human height and the associated loci are enriched for growth plate genes.

We hope you will enjoy the chapter and especially the excellent science and articles highlighted within it.

Novel Treatments for Rare Skeletal Disorders

3.1. Safety and efficacy of denosumab for fibrous dysplasia of bone

de Castro LF, Michel Z, Pan K, Taylor J, Szymczuk V, Paravastu S, Saboury B, Papadakis GZ, Li X, Milligan K, Boyce B, Paul SM, Collins MT, Boyce AM

N Engl J Med. 2023 Feb 23;388(8):766–768.

doi: 10.1056/NEJMc2214862. PMID: 36812441.

https://pubmed.ncbi.nlm.nih.gov/36812441/

In brief: This phase 2 study investigated the effect of the RANKL inhibitor denosumab on fibrous dysplasia lesion activity, as well as the rebound in bone turnover after treatment discontinuation.

Commentary: Denosumab is a humanized monoclonal antibody that inhibits RANKL with potent but transient antiosteoclastic effects, and discontinuation of denosumab treatment is associated with a rebound in bone turnover. In this study, eight women received high dose denosumab for 6 months and were observed for a further 8 months after treatment discontinuation. The effect of denosumab on lesion activity was assessed by measuring reduction in serum levels of bone-formation and bone-resorption markers. In addition, combined positron-emission tomography–computed tomography (PET–CT), analysis of bone biopsies and assessment of pain score.

Denosumab therapy dramatically reduced in the serum levels of bone-formation marker procollagen type 1 N-terminal propeptide and bone-resorption marker C-terminal telopeptide. Consistently, there was a marked decrease in lesion activity assessed by PET-CT with uptake of ^{18}F-sodium fluoride tracer. Participants also reported amelioration of complications associated with fibrous dysplasia, such as increased pulmonary function in a participant with severe thoracic fibrous dysplasia. Participants also reported reduced pain. One participant developed severe hypercalcemia 12 weeks after discontinuation of denosumab, and three additional participants had a rebound in bone turnover that was above the pretreatment level and was associated with mild-to-moderate asymptomatic hypercalcemia.

This is a small phase 2 study on selected patients with severe fibrous dysplasia that provides important information on the use of denosumab in this condition and strategies to manage rebound hypercalcemia. It confirms earlier reports that denosumab dramatically reduces bone formation and resorption markers and also demonstrates clinical benefits in selected patients with fibrous dysplasia. However, the risk of severe side-effects needs to be considered, e.g. osteonecrosis of the jaw with long-term treatment, as well as malignant hypercalcemia if treatment is interrupted or discontinued. Further studies are needed to determine the clinical situations in which the benefits justify the risks of denosumab treatment of fibrous dysplasia.

3.2. Phase 1 safety, tolerability, pharmacokinetics and pharmacodynamics results of a long-acting C-type natriuretic peptide prodrug, TransCon CNP

Breinholt VM, Mygind PH, Christoffersen ED, Zhang Y, Ota S, Will Charlton R, Viuff D
Br J Clin Pharmacol. 2022 Nov;88(11):4763–4772.
doi: 10.1111/bcp.15369. PMID: 35481707.
https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/bcp.15369

In brief: This phase 1 study in healthy adults assessed the safety and feasibility of transcon-CNP, a novel prodrug that releases native C-type natriuretic peptide (CNP). The novel drug was well tolerated and CNP remained in systemic circulation for >7 days following a single dose.

Commentary: Achondroplasia is caused by autosomal activating mutation in the fibroblast growth factor receptor 3 gene (*FGFR3*) resulting in constitutive receptor activation and signalling through the mitogen-activated protein kinase (MAPK) pathway in chondrocytes, resulting in inhibited longitudinal bone growth. C-type natriuretic peptide, encoded by *NPPC*, and its receptor, natriuretic peptide receptor 2 (*NPR2*), are potent stimulators of endochondral ossification at the growth plate. Transcon-CNP is a long-acting prodrug that releases native CNP and has demonstrated positive effects on bone growth in both murine and non-human primate models.

This study was funded and performed by Ascendis pharmaceutical and is the first in-human trial with transcon-CNP. The study drug was administered SC in single doses to ascending-dose cohorts (3, 10, 25, 75 and 150 μg CNP/kg). A total of 49 healthy men were randomized to the study drug in a 4:1 ratio (active:placebo). The average half-life was $\sim$120 hours, which is substantially longer than the half-life of endogenous CNP[1] (only 2–3 minutes) and vosoritide[2] (19–46 minutes).

Transcon CNP administration caused sustained increases of cGMP levels in both plasma and urine indicating that the CNP released from the PEG carrier is active and that the level of released CNP affords a sustained activation of NPR2 for at least 7 days post-administration. Interestingly, there was no change in endogenous levels of NTproCNP, indicating that CNP released from transcon-CNP does not interfere with endogenous CNP biosynthesis. Transient, symptomatic vasodilatory adverse events were reported at higher doses and symptoms included mild postural dizziness, feeling lightheaded on standing, and palpitations. However, no major safety or tolerability concerns were observed.

Ongoing and future phase 2 and phase 3 trials with subsequent extensions will determine if transcon-CNP is an efficacious and safe treatment to improve growth and possibly also mitigate other complications of achondrondroplasia. If so, it would represent a once-a-week alternative to vosorotide for growing children with achondroplasia.

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3.3. Burosumab treatment for autosomal recessive hypophosphatemic rickets type 1 (ARHR1)

Bai X, Levental M, Karaplis AC

J Clin Endocrinol Metab. 2022 Sep 28;107(10):2777–2783.

doi: 10.1210/clinem/dgac433. PMID: 35896139.

https://pubmed.ncbi.nlm.nih.gov/35896139/

In brief: Autosomal recessive hypophosphatemic rickets type 1 (ARHR1) is caused by inactivating pathogenic variants in the *DMP1* gene. Fibroblast growth factor 23 (FGF23) concentration is elevated, which causes hypophosphatemic rickets. This study showed benefits of burosumab (Crysvita), a humanized monoclonal antibody to FGF23, on biochemical and clinical outcomes in two patients with ARHR1.

Commentary: ARHRs are rare, heritable renal phosphate-wasting disorders that arise from overexpression of the bone-derived phosphaturic hormone FGF23 leading to impaired bone mineralization (rickets and osteomalacia). Inactivating mutations in Dentin matrix protein 1 (*DMP1*) give rise to ARHR type 1 (ARHR1). Short stature, prominent bowing of the legs, fractures/pseudofractures, and severe enthesopathy are prominent in this patient population. Traditionally, treatment consists of oral phosphate replacement and calcitriol, but this approach is limited by modest efficacy and potential renal and gastrointestinal side effects.

The advent of burosumab (Crysvita), a fully humanized monoclonal antibody to FGF23, to treat X-linked hypophosphatemia and tumour-induced osteomalacia, offers a unique opportunity to evaluate its safety and efficacy in patients with ARHR1. This study reports monthly administration of burosumab to two brothers (age 42 and 52 years old) affected by ARHR1, resulting in normalized serum phosphate, pseudofracture healing, diminished fatigue, less bone pain, and reduced incapacity arising from the extensive enthesopathy and soft tissue fibrosis/calcification that characterizes this disorder. No adverse effects were reported.

This report highlights the beneficial biochemical and clinical outcomes of burosumab in patients with ARHR1. A multicenter cohort study is needed to fully assess its safety and long-term efficacy in this patient population, albeit the rarity of this disorder will undoubtedly pose a major obstacle to such an endeavour.

Advances in Clinical Practice

3.4. Skeletal and extraskeletal disorders of biomineralization

Collins MT, Marcucci G, Anders HJ, Beltrami G, Cauley JA, Ebeling PR, Kumar R, Linglart A, Sangiorgi L, Towler DA, Weston R, Whyte MP, Brandi ML, Clarke B, Thakker RV

Nat Rev Endocrinol. 2022 Aug;18(8):473–489.

doi: 10.1038/s41574-022-00682-7. PMID: 35578027.

https://www.nature.com/articles/s41574-022-00682-7

In brief: This is a timely and well-written review on disorders of biomineralization and their fundamental mechanisms and by leading experts in the field. This is mandatory reading for any aspiring endocrinologist.

Commentary: Biomineralization is a critical physiological process, and deviations from it can cause various diseases. Recent progress has furthered our understanding of the genetic, molecular, and cellular underpinnings of the disorders of biomineralization. The review explains the principal regulators of mineralization and crystallization, including the generation of pyrophosphate and matrix vesicles, which are crucial for forming

hydroxyapatite, the primary building block of all mineralized tissues. Tissue-level pyrophosphate degradation by tissue non-specific alkaline phosphatase, expressed by ossifying tissues, permits hydroxyapatite formation and subsequent calcification.

Fibroblast growth factor 23 (FGF23) is another important player with a crucial role in the regulation of phosphate and 1,25 dihydroxy vitamin D with deficiency causing rickets or osteomalacia and excess resulting in ectopic calcification. Additionally, exposure to excessive fluoride can substitute the hydroxyl group of hydroxyapatite, leading to altered tooth enamel and skeletal complications, such as osteomalacia and fractures (fluorosis). This review also sheds light on non-hydroxyapatite crystal formation (crystallopathies) often promoted by adhesive proteins or neutrophil extracellular traps, especially in excretory organs, which can stimulate inflammation-related pathogenic responses.

Disorders of biomineralization cause alterations in mineral quantity and quality, as well as extraskeletal mineralization, such as hyperphosphataemic familial tumoural calcinosis. Disorders of alkaline phosphatase (hypophosphatasia) and phosphate homeostasis are highlighted, including X-linked hypophosphataemic rickets, fluorosis, rickets, and osteomalacia. There is also a discussion on arterial and renal calcification, emphasizing that dysregulation of the hormonally controlled critical process of urinary calcium and phosphate reabsorption in the kidney can lead to pathological calcification (nephrocalcinosis and nephrolithiasis). The newly attained understanding summarized in this review is the basis for future studies that promise new therapeutic approaches for biomineralization disorders.

3.5. High bone mass disorders: New insights from connecting the clinic and the bench

Bergen DJM, Maurizi A, Formosa MM, McDonald GLK, El-Gazzar A, Hassan N, Brandi ML, Riancho JA, Rivadeneira F, Ntzani E, Duncan EL, Gregson CL, Kiel DP, Zillikens MC, Sangiorgi L, Högler W, Duran I, Mäkitie O, Van Hul W, Hendrickx G
J Bone Miner Res. 2023 Feb;38(2):229–247.
doi: 10.1002/jbmr.4715. PMID: 36161343.
https://asbmr.onlinelibrary.wiley.com/doi/10.1002/jbmr.4715

In brief: This comprehensive review classifies the known high bone mass (HBM) disorders based on Gene Ontology (GO) nomenclature. The authors emphasize the importance of functional genomics in the discovery of new HBM genes and discuss strategies to improve understanding of the underlying pathogenic mechanisms and inform the development of therapeutic approaches.

Commentary: HBM disorders are typically defined by a high areal bone marrow density (BMD) *Z*-score $> +$ 2.5 in at least 2 skeletal sites. Increased bone mass may be due to increased osteoblastic bone formation, decreased osteoclastic bone resorption or imbalance in bone formation and resorption. It is important to classify the different HBM disorders based on the genes involved and the implicated signalling pathways/biological processes.

The authors classified the known HBM disorders into 10 distinct sub-groups based on the biological function of the mutated genes, using Gene Ontology nomenclature. Some sub-groups are further classified based upon genes/disorders involving a particular signalling pathway. The authors believe that 'reverse genetics' is a future direction, using different functional genomics methodologies to study novel genes implicated in the pathogenesis of HBM disorders.

This classification of HBM disorders will be very useful for both researchers and clinicians. There are relatively few functional genomics studies on HBM disorders. The authors emphasize the importance of reverse genetics approaches, using appropriate model systems to identify novel HBM genes. Further, amalgamation of both clinical genetics and experimental approaches will be crucial to identify pathological mechanisms and guide the design of therapeutic strategies to existing and novel HBM conditions.

3.6. Quality of life of pediatric and adult individuals with osteogenesis imperfecta: a meta-analysis

Wehrli S, Rohrbach M, Landolt MA

Orphanet J Rare Dis. 2023 May 24;18(1):123.
doi: 10.1186/s13023-023-02728-z. PMID: 37226194.
https://ojrd.biomedcentral.com/articles/10.1186/s13023-023-02728-z

In brief: Osteogenesis imperfecta (OI) is a genetically and phenotypically heterogeneous disorder which leads to significantly reduced quality of life (QoL) and high morbidity primarily due to frequent fractures, musculoskeletal pain, reduced mobility and loss of ambulation. This meta-analysis shows that QoL is significantly lower in children and adults with OI compared to normative data and controls.

Commentary: Quality of life (QoL) is a multidimensional concept that includes wide-ranging constructs such as functional status, emotional functioning, health perceptions, and social functioning. Measuring QoL in OI is important because it captures personal experiences and therefore is vital to guide the successful implementation of interventions and the assessment of novel treatments. Because of clinical heterogeneity, QoL is expected to vary between various subtypes of OI.

This meta-analysis investigate if and how individuals with OI differ from healthy controls regarding QoL, and if and how QoL differs among different OI subtypes. Nine databases were searched using predefined keywords, exclusion and inclusion criteria. Study quality was assessed using a risk of bias tool.

Two studies featuring children and adolescents ($n = 189$), and four studies on adults ($n = 760$) were identified for meta-analysis. Children with OI had significantly lower QoL on the pediatric quality of life inventory (PedsQL) with regards to the total score, emotional, school, and social functioning compared to controls and norms. There was insufficient data to calculate differences between OI subtypes. Studies in adults used the Short Form Health Survey Questionnaire, 12 (SF-12) and 36 items (SF-36). Adults with all OI types showed significantly lower QoL levels across all physical component subscales compared to norms. The same pattern was found for the mental component subscales namely vitality, social functioning, and emotional role functioning. The mental health subscale was significantly lower for OI type I, but not for types III and IV and was not related to clinical severity of OI. All the included studies showed low risk of bias.

The implication of poorer emotional, school, and social functioning in children with OI is that they should be supported in their schools, which should also improve their emotional and social functioning.

3.7. Prevalence of monogenic bone disorders in a Dutch cohort of atypical femur fracture patients

Zhou W, van Rooij JG, van de Laarschot DM, Zervou Z, Bruggenwirth H, Appelman-Dijkstra NM, Ebeling PR, Demirdas S, Verkerk AJ, Zillikens MC

J Bone Miner Res. 2023 Apr 19.
doi: 10.1002/jbmr.4801. PMID: 37076969.
https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/37076969/

In brief: Prolonged bisphosphonate treatment is associated with atypical femoral fractures (AFF). However, AFFs also occur in bisphosphonate-naïve patients, so bisphosphonate is not a prerequisite for AFF. This study found a higher yield of (likely) pathogenic variants in AFF patients with a clinical suspicion of monogenic bone disorder, stressing the importance of careful clinical evaluation of patients who present with this condition.

Commentary: Atypical femur fractures (AFFs) are a rare type of fractures, which have been associated with long-term bisphosphonate use. They occur with no or minimal trauma at the femoral shaft from the subtrochanteric region to just above the supracondylar flare. They can be distinguished from typical osteoporotic fractures by specific radiological features, such as a horizontal fracture line originating at the lateral side, no or minimal comminution, and localized cortical thickening.

It has been suggested that AFFs are stress or insufficiency fractures, possibly due to the accumulation of microdamage from suppressed bone remodelling and/or increased homogeneity of bone mineralization caused by long-term bisphosphonate use. Nevertheless, it remains unexplained why AFF occurs in only a minority of patients treated with bisphosphonates. Nor is it been explained why AFFs also occur in patients who never used bisphosphonates. AFF has been reported in monogenic bone disorders, where bisphosphonates are not used, e.g. hypophosphatasia (HPP), X-linked hypophosphatemia, pycnodysostosis, osteopetrosis, osteoporosis pseudoglioma syndrome (OPPG), osteogenesis imperfecta (OI), and X-linked osteoporosis.

This study recruited AFF patients from two specialist bone centers in the Netherlands. Their medical records were reviewed for clinical features of monogenic bone disorders. Genetic variants identified by whole-exome sequencing in 37 candidate genes involved in monogenic bone disorders were classified based on the American College of Medical Genetics and Genomics (ACMG) guidelines. Copy number variants overlapping the candidate genes were also evaluated using DNA array genotyping data. The cohort comprised 60 AFF patients (including two siblings), of whom 95% had received bisphosphonates. Fifteen AFF patients (25%) had clinical features of a monogenic bone disorder. Eight of them (54%), including the 2 siblings, had a (likely) pathogenic variant in *PLS3, COL1A2, LRP5*, or *ALPL*. Among patients not suspected of monogenic bone disorders, only one patient (2%) carried a likely pathogenic variant in *TCIRG1*. In total, nine AFF patients (15%) had a (likely) pathogenic variant. One patient, was found to have 12.7 Mb deletion in chromosome 6, encompassing *TENT5A*.

In summary, these findings strongly suggest that genetic variants associated with monogenic bone disorders play a role in the pathogenesis of AFF, although the underlying mechanism is still unclear.

3.8. Nosology of genetic skeletal disorders: 2023 revision

Unger S, Ferreira CR, Mortier GR, Ali H, Bertola DR, Calder A, Cohn DH, Cormier-Daire V, Girisha KM, Hall C, Krakow D, Mäkitie O, Mundlos S, Nishimura G, Robertson SP, Savarirayan R, Sillence D, Simon M, Sutton VR, Warman ML, Superti-Furga A

Am J Med Genet A. 2023 May;191(5):1164–1209.
doi: 10.1002/ajmg.a.63132. PMID: 36779427.
https://onlinelibrary.wiley.com/doi/10.1002/ajmg.a.63132

In brief: The 11th edition of the 'Nosology' is significantly expanded, now covering 771 conditions linked to 552 genes. In a major shift from previous editions, it has adopted a dyadic naming system that defines disorders based on both their phenotypic and genetic features. It continues to be a vital tool for diagnosing and communicating about genetic skeletal disorders.

Commentary: The first Nosology of genetic skeletal disorders was published in 1970 with the aim of establishing a unified naming system for the growing number and variety of skeletal phenotypes with a genetic basis. This was driven by a surge in disease identification and description in the 1960s, with distinct skeletal disorders such as diastrophic dysplasia, spondylo-epiphyseal dysplasia congenita, and 'pseudo-Morquio' disorders being identified. The role of radiology in distinguishing these disorders was crucial, and differentiation was based on radiographic features and the recognition of a gene's radiographic signature in phenotypically distinct disorders.

Since the 1980s, updates of the Nosology incorporated increasing molecular criteria, with the discovery of genetic variants in collagen 1 in osteogenesis imperfecta being a pivotal development. This led to the concept of 'bone dysplasia families,' which are disorders originating from different pathogenic variants in a single gene, with *COL2A1*- and *FGFR3*-related skeletal dysplasia being early and prominent examples.

The Nosology, now in its 11th edition, has dramatically expanded and contains 771 conditions associated with 552 genes. However, the most significant change from previous versions is that it has adopted the dyadic naming system, which means that disorders now are defined based on a combination of their phenotypic and genetic

features. Each disorder is now named by the combination (dyad) of an altered gene and specific phenotype. For example, achondroplasia is now named '*FGFR3*-related achondroplasia', and spondyloepiphyseal dysplasia congenita is now '*COL2A1*-related spondyloepiphyseal dysplasia'.

Despite the inherent complexity and limitations in classification, the Nosology remains a critical tool for diagnosing and communicating about genetic skeletal disorders and therefore an important tool for paediatric endocrinologists.

3.9. Lifetime impact of achondroplasia study in Europe (LIAISE): findings from a multinational observational study

Maghnie M, Semler O, Guillen-Navarro E, Selicorni A, Heath KE, Haeusler G, Hagenäs L, Merker A, Leiva-Gea A, González VL, Raimann A, Rehberg M, Santos-Simarro F, Ertl DA, Gregersen PA, Onesimo R, Landfeldt E, Jarrett J, Quinn J, Rowell R, Pimenta J, Cohen S, Butt T, Shediac R, Mukherjee S, Mohnike K

Orphanet J Rare Dis. 2023 Mar 15;18(1):56.
doi: 10.1186/s13023-023-02652-2. PMID: 36922864.
https://pubmed.ncbi.nlm.nih.gov/36922864/

In brief: Individuals with achondroplasia carry a lifelong burden of reduced physical and mental health. The Lifetime Impact of Achondroplasia Study in Europe (LIAISE; NCT03449368) found that, across an individual's lifetime, achondroplasia is associated with multisystem complications, reduced QoL and functionality, increased pain and increased healthcare resource utilization.

Commentary: Individuals with achondroplasia present with a range of clinical features. The retrospective, observational LIAISE study was designed to quantify the impact of achondroplasia across the age spectrum within Europe by assessing the clinical and surgical burden, healthcare resource use and QoL of affected individuals. It collected demographic, clinical and healthcare resource use data from medical records in 13 sites across six European countries. Descriptive statistics or event rates per 100 person-years were calculated and compared across age groups as well as by the history of limb lengthening. Patient-reported outcomes (quality of life [QoL], pain, functional independence, work productivity and activity impairments) were evaluated using questionnaires at the time of enrolment. An exploratory analysis investigated correlations between height (*z*-score or centimetres) and patient-reported outcomes.

Overall, 186 study patients were included (mean age 21.7 ± 17.3 years, range 5.0–84.4). At least one complication or surgery was reported for 94.6% and 72.0% of patients, respectively, at a rate of 66.6 and 21.5 events per 100 person-years. Diverse medical and surgical complications were reported for all ages in a bimodal distribution, occurring more frequently in the youngest and oldest age groups. A total of 40 patients had previously undergone limb lengthening (capped at 20% per study protocols). The most frequent surgery types varied by age, in line with complication profiles. The most common types of surgeries were middle ear procedures (28.0%) tonsillectomies/adenoidectomies (26.3%), and brainstem decompression surgeries (14.0%). Healthcare resource use was high across all age groups, especially among the youngest and oldest individuals, and did not differ substantially by history of limb lengthening. Compared to general population values, patients reported impaired QoL, particularly for physical functioning domains. In addition, patients reported difficulty carrying out daily activities independently and pain starting in childhood. Patient height correlated with multiple patient-reported outcomes.

These findings show that individuals with achondroplasia experience a range of severe complications throughout their lives, resulting in a high level of healthcare resource use and reduced QoL. Importantly, LIAISE revealed varied approaches to achondroplasia management and highlights the value of an international consensus on management practices to ensure high-quality care for all patients across geographical regions.

3.10. Neonatal and early infancy features of patients with inactivating PTH/PTHrP signaling disorders/pseudohypoparathyroidism

Del Sindaco G, Berkenou J, Pagnano A, Rothenbuhler A, Arosio M, Mantovani G, Linglart A
J Clin Endocrinol Metab. 2023 Apr 25:dgad236.
doi: 10.1210/clinem/dgad236. Online ahead of print. PMID: 37098127.
https://pubmed.ncbi.nlm.nih.gov/37098127/

In brief: Clinical symptoms of Pseudohypoparathyroidism (PHP) and related disorders present during late childhood and adulthood. This study found that newborns with this group of conditions require specific care at birth due to increased risk of neonatal complications.

Commentary: Pseudohypoparathyroidism (PHP) and related disorders, newly referred to as inactivating PTH/PTHrP signalling disorders (iPPSD), are rare endocrine diseases. Their many clinical features include obesity, neurocognitive impairment, brachydactyly, short stature, parathyroid hormone (PTH) resistance, and resistance to other hormones such as thyroid-stimulating hormone (TSH). Full development of disease manifestations is mainly described during late childhood and adulthood, associated with significant delays in diagnosis.

This study analysed a large cohort of 136 iPPSD/PHP patients. Retrospective data from birth were collected to investigate the rate of neonatal complications occurring in each iPPSD/PHP category. 36% of patients had at least one neonatal complication, far more than the general population. This proportion was as high as 47% among patients with iPPSD2/PHP1A, including neonatal hypoglycemia (10.5%) and transient respiratory distress (18.4%). The presence of neonatal features was associated with earlier onset of TSH resistance ($P < 0.001$) and with higher risk of neurocognitive impairment ($P = 0.02$) and constipation ($P = 0.04$) in later life.

These findings suggest that iPPSD/PHP, and especially iPPSD2/PHP1A, newborns require specific care at birth because of an increased risk of neonatal complications. These complications may predict a more severe course of the disease. However, they are nonspecific and, in the absence of positive family history, diagnostic delay remains likely.

3.11. Growth and disease burden in children with hypophosphatasia

Högler W, Linglart A, Petryk A, Kishnani PS, Seefried L, Fang S, Rockman-Greenberg C, Ozono K, Dahir K, Martos-Moreno GÁ
Endocr Connect. 2023 Apr 25;12(5):e220240.
doi: 10.1530/EC-22-0240. PMID: 36917043.
https://ec.bioscientifica.com/view/journals/ec/12/5/EC-22-0240.xml

In brief: Hypophosphatasia is a clinically heterogenous disorder. By analysing observational data on 215 children (54.4% girls) with hypophosphatasia from the Global Hypophosphatasia Registry, the authors found growth impairment in patients aged < 2 years. However, short stature was not a characteristic feature of children with hypophosphatasia, and height was not correlated with disease severity.

Commentary: Hypophosphatasia is an inborn error of metabolism characterized by impaired bone mineralization that often affects linear growth. A natural history study of longitudinal data collected from 101 patients over the course of 25 years at a single center in the United States showed that children with HPP may have below-average height but normal weight compared to the general population. Given that hypomineralization disorders can negatively affect the zone of hypertrophic chondrocytes of the growth plate, growth in children with HPP has often been suggested as an indicator of disease burden and overall health.

This study evaluated relationships between anthropometric parameters (height, weight, and body mass index) and clinical manifestations of hypophosphatasia in children (aged < 18 years) with hypophosphatasia. Anthropometry was evaluated by age group (< 2 years and ≥ 2 years). The frequency of hypophosphatasia manifestations was compared between children with and without short stature.

Short stature was present in 16.1% of children with hypophosphatasia aged <2 years, and in 20.4% of those aged ≥ 2 years. Among those with available data ($n=62$), height was below target height (mean -0.66 standard deviations). Substantial worsening of growth (mean delta height z score: -1.45; delta weight z score: -0.68) occurred before 2 years of age, but after age ≥ 2 years growth trajectories were maintained (delta height z score: 0.08; delta weight z score: 0.13). Broad-ranging hypophosphatasia manifestations (beyond dental) were observed in most children, with bone deformities, bone pain, failure to thrive, fatigue, and muscle weakness being the most common.

Short stature is not a consistent characteristic of children with hypophosphatasia, but growth impairment was observed in those aged <2 years, indicating that alkaline phosphatase activity is critical for growth plate function during the rapid growth of infancy. In addition, a broad range of clinical manifestations occurred in those above and below the 3rd percentile for height, indicating that height alone does not indicate hypophosphatasia severity and that weight is less affected than longitudinal growth.

Translational Highlights

3.12. Impaired bone strength and bone microstructure in a novel early-onset osteoporotic rat model with a clinically relevant PLS3 mutation

Hu J, Zhou B, Lin X, Zhang Q, Guan F, Sun L, Liu J, Wang O, Jiang Y, Xia WB, Xing X, Li M
Elife. 2023 Apr 21;12:e80365.
doi: 10.7554/eLife.80365. PMID: 37083757.
https://pubmed.ncbi.nlm.nih.gov/37083757/

In brief: The study established a novel rat model with a clinically relevant *PLS3* mutation, which replicates the osteoporotic phenotype of early-onset *PLS3*-related osteoporosis. The findings suggest that treatment with alendronate or teriparatide improves bone mass and microarchitecture, suggesting their potential as effective treatments for early-onset osteoporosis caused by *PLS3* mutations.

Commentary: This study is an essential step forward in understanding early-onset osteoporosis caused by mutations in *PLS3*, which encodes a protein crucial to bone health. The creation of a rat model with a clinically relevant *PLS*3 mutation is important as it effectively mimics the osteoporotic conditions seen in humans. This model further provided insights into the role of *PLS3* in bone microstructure and strength regulation, highlighting its critical role in maintaining bone health. By observing the decreased bone strength and thin cortical thickness in the rat model, researchers were able to gain a clearer understanding of the impacts of *PLS3* mutations on human bone health.

Importantly, the finding that alendronate and teriparatide dramatically improve bone mass and microarchitecture in this rat model offers hope for therapeutic advances in treatment of *PLS3*-induced osteoporosis. Alendronate and teriparatide have long been used in the treatment of osteoporosis, but their efficacy in the context of a *PLS3* mutation has not been established. This new evidence underscores their potential in treating early-onset osteoporosis. Ultimately, these findings add to our understanding the role of *PLS3* in bone health, and also open new avenues to develop and test novel treatment strategies for early-onset osteoporosis.

3.13. Loss-of-function variant in SPIN4 causes an X-linked overgrowth syndrome

Lui JC, Wagner J, Zhou E, Dong L, Barnes KM, Jee YH, Baron J
JCI Insight. 2023 May 8;8(9):e167074.
doi: 10.1172/jci.insight.167074. PMID: 36927955.
https://insight.jci.org/articles/view/167074.

In brief: These authors identify a frameshift truncating variant in the gene *SPIN4* (Spindlin member 4) in a patient with skeletal overgrowth, hepatosplenomegaly and macrocephaly. Using state-of-the-art mouse models

and histone peptide arrays, they delineate the underlying pathogenic mechanism and show that *SPIN4* positively regulates Wnt signalling by functioning as an epigenetic reader.

Commentary: Generalized overgrowth disorders are characterized by increased cellular proliferation leading to tall stature, macrocephaly and organomegaly. Some known overgrowth disorders, such as Weaver syndrome and Sotos syndrome, are caused by mutations in genes that function as chromatin modifiers. This paper describes a novel mutation in the gene *SPIN4* in a patient with generalized overgrowth. It shows that *SPIN4* functions as an epigenetic reader by binding to modified histones and mediating downstream effects.

The proband was a 13-year-old boy who was born large for gestational age and had excessive linear growth (+4.5 to +5 SDS) with advanced bone age. Exome sequencing identified a hemizygous frameshift truncating variant in *SPIN4* on the X chromosome. Using histone peptide arrays, normal SPIN4 protein was shown to bind modified histones, whereas this binding was abrogated by the identified *SPIN4* variant. In mouse models harboring the *SPIN4* truncating variants, skeletal and organ overgrowth was evident, thus replicating the human phenotype. Mutant mice showed expanded resting zone in the growth plate cartilage leading to increased cell proliferation and increased growth plate height. Wild-type *SPIN4* induced Wnt signaling and suppressed cell proliferation in-vitro, whereas mutant SPIN4 did not. Further, growth plate chondrocytes lacking SPIN4 showed reduced basal Wnt signaling.

The study implicates *SPIN4* gene, an epigenetic reader in, skeletal overgrowth. *SPIN4* was shown to regulate body size in mice by binding modified histones on chromatin and mediating downstream effects through the activation of Wnt signaling. This is a novel overgrowth syndrome and another example of an overgrowth syndrome caused by mutation in an epigenetic regulator of gene expression, thus highlighting the importance of epigenetic mechanisms in growth and maybe specifically its role to limit body growth and prevent overgrowth.

Advances in Growth, Bone Biology, and Mineral Metabolism

3.14. SIRT2 regulates extracellular vesicle-mediated liver-bone communication

Lin L, Guo Z, He E, Long X, Wang D, Zhang Y, Guo W, Wei Q, He W, Wu W, Li J, Wo L, Hong D, Zheng J, He M, Zhao Q
Nat Metab. 2023 May;5(5):821–841.
doi: 10.1038/s42255-023-00803-0. PMID: 37188819.
https://pubmed.ncbi.nlm.nih.gov/37188819/

In brief: These authors studied liver-specific *SIRT2* knockout mice to examine how loss of hepatocyte *SIRT2* (Sirtuin 2) prevents bone loss in aged mice. Hepatocyte *SIRT2* deficiency led to upregulation of Leucine rich α2 glycoprotein (LRG1) in hepatocyte-derived small extracellular vesicles (sEVs) which inhibited osteoclastogenesis in bone marrow.

Commentary: Liver-bone communication has been implicated in bone homeostasis. Patients with chronic liver disease show altered bone metabolism and often develop osteoporosis. *SIRT2* encodes a deactelyase that is abundantly expressed in the liver and is involved in the regulation of multiple cellular processes such as aging, metabolism and inflammation, but its role in bone is unknown.

In this study, hepatocyte SIRT2 levels were found to be upregulated in aged mice and elderly humans. Liver-specific *SIRT2* knockout mice (SIRT2-KO^{hep}) mice at 18 months of age showed increased BV/TV, increased trabecular number and decreased trabecular separation than their control littermates which showed obvious bone loss. Plasma from aged SIRT2-KO^{hep} mice suppressed *in-vitro* osteoclast differentiation and reduced the number of TRAP+ osteoclasts. Probing further, small extracellular vesicles (sEVs) isolated from SIRT2-KO^{hep} plasma and co-cultured with bone marrow derived monocytes (BMDMs) inhibited osteoclast differentiation. Combining mass spectrometry and RNA sequencing to compare global plasma protein profiles, LRG1 was found to be increased in SIRT2-KO^{hep} plasma sEVs and in the osteoclast progenitors of SIRT2-KO^{hep} mice. Hepatic SIRT2-KO^{hep} upregulated sEV-LRG1 transfer to BMDMs to suppress osteoclastogenesis

by preventing NF-κβ p65 activation. Moreover, pharmacological treatment with a SIRT2 inhibitor or sEV-LRG1 was therapeutically beneficial in mouse models of osteoporosis and human primary cell cultures.

These findings illustrate a novel mechanism of hepatocyte-osteoclast communication, involving sEVs-LRG1 and SIRT2, wherein hepatic SIRT2 regulates osteoclastogenesis in the bone marrow. This insight may provide a novel therapeutic approach to treat osteoporosis.

3.15. Lymphatic vessels in bone support regeneration after injury

Biswas L, Chen J, De Angelis J, Singh A, Owen-Woods C, Ding Z, Pujol JM, Kumar N, Zeng F, Ramasamy SK, Kusumbe AP
Cell. 2023 Jan 19;186(2):382–397.e24.
doi: 10.1016/j.cell.2022.12.031. PMID: 36669473.
https://pubmed.ncbi.nlm.nih.gov/36669473/

In brief: Current dogma is that lymphatic vessels are absent in bone and bone marrow. Using advanced 3D-imaging and mouse genetics, these authors show the presence of lymph vessels in bone. Moreover, they show that genotoxic stress causes lymph vessels expansion and lymphangiogenesis in bone, which in turn promotes bone and hematopoietic regeneration.

Commentary: The lymphatic system maintains fluid homeostasis, removes cellular waste products and produces lymphocytes, which are important players in immune defence. Until recently, it was believed that bone, bone marrow and other organs, such as brain and eye, lack lymphatic vessels.

Here, using modified tissue clearing and processing steps and advanced 3D light sheet imaging, these authors uncovered the presence of lymphatic vessels in bone. Immunolabeling and 3D imaging of skeletal tissue is technically challenging due to the calcified nature of bones. Lymphatic vessels, immunolabeled with lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) and prospero-related homeobox 1 (PROX1), were found across different mouse bones and human bone biopsies, including tibia, femur, sternum, vertebral column, calvarium and hip bones. Irradiation caused proliferation of lymphatic endothelial cells (LECs) expansion of lymphatic vessels. Selective depletion of PROX1+ LECs (PROX1 Cre-iDTA) decreased expansion of lymphatic vessels and reduced numbers of hematopoietic stem cell (HSC) in irradiated bones. Competitive transplantation of bone marrow cells from PROX1 Cre+iDTA mice reduced their reconstituting activity. LECs are known to secrete factors and regulate other cell types. C-X-C motif chemokine 12(CXCL12) was upregulated in LECs after irradiation. Moreover, competitive secondary transplantation of bone marrow cells from LEC specific CXCL12 knockout mice decreased reconstitution ability of HSCs. Radiation injury led to expansion of myosin heavy chain 11 (Myh11) expressing pericyte cells in bones, which contributed to bone regeneration by giving rise to osteoblasts, chondrocytes and adipocytes.

This study establishes the presence of lymphatic vessels in murine and human bones. Further, the study shows that lymphatic vessels are crucial drivers of HSC and bone regeneration following genotoxic injury. Radiation injury caused expansion of lymphatic vessels in bone, promoted lymphangiogenesis and supports hematopoietic and bone regeneration. Taken together, these findings uncover a novel fundamental role of the lymphatic system in skeletal and hematopoietic regeneration post injury and adds to the broad array of its existing physiologic functions.

3.16. A saturated map of common genetic variants associated with human height

Yengo L, Vedantam S, Marouli E, Sidorenko J, Bartell E, Sakaue S, Graff M, Eliasen AU, Jiang Y, Raghavan S, Miao J, Arias JD, Graham SE, Mukamel RE, Spracklen CN, Yin X, Chen SH, Ferreira T, Highland HH, Ji Y, Karaderi T, Lin K, Lüll K, Malden DE, Medina-Gomez C, Machado M …(See abstract for full author list)
Nature. 2022 Oct;610(7933):704–712.
doi: 10.1038/s41586-022-05275-y. PMID: 36224396.
https://www.nature.com/articles/s41586-022-05275-y

In brief: This comprehensive genome-wide association study (GWAS) identified over 20 000 loci associated with adult height. This groundbreaking research revealed that up to 21% of the human genome can be linked to variation in human height, providing a deeper understanding of the complex regulation of human height.

Commentary: This one of the largest GWAS to date involving 5.4 million individuals. It discovered 12 111 independent SNPs associated with adult height, which collectively account for nearly all of the estimated common SNP-based heritability for this trait. These SNPs clustered within 7209 non-overlapping genomic segments with a mean size of around 90 kb, covering roughly 21% of the genome. The density of independent associations was uneven across the genome, with regions of increased density being enriched for growth plate genes[1]. Intriguingly, the findings also showed disparities in the amount of phenotypic variance explained by these SNPs across different ancestral populations – about 40–45% in populations of European ancestry, but only 10–20% in populations of non-European ancestries.

Effect sizes, associated regions, and gene prioritization were consistent across ancestries. Therefore, the diminished prediction accuracy for adult height observed in non-European ancestries is due to differences in linkage disequilibrium and allele frequency rather than differences in the underlying genetic architecture. Importantly, relevant biological pathways associated with height could be identified with smaller sample sizes than those needed to implicate causal genes and variants, highlighting the importance of pathway-level analyses. However, while the study provides a comprehensive map of specific genomic regions containing the majority of common height-associated variants, it also emphasizes that the map is saturated for populations of European ancestry and more work is required to achieve equivalent saturation for other ancestries. This conclusion underscores the importance of diversity in genetic studies and the need for further research to comprehensively understand the genetic architecture of human height across all ancestries.

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1. Synthesizing genome-wide association studies and expression microarray reveals novel genes that act in the human growth plate to modulate height. Lui JC, Nilsson O, Chan Y, Palmer CD, Andrade AC, Hirschhorn JN, Baron *J. Hum Mol Genet*. 2012 Dec 1;21(23):5193–5201. PMID: 22914739

3.17. Nutrient-regulated dynamics of chondroprogenitors in the postnatal murine growth plate

Oichi T, Kodama J, Wilson K, Tian H, Imamura Kawasawa Y, Usami Y, Oshima Y, Saito T, Tanaka S, Iwamoto M, Otsuru S, Enomoto-Iwamoto M

Bone Res. 2023 Apr 21;11(1):20.
doi: 10.1038/s41413-023-00258-9. PMID: 37080994.
https://pubmed.ncbi.nlm.nih.gov/37080994/

In brief: The authors use cell-tracing of Axin2-positive chondroprogenitor cells in the resting zone during and after food restriction to demonstrate that nutrient availability influences the balance between accumulation and differentiation of resting zone chondrocytes and that this is influenced by Igf-1.

Commentary: Catch-up growth is the rapid growth that occurs after growth-inhibiting conditions have been cured or removed. The molecular mechanism for catch-up growth has not been fully elucidated. According to the growth plate senescence model, growth-inhibiting conditions slow growth plate senescence. When the growth-inhibiting condition is removed, the growth plates are less senescent and therefore grow faster than normal for age, leading to catch-up growth[1].

These authors studied growth plate chondrocyte cell dynamics using an Axin2-Cre^{ERT} and different reporter strains to label and trace resting zone chondrocytes during and after food-restriction. Their findings confirm previous data that food-restriction delays the rate of senescent decline, growth plate height and the number of resting zone chondrocytes[2]. Using cell-tracing techniques, they specifically showed that resting zone chondrocyte proliferation was maintained during food-restriction, but the transition from resting to proliferative chondrocytes was dramatically decreased, resulting in accumulation of resting zone chondrocytes. When food

restriction was stopped, the accumulated resting zone chondrocytes were committed to the proliferative pool and contributed to catch-up growth.

This finding suggests that growth plates retain growth potential during growth-inhibiting conditions by maintain resting zone chondrocyte proliferation and simultaneously blocking their commitment to the proliferative pool, thus resulting in accumulation of resting zone chondrocytes. In addition, they show that the block in differentiation of resting to proliferative chondrocytes that occurs with food restriction can be overcome by treatment with exogenous IGF1, suggesting that IGF1 is required for differentiation of resting to proliferative chondrocytes and thus that decreased IGF1 may be the nutritional cue that the growth plate uses to conserve resting zone chondrocytes and thereby growth potential during food restriction.

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3.18. Periosteal stem cells control growth plate stem cells during postnatal skeletal growth

Tsukasaki M, Komatsu N, Negishi-Koga T, Huynh NC, Muro R, Ando Y, Seki Y, Terashima A, Pluemsakunthai W, Nitta T, Nakamura T, Nakashima T, Ohba S, Akiyama H, Okamoto K, Baron R, Takayanagi H

Nat Commun. 2022 Jul 18;13(1):4166.
doi: 10.1038/s41467-022-31592-x. PMID: 35851381.
https://pubmed.ncbi.nlm.nih.gov/35851381/

In brief: The authors use cell tracing approaches in a PRMT5$^{flox/\Delta}$ Ctsk-Cre strain to label periosteal stem cells (PSCs) and show that PSCs are not only essential for intramembranous bone formation but also for endochondral bone formation. The study identifies the role of PSC derived indian hedgehog (Ihh) in maintaining growth plate homeostasis and postnatal skeletal growth.

Commentary: The skeleton harbors stem cells in three independent niches: the growth plate resting zone, the periosteum/perichondrium, and the bone marrow. Periosteal stem cells (PSCs) were identified recently and shown to play a role in intramembranous bone formation. In this study, PSCs were shown to be important for endochondral bone formation through the secretion of Ihh.

While studying the role of the enzyme protein arginine methyltransferase 5 (PRMT5) in osteoclastogenesis, the authors generated a mouse strain (PRMT5$^{flox/\Delta}$ Ctsk-Cre) wherein cre enzyme is expressed in osteoclasts as well as PSCs. These mice showed reduced bone volume, calvarial volume and abnormal growth plate architecture with reduced column length suggesting defective intramembranous and endochondral bone formation. The altered skeletal phenotype became prominent with aging. Using a well-defined FACS gating strategy, the authors isolated PSCs from these mice and found that the number of PSCs were markedly reduced. The role of PSCs ablation in endochondral bone formation was confirmed by studying diptheria toxin mediated selective depletion of Ctsk + cells. The resulting mice had reduced body size, bone length, bone width and trabecular bone volume. Further, the authors specifically ablated Ihh in PSCs (Ihh $^{flox/flox}$ x Ctsk-Cre) and found that these mice had reduced body size, bone length and body weight, indicating that endochondral bone formation was indeed affected. The phenotype was not evident until 3 weeks of age but manifested strongly as the animals became older and displayed complete loss of trabecular bone at 11 weeks of age. Additionally, both calvarial bone volume and bone width were decreased in these animals indicating that intramembranous ossification was also affected.

These findings show that PSCs are essential for both intramembranous and endochondral bone formation. In contrast to the role of Ihh/PTHrP in maintaining chondrocyte proliferation by regulating the fate of resting zone stem cells during early development, PSC-derived Ihh acts on resting zone stem cells to maintain postnatal skeletal growth. The study therefore establishes a functional link between periosteal stem cells and growth plate resting zone stem cells and further shows that both intramembranous and endochondral ossification processes co-operate to ensure proper bone development and maintenance.

3.19. The synovial microenvironment suppresses chondrocyte hypertrophy and promotes articular chondrocyte differentiation

Chau M, Dou Z, Baroncelli M, Landman EB, Bendre A, Kanekiyo M, Gkourogianni A, Barnes K, Ottosson L, Nilsson O
NPJ Regen Med. 2022 Sep 16;7(1):51.
doi: 10.1038/s41536-022-00247-2. PMID: 36114234.
https://www.nature.com/articles/s41536-022-00247-2

In brief: Growth plate and articular cartilage have similar structures composed of distinct chondrocyte layers but they differ substantially with respect to their function and fate. This study combines transplantation experiments with cell tracing in rat with *in vitro*studies to identify a novel mechanism by which synoviocytes act directly on chondrocytes to suppress endochondral and promote articular cartilage at the joint surface.

Commentary: Growth plate cartilage is responsible for elongation of the appendicular skeleton, whereas articular cartilage provides a low friction surface that protects the ends of long bones, despite their common origin from the early cartilage template. However, the key cellular and molecular mechanisms responsible for their divergent differentiation are unknown.

These authors investigated the role of the synovial microenvironment in the development of articular cartilage by transplanting osteochondral biopsies from distal femur intercondylar groove from donor rats and inserting them in distal femur in recipient rats, either with original orientation or inverted orientation placing the growth plate cartilage at the joint surface. Cells in the transplants could be traced using donor rats with ubiquitous expression of eGFP and eGFP-negative recipient rats. Interestingly, growth plate cartilage ectopically transplanted to the articular surface remodelled into articular-like cartilage with cells expressing *Prg4* at the surface, whereas articular cartilage placed into the recipient growth plate was gradually remodelled into bone. These findings indicate that the synovial microenvironment acts directly on the articular cartilage to prevent endochondral bone formation and maintain the articular cartilage phenotype.

To further investigate this finding the authors used chondrocyte pellet cultures and showed that synoviocytes produce a soluble factor that directly prevents hypertrophic differentiation and promotes differentiation towards the articular phenotype.

This novel mechanism provides an answer to the long-standing question, why are the chondrocytes closest to the synovial joint space protected from endochondral bone formation and instead form articular cartilage. These findings have important implications for the understanding of skeletal development and joint formation, and also for the pathogenic mechanism of osteoarthritis and other degenerative joint diseases, as well as for tissue engineering of articular cartilage.

4. Differences of Sexual Development (DSD) and Gender Incongruence (GI)

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Preface

In the past 12 months, between June 1, 2022 and May 31, 2023, the search for 'Differences of Sexual Development' or 'disorders of sex development' or 'ambiguous genitalia' or 'gonadal development' or 'DSD' in PubMed yielded 680 publications published in English. A similar search for gender incongruence revealed more than 600 papers. Among those, 15 are summarized in this chapter. The selection process has been very challenging given the space available but we prioritized the key publications chosen on the quality of methodology, the significance of the outcome, and particularly the impact on clinical practice. We have endeavored to balance basic research and clinical articles.

Emerging themes of DSD research for this year's chapter include: i) Novel genomic and epigenomic insights in gonadal development; ii) Novel technologies for fertility augmentation and; iii) New modifiers of androgen receptor. The selection of gender incongruence articles report clinical studies with potential impact on growth, metabolic and psychological outcomes of transgender individuals. We hope these selected publications will help with understanding and improve both knowledge and the clinical care of patients.

Sexuality, Fertility and Fertility Optimization in DSD

4.1. Sexuality and fertility desire in a large cohort of individuals with 46, XY differences in sex development

Batista RL, Inácio M, Brito VN, Sircili MHP, Bag MJ, Gomes NL, Costa EMF, Domenice S, Mendonca BB
Clinics (Sao Paulo). 2023 Mar 23;78:100185.
doi: 10.1016/j.clinsp.2023.100185. PMID: 36965237; PMCID: PMC10091460

Brief summary: This cross-sectional single tertiary center study analyzed the aspects of sexual life and fertility desire among a large cohort of adults with 46,XY DSD in Brazil. The influence of critical variables such as external genital appearances, prenatal androgen exposure, and gender change in the sexuality of 46,XY DSD people were investigated.

There is sparse literature on the sexual function of 46,XY DSD conditions due to their rarity and heterogeneity and sub-optimal long-term follow-up. Such studies were conducted in different countries with cultural variations in expectations for sexual life and sexual function. Therefore, they report discrepant factors in adults with 46,XY DSD who feel dissatisfaction with their sexual lives including external genitalia appearance, prenatal steroids exposure, the incongruence between gender identity and sex assignment, negative body image, traumatic sexual experiences, social stigma, poor familial environment, and previous genital surgery. Understanding their unique needs is crucial to helping individuals achieve good sexual health.

In this study, Batista RL *et al*. included 127 adults with 46 XY, DSD and investigated sexual life parameters and fertility desire according to subgroups: gender in adulthood: females ($n=83$) vs. males ($n=44$); external genitalia appearance at birth in female 46,XY DSD individuals: typical female genitalia ($n=60$) vs. atypical

external genitalia ($n=35$); male gender in adulthood: 46,XY DSD individuals who were assigned male at birth ($n=20$) vs. 46,XY DSD individuals who changed from female to male gender ($n=24$). They found that fertility desire was reported approximately 80% among 46,XY DSD people regardless of gender. Prenatal androgen exposure affected fertility desire negatively in 46,XY women. The onset of sexual life in both genders was later than the age reported in the Brazilian population. Sexual frequency and satisfaction with sexual life were similar in both genders except for more active sexual life in adult males who changed from female to male than those assigned as male. Virilized external genital appearance at birth did not have a negative effect on sexual relations and sexuality among 46,XY DSD women once all feminizing genital surgeries have been performed. More women than men had difficulties in establishing long-term romantic relationships due to concerns about infertility. Collectively, these results expand the knowledge about sexuality in 46,XY DSD people.

4.2. Generation of functional oocytes from male mice *in vitro*

Murakami K, Hamazaki N, Hamada N, Nagamatsu G, Okamoto I, Ohta H, Nosaka Y, Ishikura Y, Kitajima TS, Semba Y, Kunisaki Y, Arai F, Akashi K, Saitou M, Kato K, Hayashi K

Nature. 2023 Mar;615(7954):900–906.
doi: 10.1038/s41586-023-05834-x. Epub 2023 Mar 15. PMID: 36922585

Brief summary: This *in vitro* study reports that fully potent oocytes were generated from pluripotent stem cells of the tail of a sexually mature male mouse. These oocytes were able to give rise to offspring after fertilization.

A particular challenge in the care of DSD patients in adulthood is the optimization of fertility potential. Fertility outcome is significantly reduced in all types of DSD depending on the underlying etiology as well as the severity of the condition. When considering fertility potential, it is also important not to assume heterosexual orientation and to be open-minded about the many ways in which fertility can be achieved for individuals. Notably, it should not be assumed that an individual's gametes must match his or her gender to discuss biological fertility potential. Advancing technology and innovative thinking may enable future fertility for individuals with DSD currently considered to be infertile.

In such an attempt, Murakami K, *et al*. converted the sex of ES and iPS cells (embryonic stem cells and induced Pluripotent stem cells) by removing the Y chromosome and duplicating the X chromosome. Knocking-in of DsRed reporter into the X chromosome by CRISPR-Cas9 system facilitated the generation of sex-converted ES and iPS cells that embody maternally uniparental disomy of the X chromosome. Uniparental disomy of X chromosome can be caused by unequal segregation of the X chromosomes into daughter cells, a similar machinery would be effective for generating XX ES cells from XO ES cells, but such segregation is quite rare. Fluorescence-activated cell sorting (FACS) followed by fluorescence *in situ* hybridization (FISH) sorted XX ES clones and DNA sequencing confirmed that XX ES cells were euploid, without any large insertions or deletions. These sex-converted XX ES and iPS cells then underwent germ cell differentiation and oocyte production in differentiation, growth, and maturation cultures in the reconstituted ovary, making it possible to analyze the effect of uniparental disomy in oogenesis. lUniparental maternal disomy of the X chromosome had no effect on oocyte development or gene expression. XX iPS cell-derived oocytes were capable of fertilization with wild-type sperm by *in vitro* fertilization, and development to two-cell embryos which were transferred to pseudopregnant mice and consequently gave rise to pups. Furthermore, offspring were generated from a single male iPS line via sex conversion *in vitro*. These results were the gold standard evidence of the proper function of oocytes from the sex-converted PS cells.

Using similar chromosomal alteration techniques Murakami K, *et al*. successfully eradicated trisomy 16, a model of Down's syndrome, in iPS cells. This study provides insights that could ameliorate infertility caused by sex or autosomal chromosome disorders, or 46,XY DSD, and opens the possibility of bipaternal reproduction. Nevertheless, a major concern seems a need for detailed monitoring for other conditions related to uniparental disomy and chromosomal rearrangements before human applications.

4.3. AAV-mediated gene therapy produces fertile offspring in the Lhcgr-deficient mouse model of Leydig cell failure

Xia K, Wang F, Lai X, Dong L, Luo P, Zhang S, Yang C, Chen H, Ma Y, Huang W, Ou W, Li Y, Feng X, Yang B, Liu C, Lei Z, Tu X, Ke Q, Mao FF, Deng C, Xiang AP

Cell Rep Med. 2022 Nov 15;3(11):100792.

doi: 10.1016/j.xcrm.2022.100792. Epub 2022 Oct 20. PMID: 36270285; PMCID: PMC9729833

Brief summary: In this *in vivo* study, Xia *et al*. demonstrate that AAV-mediated gene therapy recovers testosterone levels, restarts sexual development, restores spermatogenesis, and produces fertile offspring in a mouse model of Leydig cell failure (LCF).

A null mutation in the gene encoding luteinizing hormone/choriogonadotrophin receptor (Lhcgr) causes a hereditary LCF in mice which is characterized by a reduction in testosterone levels, stunted sexual development, defective spermatogenesis, and infertility. Mice, homozygous for the mutation in Lhcgr ($Lhcgr^{-/-}$) mimic the phenotype of LCF in humans.

In this study, Xia K *et al*. used recombinant adeno-associated virus (AAV) gene delivery vectors for *in vivo* gene therapy. AAV8 was identified as an efficient vector to drive exogenous Lhcgr expression in progenitor Leydig cells through interstitial injection in the testis of $Lhcgr^{-/-}$ pubertal mice.

AAV8-mediated gene therapy in pubertal $Lhcgr^{-/-}$mice partially increased serum testosterone levels and substantially improved sexual development. Testosterone restoration of approximately 30% in AAV8-Lhcgr-injected $Lhcgr^{-/-}$ mice recovered full spermatogenesis, and effectively gave rise to offspring. Furthermore, these AAV8-Lhcgr gene therapy-derived mice were able to produce a second generation by natural mating.

AAV8 showed a clear tropism to target progenitor Leydig cells and showed an absence of vector infection in germ cells and Sertoli cells by interstitial injection in the testis of $Lhcgr^{-/-}$ pubertal mice. Furthermore, tail DNA from the offspring born after AAV8-Lhcgr treatment did not contain any vector sequence signals, suggesting that AAV8 did not integrate into the genomes of the offspring which would otherwise result in off-target genomic effects.

Adult mice responded to AAV-mediated gene therapy as effectively as pubertal mice, as demonstrated by similar increments of testosterone production and restart of sexual development. The researchers also showed tropism of the AAV8 vector to progenitor Leydig cells but not germ cells or Sertoli cells in monkey testes.

Overall, the findings of this study suggest that *in vivo* gene therapy seems a promising future treatment for the group of DSD conditions associated with impaired testosterone biosynthesis and LCF such as 3ß-hydroxysteroid dehydrogenase type 2 (3ß-HSD2) deficiency, cytochrome P450 oxidoreductase (POR) deficiency, CYP17A1 deficiency, or 17ß-hydroxysteroid dehydrogenase type 3 (17ß-HSD3) deficiency. One open question remains about the short effect o such treatment which would be due to the loss of the vector genome during progenitor Leydig cell proliferation because of the nonintegrating nature of AAV vectors.

Novel Players in the Pathogenesis of DSD

4.4. Elevated plasma miR-210 expression is associated with atypical genitalia in patients with 46,XY differences in sex development

Elias FM, Nishi MY, Sircili MHP, Bastista RL, Gomes NL, Ferrari MTM, Costa EMF, Denes FT, Mendonca BB, Domenice S

Mol Genet Genomic Med. 2022 Dec;10(12):e2084.

doi: 10.1002/mgg3.2084. Epub 2022 Nov 11. PMID: 36369742; PMCID: PMC9747552

Brief summary: This translational study showed an increased plasma expression of miR-210 in individuals with 46,XY DSD compared to the control population and also showed a positive association between the presence of atypical genitalia and plasma levels of miR-210 expression in individuals with 46,XY DSD. The findings of this study contribute to a novel perspective on the possible role of miRNAs in the development of the male external

genitalia and in the development of phenotypical variability in individuals with 46,XY DSD due to same genetic defect.

Atypical external genitalia is identified in approximately 1:4500 newborns. However, more than half of affected individuals, especially the ones with 46,XY DSD have an unknown genetic etiology and cannot be given an accurate diagnosis. Furthermore, phenotypical variability for the same allelic variant of one DSD gene in affected members of the same 46,XY DSD family is possible. These complex presentations may suggest yet unknown modulating factors regulating gene expression involved in 46,XY DSD phenotype.

MiRNAs, which are small noncoding RNAs, generally interact with the 3'UTR segment of mRNAs and subsequently inhibit protein expression. In this study, Elias FM *et al*. evaluated the plasma expression of miR-210 in 18 individuals with 46,XY DSD of unknown etiology compared to 36 healthy controls. MiRNA-210 was demonstrated to be differentially regulated in various male reproductive disorders. They found that plasma miR-210 expression was higher in 46,XY DSD individuals, and is positively correlated with higher EMS scores/severity of atypical genitalia independent of age, pubertal status, or hormone replacement therapy.

Although the results of this study reveal that there is increased miR-210 expression in 46, XY DSD, it is not clear with this data whether this might be a primary etiology or represent a secondary effect of abnormal male sex development.

Further investigation with a large number of 46,XY DSD individuals will be necessary to establish the role of miR-210 in the development of 46,XY DSD and variable phenotypes.

4.5. CDYL reinforces male gonadal sex determination through epigenetically repressing Wnt4 transcription in mice

Okashita N, Maeda R, Tachibana M

Proc Natl Acad Sci U S A. 2023 May 16;120(20):e2221499120.
doi: 10.1073/pnas.2221499120. Epub 2023 May 8. PMID: 37155872

Brief summary: This *in vivo* study identifies a new gene, CDYL (Chromodomain Y-like protein) in the epigenetic control of sex determination in mice.

Recent findings on mammalian sex development suggest that the fate decision of bipotential gonads depends not only on transcriptional activation of Y-linked testis-determining gene (SRY/Sry) but also on a delicate expression balance between the mutually antagonistic pro-testis and pro-ovarian genes. Particularly, epigenetic regulatory genes which are involved in the covalent modifications of DNA and histones in chromatin at the SRY locus emerged to reinforce or repress these pathways.

CDYL is a reader protein for repressive histone H3 methylation marks at the SRY locus. To test the role of CDYL in sex determination Okashita N, *et al*. introduced a mutant heterozygous allele of the Cdyl gene by CRISP-R Cas9 method into an Jmjd1a$^{\Delta/\Delta}$ XY sex reversal mice model which has ovotestis containing both SOX9+ cells and FOXL2+ cells. They found that Cdyl heterozygous mutation further enhanced the XY sex reversal phenotype of Jmjd1a-deficient mice. Importantly, Cdyl mutation led to a remarkable increase in the ratio of FOXL2+ cells in the gonads of XY Jmjd1a$^{\Delta/\Delta}$ mice. They also demonstrated XY sex reversal in a subpopulation of XY Cdyl$^{\Delta/\Delta}$ mice in the Jmjda wild-type background. These transgenic mice showed increased Wnt4 expression and decreased Sox9 expression in the gonadal somatic cells.

By mechanism, this study demonstrated that Cdyl deficiency leads to the failure of H3K27 methylation at the Wnt4 promoter, which induces pro-ovarian Wnt4 derepression and subsequent pro-testis Sox9 repression. This suggests that Cdyl represses Wnt4 transcription through tethering H3K27 methyltransferase to its promoter as a mechanism promoting male sex determination and repressing ovarian development, independent of Sry.

Overall, these data provide further insights into the epigenetic modification of sexual differentiation.

4.6. Formin-mediated nuclear actin at androgen receptors promotes transcription

Knerr J, Werner R, Schwan C, Wang H, Gebhardt P, Grötsch H, Caliebe A, Spielmann M, Holterhus PM, Grosse R, Hornig NC
Nature. 2023 May;617(7961):616–622.
doi: 10.1038/s41586-023-05981-1. Epub 2023 Mar 27. PMID: 36972684

Brief summary: This *ex vivo*/ *in vitro* study describes a novel regulatory mechanism of androgen receptor (AR) gene transcription by intranuclear actin assembly in droplets upon dihydrotestosterone (DHT) stimulation mediated by DAAM2 (Dishevelled-associated activator of morphogenesis 2) gene.

Androgen insensitivity syndrome (AIS) is a common etiology in individuals with 46, XY disorder/differences of sex development, AIS has diverse genital phenotypes. Complete androgen insensitivity syndrome (CAIS), is presented with XY sex reversal with normal female phenotype, whereas phenotype diversity is variable with residual androgen receptor activity, which leads to partial androgen insensitivity syndrome (PAIS). Various pathogenic variants within the androgen receptor (AR) gene on the X chromosome are the primary pathogenesis of AIS. The majority of clinically diagnosed CAIS can be explained by inactivating mutations in the AR, this is true for less than half of cases with the clinical diagnosis of PAIS. The AR mutation-negative group of AIS characterized by significantly lower AR activity in its human target tissues such as genital skin fibroblasts (GSFs), is named AIS type II. Knerr J *et al*. identified heterozygous variants in the DAAM2 gene in two unrelated individuals with AIS type II. They have studied the role of DAAM2 in the pathogenesis of AIS.

DAAM2 belongs to the formin family of cytoskeletal regulators which is involved in nucleation and polymerization of actin. In this study, Knerr J *et al*. showed that DAAM2 appear as small spots dispersed within the nuclear compartment which increase in both number and size following stimulation with DHT. DHT signalling drives actin-dependent colocalization of DAAM2, AR and active RNA Polymerase II. DAAM2 and AR colocalize to form droplet-like assemblies in both a signal- and actin polymerization-dependent manner which subsequently associate with active RNA Polymerase II to facilitate testosterone-stimulated gene expression. Overall this study demonstrates that DAAM2-mediated actin assembly drives AR clustering for transcriptionally active droplet formation in response to DHT.

This mechanism provides further insight for the pathogenesis of AIS and also highlight DAAM2 as a potential pharmacological target for AR-related diseases.

4.7. Genomic variants reducing expression of two endocytic receptors in 46,XY differences of sex development

Marko HL, Hornig NC, Betz RC, Holterhus PM, Altmüller J, Thiele H, Fabiano M, Schweikert HU, Braun D, Schweizer U
Hum Mutat. 2022 Mar;43(3):420–433.
doi: 10.1002/humu.24325. Epub 2022 Jan 15. PMID: 34979047

Brief summary: This translational study highlights the importance of 2 novel endocytic receptors that are involved in cellular androgen uptake and in the pathogenesis of androgen insensitivity syndrome (AIS) type II.

Steroids circulate in complex with plasma transfer proteins, and specific endocytic receptors can mediate cellular uptake of transfer protein/ligand complexes. Reduced intracellular hormone concentrations resulting from impaired hormone uptake might underlie reduced androgen-mediated gene expression. Therefore, some genetic defects which are associated with cellular androgen uptake may explain the etiology of DSD, especially in individuals with clinical AIS without an AR mutation (AIS Type II).

In this study, Marko HL *et al*., identified pathogenic variants in two genes encoding two endocytic receptors namely LDL receptor related protein 2 (LRP2) and limb development membrane protein 1 like (LMBR1L) genes, in individuals with AIS type II. Besides clinical PAIS phenotype, genital skin fibroblasts obtained from these individuals showed reduced cell membrane expression of these receptors and reduced AR activity.

The androgens circulate with sex hormone-binding globulin (SHBG) in plasma. In mice, LRP2 acts as an endocytic receptor for the SHBG/androgen complex to transmit the signal to the androgen receptor in the cytoplasm of specific hormone target cells. LRP2 is known to mediate testosterone-dependent testicular descend in mice which is similar in individuals with LRP2 mutations. This study showed that endocytosis of SHBG conjugated to a fluorescent dye is reduced in genital skin fibroblasts from 2 individuals with AIS type II carrying mutations in LRP2. Furthermore, mutations were identified in the lipocalin receptor gene, LMBR1L, in 3 patients with AIS type II. LMBR1L knockout cell line which were transiently transfected with expression vectors encoding LMBR1L wildtype as well as LMBR1L variants showed reduced cell surface expression of the receptor by 65–75%. Reduced androgen receptor activity related to LRP2 and LMBR1L mutations was shown by reduced androgen-responsive APOD gene mRNA expression in genital skin fibroblasts after incubation with dihydrotestosterone.

Although a few patients were described with these mutations, this study illustrates impaired cellular androgen uptake in the pathogenesis of androgen insensitivity syndrome type II. The molecular etiology in the majority of the patients with androgen insensitivity syndrome type II is yet to be identified.

Risk for Gonadal Malignancy and Gonadectomy in DSD

4.8. Consensus guide on prophylactic gonadectomy in different sex development

Guerrero-Fernández J, González-Peramato P, Rodríguez Estévez A, Alcázar Villar MJ, Audí Parera L, Azcona San Julián MC, Carcavilla Urquí A, Castaño González LA, Martos Tello JM, Mora Palma C, Moreno Macián MF, Yeste Fernández D, Nistal M
Endocrinol Diabetes Nutr (Engl Ed). 2022 Oct;69(8):629–645.
doi: 10.1016/j.endien.2022.10.002. Epub 2022 Nov 8. PMID: 36369235

Brief summary: This review article offers an update on knowledge on prophylactic gonadectomy in differences of sex development (DSDs) and evaluates the latest existing clinical evidence, which is generally limited, on risk of gonadal neoplasia potential in each group of DSDs.

On average 15% of DSD patients with Y chromosome material predispose to gonadal neoplasms especially gonadal germ cell tumors (GGCTs); the overall risk ranges between 0.8% and 40% depending on age and etiological diagnosis [1, 2]. However, the conditions without Y chromosome, and gonads displaying ovarian differentiation confer the lowest risk. The diagnosis of gonadal tumors is based on certain combined histopathological findings, and expression of certain immunohistochemistry markers of pluripotentiality. Prophylactic gonadectomy can only be proposed in DSD patients at risk of GGCTs. This risk, however, varies considerably depending on a multitude of factors that make the decision for prophylactic gonadectomy extremely difficult. In order to make informed recommendations on the convenience of this procedure in cases where there is potential for malignancy, this article evaluates the latest clinical evidence, which is generally low, and updates the existing knowledge in this field.

Two studies from last year provided further insight into gonadal neoplasia potential in various groups of DSDs. Costanzo M, *et al*. reported histological characteristics and immunoexpression patterns of gonadal parenchyma in 16 patients with SRY-negative 46,XX testicular and ovotesticular DSD at median age of 1.46 yrs (range 0.16–16 years) (3). 12 of these individuals had bilateral ovotestes, with normal ovarian tissue and some dysgenesis in the testicular components. They found significantly increased early prepubertal preinvasive and invasive malignancies in this cohort (five patients had undifferentiated gonadal tissue, five gonadoblastoma, and one dysgerminoma). This may suggest the need for assessment of gonadal histology in 46,XX testicular and ovotesticular DSD.

In a four-center study Peard LM, evaluated the incidence of germ cell neoplasia *in situ* (GCNIS) and germ cell tumor (GCT) in a large cohort of 83 patients with DSD, who undergone gonadectomy or gonadal biopsy [4]. Eight out of 83 patients (9.6%) had GCNIS or GCT at a median age of 2.95 yrs. Among those, 4 had mixed gonadal dysgenesis, 3 patients had Turner with Y chromosome material, 1 patient had partial gonadal dysgenesis. All of them were cured by surgery alone, none of them had a recurrence or tumor related

complications during the mean follow up duration of 6.4 yrs. The results of this study highlight the importance of gonadal surveillance of retained gonads based on risk stratification of DSD etiology for detection of malignant and premalignant gonadal pathologies. Nevertheless long-term oncologic outcomes for these patients are favourable.

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Inequities and Inadequacies in DSD Diagnosis and Care

4.9. Disorders of sex development: Challenges in a low-resource country

Ehua AM, Moulot MO, Agbara KS, Enache T, Bankole SR

Arch Pediatr. 2023 Jan;30(1):10–13.

doi: 10.1016/j.arcped.2022.09.002. Epub 2022 Nov 30. PMID: 36462990

Brief summary: In this retrospective clinical study Ehua AM *et al*., reports the diagnostic and management characteristics of 13 individuals whose karyotype could be studied out of 33 DSD patients, followed over 17 years by 2 pediatric surgery departments in Abidjan.

Serum testosterone could be measured only in 9 of 13 patients. Four patients were treated medically with topical androgen only. One patient had feminizing genitoplasty, and 2 had masculinizing genital surgeries. Six of the 13 patients were lost to follow-up. The average age of patients at the time of consultation was 7.95 years (range: 1 day–37 years). There was a karyotype-sex of rearing mismatch in 5 out of 13 patients in this cohort.

The incidence of DSD was reported much lower in majority of African countries. Reasons for lower incidence and later ages of presentation may include lack of registry system, lack of medical care access, and beliefs and taboos of families regarding malformations in general. The authors explain later diagnosis of DSD also due to lack of detailed examination in delivery room, lack of pediatric surgeons and urologists for referral. They report that the main determinant of male sex of rearing is the feeling at least one palpable gonad in the genital swelling. Current diagnostic algorithms including ultrasound or magnetic resonance imaging for the detection of Mullerian duct remnants, or analysis of hormonal levels, especially of the anti-Mullerian hormone, could not be performed in their cohort due to the lack of resources. Unavailability and high costs of hormone treatment and molecular tests, lack of multidisciplinary team and expert centers were the main limitations for etiological diagnosis and the management of DSDs.

In the contemporary world, it is critically important to provide access to comprehensive, expert, and multidisciplinary holistic care throughout the lifespan for individuals with DSD. A quality of life study may precede to determine the current conditions of children and adults with DSD among countries of different socioeconomic backgrounds.

To this end, the development of multicenter collaboration and standardization of hormonal and genetic evaluation, the training of caregivers, and the development of clear clinical management algorithms and protocols to improve care for patients with DSD living in countries with limited sources should be promoted.

4.10. Standards of Care for the Health of Transgender and Gender Diverse People, Version 8

Coleman E, Radix AE, Bouman WP, Brown GR, de Vries ALC, Deutsch MB, Ettner R, Fraser L, Goodman M, Green J, Hancock AB, Johnson TW, Karasic DH, Knudson GA, Leibowitz SF, Meyer-Bahlburg HFL, Monstrey SJ, Motmans J, Nahata L, Nieder TO, Reisner SL, Richards C, Schechter LS, Tangpricha V, Tishelman AC, Van Trotsenburg MAA, Winter S, Ducheny K, Adams NJ, Adrián TM, Allen LR, Azul D, Bagga H, Başar K, Bathory DS, Belinky JJ, Berg DR, Berli JU, Bluebond-Langner RO, Bouman MB, Bowers ML, Brassard PJ, Byrne J, Capitán L, Cargill CJ, Carswell JM, Chang SC, Chelvakumar G, Corneil T, Dalke KB, De Cuypere G, de Vries E, Den Heijer M, Devor AH, Dhejne C, D'Marco A, Edmiston EK, Edwards-Leeper L, Ehrbar R, Ehrensaft D, Eisfeld J, Elaut E, Erickson-Schroth L, Feldman JL, Fisher AD, Garcia MM, Gijs L, Green SE, Hall BP, Hardy TLD, Irwig MS, Jacobs LA, Janssen AC, Johnson K, Klink DT, Kreukels BPC, Kuper LE, Kvach EJ, Malouf MA, Massey R, Mazur T, McLachlan C, Morrison SD, Mosser SW, Neira PM, Nygren U, Oates JM, Obedin-Maliver J, Pagkalos G, Patton J, Phanuphak N, Rachlin K, Reed T, Rider GN, Ristori J, Robbins-Cherry S, Roberts SA, Rodriguez-Wallberg KA, Rosenthal SM, Sabir K, Safer JD, Scheim AI, Seal LJ, Sehoole TJ, Spencer K, St Amand C, Steensma TD, Strang JF, Taylor GB, Tilleman K, T'Sjoen GG, Vala LN, Van Mello NM, Veale JF, Vencill JA, Vincent B, Wesp LM, West MA, Arcelus J

Int J Transgend Health. 2022 Sep 6;23(Suppl 1):S1–S259.
doi: 10.1080/26895269.2022.2100644. PMID: 36238954; PMCID: PMC9553112

Brief summary: The World Professional Association for Transgender Health (WPATH) has issued an updated Standards of Care version 8 which contains important guidelines on how the assessment of gender variant children and adolescents should be considered, and by whom, and provides guidance around the consideration of young people for hormonal intervention.

This long and detailed document has had considerable input from many experts involved in transgender care and has incorporated what evidence is available, and when clinical experience is available, then the Delphi concensus approach was used and this process required 75% concordance to provide recommendations. There are now separate chapters dealing with children and adolescents. Key points for paediatric endocrinologists start with a recommendation to check that the mental health professionals making the diagnosis of gender incongruence are appropriately qualified to do so, and are appropriately experienced and licensed to practise in this field in their own country.

Further recommendations state that health care professionals assessing transgender and gender diverse adolescents should ensure the young people are only referred for gender-affirming medical treatments when they meet the diagnostic criteria of gender incongruence as per the WHO ICD-11 (or other appropriate diagnostic systems) and when the experience of gender incongruence is marked and sustained over time. The adolescent must demonstrate the emotional and cognitive maturity required to provide informed consent for treatment and also that their mental health concerns (if any) that could interfere with the diagnostic clarity and capacity to consent to gender-affirming medical treatments have been addressed. These recommendations emphasise assessment of maturity rather than using age-specific cut-offs. The document also emphasises the need for ongoing psychosocial support alongside endocrine treatments.

Young people must have been informed of the reproductive effects of hormonal interventions, including the potential loss of fertility and the available options to preserve fertility, and these must have been discussed in the context of the adolescent's stage of pubertal development. They must have reached Tanner stage 2 for pubertal suppression to be initiated. Although surgical referral is not common in paediatric and adolescent practice, the adolescent must have had at least 12 months of gender-affirming hormone therapy or longer, if required, to achieve the desired result for gender-affirming procedures, such as breast removal or augmentation, orchidectomy, vaginoplasty, hysterectomy, phalloplasty, metoidioplasty, and facial surgery as part of gender-affirming treatment.

4.11. Psychosocial functioning in transgender youth after 2 years of hormones

Chen D, Berona J, Chan YM, Ehrensaft D, Garofalo R, Hidalgo MA, Rosenthal SM, Tishelman AC, Olson-Kennedy J
N Engl J Med. 2023 Jan 19;388(3):240–250.
doi: 10.1056/NEJMoa2206297. PMID: 36652355; PMCID: PMC10081536

Brief summary: A four centre prospective longitudinal study of 315 transgender and non-binary adolescents in the USA showed improvement in gender congruence and appearance and some reduction of depression and anxiety (but not in birth-registered males) after 2 years of gender affirming hormone (GAH) treatment.

The race is on to provide clear evidence to support hormonal intervention for transgender adolescents. This longitudinal study employed a number of standard measures (Transgender Congruence Scale, the Beck Depression Inventory–II, the Revised Children's Manifest Anxiety Scale (Second Edition), and the Positive Affect and Life Satisfaction measures from the NIH (National Institutes of Health) Toolbox Emotion Battery. Although there was an overall increase in functional measures, this was not universal with an unexplained greater effect in those registered female at birth (60.3%). 24 subjects were in early puberty when GAH were commenced, and they showed a greater increase in life satisfaction.

In a similar but smaller study, Lavender *et al*. reported retrospectively on 38 transgender adolescents referred to endocrinology younger than 15 years who received GnRHa followed by GAH treatment (1). Measures included the Youth Self Report, the Body Image Scale, and the Utrecht Gender Dysphoria Scale, and caregivers completed the Child Behaviour Checklist and the Social Responsiveness Scale-2. Similar to the US study, dissatisfaction with primary sexual characteristics, gender dysphoria, and social motivation improved significantly over time. Self-harm and suicidality decreased. Caregivers reported a significant reduction in internalizing behaviours after GnRHa.

In another study, Morningstar *et al*. attempted to explore how GAH influence neurocognitive development by using functional MRI scanning to assess responses to verbal cues in 44 transgender boys with a mean age of 15.8 years, and encountered a direct effect of testosterone sensitising emotional responses to caregivers and a causing a quicker rise to anger to their peer challenges, these effects not being found in the control cohort (2).

Concerns are also raised on the negative potential effects of hormonal intervention on academic performance. Arnoldussen *et al*. performed IQ tests on 72 trans adolescents at mean of 12.8 years and compared this with their educational achievement at 20.4 years, all having received GnRHa and GAH (3). They found a positive association between IQ and education status with no discernible negative effect of treatment on academic performance.

All these papers, amongst others, are attempting to study the neuropsychological effects of hormonal intervention in transgender adolescents, but it is clear there is no one single assessment that can give answers to all the questions in this area.

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Changes to Growth Patterns

4.12. Just as tall on testosterone; a neutral to positive effect on adult height of GnRHa and testosterone in trans boys

Willemsen LA, Boogers LS, Wiepjes CM, Klink DT, van Trotsenburg ASP, den Heijer M, Hannema SE

J Clin Endocrinol Metab. 2023 Jan 17;108(2):414–421.
doi: 10.1210/clinem/dgac571. PMID: 36190924; PMCID: PMC9844962

Brief summary: This paper investigated the effect of pubertal suppression with GnRHa and gender affirming treatment with testosterone on the growth and final height of transgender boys. They found no negative effects, and even possibly a slight boost to adult height in comparison with target and predicted heights for birth-registered sex.

One of the concerning features of transgender males is being able to identify with their cisgender peers in stature. In the ESPE Yearbook 2022 we reported a paper from the same authors finding that the adult height of transgender girls in relation to their birth-registered sex was not affected by hormonal interventions (1). This study reports adult height outcomes in 146 transgender boys who received GnRHa then testosterone treatment. The younger ones (bone age below 14 years at start ($n = 61$) had an adult height SD score similar to the baseline and that adult height was greater than mid-parental height and the predicted adult height at the start of treatment. Further sub-analysis showed that those with a younger bone age when treatment was started had adult heights which were significantly above their predicted adult height. Not only are these findings reassuring, but they also provide insights into the control of growth in healthy adolescents and how hormonal manipulations can change the ultimate growth trajectory by manipulating puberty, in this case to achieve a desired endpoint.

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Effects of Hormone Intervention on the Immune System

4.13. Investigating sex differences in T regulatory cells from cisgender and transgender healthy individuals and patients with autoimmune inflammatory disease: a cross-sectional study

Robinson GA, Peng J, Peckham H, Butler G, Pineda-Torra I, Ciurtin C, Jury EC

Lancet Rheumatol. 2022 Aug 31;4(10):e710–e724.
doi: 10.1016/S2665-9913(22)00198-9. PMID: 36353692; PMCID: PMC9633330

Brief summary: This study further explores the sex differences in autoimmune regulation and the control of normal inflammatory responses, and also helps us understand why there are sex differences in the aetiology of autoimmune diseases such as systemic lupus erythematosus (SLE). It demonstrates the changes that occur in cisgender pubertal development on the T regulatory cell (Treg), B cell and monocyte population, and using samples from transgender adolescents undergoing GnRHa then GAH treatment, how certain regulatory genes appear to be under the control of both sex hormones and sex chromosomes following observations of significant changes in gene expression with transgender hormonal interventions.

The authors identified key sex differences in Treg phenotype and function between healthy individuals, which might explain differences in autoimmune disease susceptibilities and the response to infection. Specifically, they found that the global immune profile was altered by sex, with circulating Tregs more numerous and suppressive in young post-pubertal cisgender men compared with cisgender women. Tregs had a transcriptomic profile which differed between young cisgender men and cisgender women, and this was associated with increased secondary messenger signalling in cisgender men. Naturally secreted sex hormones pre-and post-puberty altered the Treg frequency and transcriptomic functional profile between cisgender men and women, and this finding was further explored in postpubertal transgender adolescents undergoing GAH therapy. The information reported in the study

contributes to the understanding of immunopathological mechanisms of sexually dimorphic autoimmune disease development and contributes to the basic understanding of immunology by sex and gender. As observed in cisgender men, transgender men had increased Treg frequencies following GnRHa treatment and early-stage gender-affirming testosterone administration. Sex differences in Treg frequencies in pre-pubertal children were similar to those seen post-puberty. Treg cells in young cisgender men were more suppressive than those in transgender men, cisgender and transgender women, suggesting that both sex hormones and sex chromosomes play a role in driving the increased suppressive function of Tregs noted in cisgender men. The study identified multiple novel genes that could be associated with Treg function by sex. T-responder (Tresp) cells could also be important for the inflammatory balance between cisgender men and women, but this would require additional phenotype and functional analyses. The increased suppressive capacity of Treg cells in cisgender men could also be due to increased proliferation of Tresp cells in cisgender women. The information gathered helps to begin to understand the mechanistic pathogenesis of autoimmune disease and the bias towards cisgender women.

Longer Term Outcomes

4.14. Continuation of gender affirming hormones in transgender people starting puberty suppression in adolescence: a cohort study in the Netherlands

van der Loos MATC, Hannema SE, Klink DT, den Heijer M, Wiepjes CM

Lancet Child Adolesc Health. 2022 Dec;6(12):869–875.

doi: 10.1016/S2352-4642(22)00254-1. Epub 2022 Oct 21. PMID: 36273487

Brief summary: Concerns exist that transgender adolescents opting for gender affirming treatments may subsequently regret their decision. This large study from Amsterdam provides information that in early adulthood, 98% of their cohort continued to access these treatments.

The Amsterdam Cohort of Gender Dysphoria is one of the largest ongoing studies on the long-term outcomes of gender affirming hormone treatments (GAH). The authors presented outcomes at a mean age of 20.2 years on 720 transadolescents (69% birth-registered female) starting GnRHa at 14.1 yr in the transgirls and 16.0 yrs in the transboys. All subsequently moved on to GAH. The authors report a very high proportion continuing to access GAH as adults. The continued GAH use was not associated with age at first clinic visit, nor was age at start of GnRHa treatment, age at start of GAH treatment, nor puberty stage at start of GnRHa treatment, nor whether gonadectomy had been performed.

Butler *et al*. reported the status of access to treatment and transgender identification in 1089 young adults at the time of discharge from child and adolescent services in England (1). 92% continued to identify as transgender or gender variant, 87% seeking ongoing care through National Health Service adult gender identity clinics. 2.9% ceased identifying as transgender after an initial consultation prior to any endocrine intervention and 5.3% had stopped treatment either with GnRHa or GAH, a higher proportion in those starting interventions under 16 years of age compared with those over 16 years. Using a different approach, Roberts *et al*. examined medical records from the US Military Healthcare System and found that at a mean age of 19.2 years, the 4 year GAH continuation rate in 627 trans males and 325 trans females was 70%, but with a higher continuation rate in those initially starting treatment under 18 years (74%) compared with adult starts (64%), a finding in contrast to that of the UK study (2). An additional retrospective report from the US Military Healthcare System of 434 transadolescents, mean age 15.4 years, found no increased use of GAH in those initially starting with GnRHa interventions (3).

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5. Puberty

Caroline Gernay[1], Sara Moline[2], Anne-Simone Parent[1,2]

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Introduction

This year, cohort studies have brought new insights regarding prepubertal and pubertal markers of future gonadal function and illustrated further the influence of physical activity on puberty timing. Basic studies have identified new candidate genes for congenital hypogonadotropic hypogonadism such as jagged-1 and NOS1. They also exposed new data illustrating the role of MKRN3 as a factor involved in progressive plastic changes rather than a sudden switch of puberty and GnRH activity. Some of the most fascinating studies this year discovered new roles for GnRH and its regulator nitric oxide in higher functions such as cognition, opening new perspectives for GnRH treatment and new questions regarding GnRH agonist treatment.

Clinical Guidance and Studies

5.1. Leuprolide and triptorelin treatment in children with idiopathic central precocious puberty: an efficacy/tolerability comparison study

Valenzise M, Nasso C, Scarfone A, Rottura M, Cafarella G, Pallio G, Visalli G, Di Prima E, Nasso E, Squadrito V, Wasniewska M, Irrera P, Arcoraci V, Squadrito F

Front Pediatr. 2023;11:1170025.

PMID: 37266535.

https://www.frontiersin.org/articles/10.3389/fped.2023.1170025/full

Brief summary: This retrospective study compared the efficacy and tolerability profiles of leuprolide and triptorelin in patients with central precocious puberty and did not find any significant difference between the two drugs.

Treatment with GnRH analogues represents the standard of care for central precocious puberty (CPP) in order to preserve adult height potential[1]. Leuprolide and Triptorelin are the most prescribed drugs (1,2). They are generally considered to be safe (3) despite some mild side effects (4). This study retrospectively analyzed the treatment of 110 girls affected with CPP; 48 received Leuprolide and 62 received Triptorelin. Height at the end of treatment (P-value $=0.3$), gain in predicted adult height and number of observed side effects did not differ between treatment. 41.8% of patients reported at least on side effect. The most frequent were pain at injection site, headache, appetite increase and mood swings, consistently with previous studies (5). A novel aspect of this study is the focus on factors that could be related to the onset of side effects. A negative correlation was found between the risk of side effects and bone age at treatment initiation (P-value $=0.038$): the probability of side effects appearance significantly reduced with the increase of the initial bone age. Other parameters (duration of treatment, weight, height, chronological age, hormonal levels and radiological pelvic parameters) did not correlate with the risk of side effects.

In conclusion, Leuprolide and Triptorelin treatment appear to be effective and safe, without significant difference between the 2 drugs.

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5.2. Timing of puberty in relation to semen characteristics, testicular volume, and reproductive hormones: a cohort study

Brix N, Gaml-Sorensen A, Ernst A, Arendt LH, Harrits Lunddorf LL, Toft G, Tottenborg SS, Haervig KK, Hoyer BB, Hougaard KS, Bonde JPE, Ramlau-Hansen CH

Fertil Steril. 2023; S0015-0282(23)00540-X.

PMID: 37257718.

https://www.fertstert.org/article/S0015-0282(23)00540-X/fulltext

Brief summary: This cohort study explored the potential link between self-reported age at puberty and markers of male fecundity.

In this article, the authors explored pubertal timing as a determinant of male fecundity. This cohort study was based on the Danish National Birth Cohort which consisted of around 100 000 mother-child pairs recruited during 1996–2002. A little more than 1000 men aged 19 were evaluated for semen volume, sperm concentration, total sperm count, sperm motility, percentage of morphologically normal spermatozoa, testicular volume and reproductive hormones according to self-reported timing of pubertal development.

Older age at voice break was associated with higher FSH and lower testosterone. Later puberty also tended to be associated, although not significantly, with slightly lower total sperm count, poorer motility, and lower testicular volume. These results are consistent with previous smaller studies reporting that men completing their puberty later tended to have lower sperm count, sperm concentration and testosterone levels (1–3). Although a recruitment bias might exist, it is unlikely to be major as 19-year-old men are usually not aware of their fecundity status. Stronger effects might have been identified if men had been evaluated later in life. As stated by the authors, these results lend weak support to the hypothesis that older age at pubertal development is associated with markers of reduced male fecundity, especially in reproductive hormones. The mechanisms through which later puberty would be associated to potentially impaired Sertoli cell function remain elusive.

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5.3. Pre-pubertal accelerometer-assessed physical activity and timing of puberty in British boys and girls: the Millennium Cohort Study

Cheng TS, Brage S, van Sluijs EMF, Ong KK

Int J Epidemiol. 2023, dyad063.

PMID: 37208864.

https://academic.oup.com/ije/advance-article/doi/10.1093/ije/dyad063/7174316?login=false

Brief summary: This longitudinal UK cohort study examined the prospective associations between objectively measured physical activity, independently of BMI, and pubertal timing in boys and girls.

This large study analyzed device-measured physical activity and its intensity in relation to puberty timing, using accelerometers worn on the right hip. A previous meta-analysis reported that female athletes had later age at menarche than non-athletes (1), but it was unclear whether these effects were mediated by BMI and whether total physical activity or any particular intensity influence puberty timing.

This study included 5610 children, comprising 2531 boys and 3079 girls. Higher total daily activity counts at age 7 years were associated with lower risks for earlier skin changes and voice breaking in boys and lower risks for earlier growth spurt, body hair growth, skin changes and menarche in girls. All observed associations were independent of baseline BMI at age 7 years and persisted after further adjustment for BMI at 11 years. Beyond total movement volume, no specific intensity of physical activity was associated with puberty timing.

These findings substantially extend existing evidence (2,3) regarding the potential role of physical activity on pubertal timing by analyzing robust and comprehensive data. The authors suggest that promoting overall physical activity may be a potential strategy to avoid early puberty timing, especially in girls.

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5.4. Reproductive markers of testicular function and size during puberty in boys with and without a history of cryptorchidism

Rodprasert W, Koskenniemi JJ, Virtanen HE, Sadov S, Perheentupa A, Ollila H, Albrethsen J, Andersson A-M, Juul A, Skakkebaek NE, Main KM, Toppari J

J Clin Endocrinol Metab. 2022;107(12):3353–3361.

PMID: 36073163.

https://academic.oup.com/jcem/article/107/12/3353/6693919

Brief summary: This prospective longitudinal study compared serum reproductive hormone levels and testicular volume around the onset of puberty between cryptorchid and healthy boys.

Congenital cryptorchidism is the most common congenital anomaly among newborn boys, and still little is known about pubertal testicular function and serum reproductive hormone levels during puberty in this population.

Pubertal follow-up with clinical examination every 6 months from the age of 8.5 years was performed in a cohort of 109 patients divided in five groups: bilateral cryptorchidism with orchidopexy (OpBC, $n=9$), bilateral cryptorchidism with spontaneous testicular descent (SpBC, $n=7$), unilateral cryptorchidism with orchidopexy (OpUC, $n=15$), unilateral cryptorchidism with spontaneous testicular descent (SpUC, $n=15$) and controls ($n=63$). Serum samples were collected at the first visit and then every 6 months for hormone measurements (FSH, LH, total testosterone, inhibin B, SHBG, estradiol, IGF1, IGFBP-3).

Boys with bilateral cryptorchidism had higher FSH and lower inhibin B levels indicating Sertoli cell dysfunction (1). In boys with unilateral cryptorchidism, only FSH level were higher after the onset of puberty. Testosterone and LH levels were similar between groups, suggesting preserved Leydig cell function during puberty. IGF1 and IGFBP-3 levels were similar between groups.

Testicular volume was lower only in cryptorchid boys who underwent surgery, suggesting lower germ cell numbers in these boys compared to those who had spontaneous testicular descent. Lack of spontaneous testicular descent and therefore need for intervention may be a sign of testicular dysgenesis, explaining the decreased testicular volume. However, the longer time spent in an undescended position could also contribute to the progressive loss of germ cells and Sertoli cells (2).

In conclusion, cryptorchid boys, particularly after bilateral orchidopexy, showed lower levels of Sertoli and germ cell markers, whereas Leydig cell function seemed to be well-preserved during puberty.

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5.5. AMH concentrations in infancy and mid-childhood predict ovarian activity in adolescence: a long-term longitudinal study of healthy girls

Hagen CP, Fischer MB, Wohlfahrt-Veje C, Assens M, Busch AS, Pedersen AT, Juul A, Main KM

EClinicalMedicine. 2022; 55:101742.

PMID: 36386030.

https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(22)00471-0/fulltext

Brief summary: This long-term longitudinal study of 437 Danish girls shows that AMH level measured in infancy is a useful tool to predict future ovarian activity.

Anti-Müllerian hormone (AMH) is produced by granulosa cells in small ovarian follicles and thus reflects the ovarian reserve of resting primordial follicles (1). High AMH concentrations are observed in women with polycystic ovarian syndrome (PCOS) (2), while low age-specific AMH could be associated with an increased risk of early menopause (3), although longitudinal studies are needed to confirm this hypothesis.

The first aim of this study was to evaluate the potential correlation between AMH concentrations in infancy (median age 0.3 yrs) and mid-childhood (7.2 yrs) and concentrations in puberty (11.3 yrs) and adolescence (15.9 yrs). Participants needed at least two AMH measurements to be included in the study ($n = 437$). Some of these girls were also assessed for reproductive hormones and underwent transabdominal ultrasound (TAUS) and magnetic resonance imaging (MRI) of the ovaries at puberty (TAUS $n = 83$ and MRI $n = 78$) and adolescence (TAUS, $n = 137$) to evaluate ovarian morphology.

Each girl maintained her relative AMH concentration over time. AMH serum concentrations in mid-childhood predicted the number of ovarian follicles during puberty and adolescence.

Because AMH levels in childhood could predict hypergonadotropic hypogonadism at time of expected puberty (4), the authors propose to monitor early AMH levels in patients at risk of premature ovarian insufficiency to offer early fertility preservation strategies. Another potential clinical application relies on the demonstration that AMH in early childhood was associated with adolescent hormone concentrations, ovarian morphology and menstrual cycle patterns resembling PCOS in adulthood.

In conclusion, this article documents a strong correlation between AMH concentrations in infancy/mid-childhood and concentrations in puberty/adolescence. In mid-childhood, AMH concentrations also reflected the number of ovarian follicles in puberty and adolescence, and could therefore be used as a clinical tool to predict future ovarian activity.

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5.6. Neuroimaging in 205 consecutive children diagnosed with central precocious puberty in Denmark

Hansen AB, Renault CH, Wøjdemann D, Gideon P, Juul A, Jensen RB

Pediatr Res. 2023; 93(1):125–130.

PMID: 35365758.

https://pubmed.ncbi.nlm.nih.gov/35365758/

Brief summary: This Danish single-center retrospective study evaluated the occurrence of pathological findings on neuroimaging among children diagnosed with central precocious puberty (CPP).

CPP incidence has been increasing over the last four decades (1) and is more prevalent in females than in males. Most cases are idiopathic (2) but brain magnetic resonance imaging (MRI) is routinely performed in order to exclude rare pathological causes.

This study evaluated 205 children (176 girls, 29 boys) with clinical and biological signs of CPP over a period of 10 years. On their brain MRI, 6 patients, 3/164 (1.9%) girls and 3/24 (12.5%) boys, had a newly diagnosed intracranial pathology. Most of these findings were hypothalamic hamartomas (4/6). All 6 of these patients had pubertal onset before 6.1 years of age, significantly younger than children with idiopathic CPP.

These findings are in concordance with previous studies (3,4), which reported tendencies towards a lower age at pubertal onset in patients with newly found intracranial pathologies causing CPP in both sexes. The authors cite a meta-analysis that included 1853 patients and reporting a low incidence (1.6%) of tumors that required any intervention. Based on their observation and the cited meta-analysis, the investigators suggest to lower the age threshold for performing a routine brain MRI for CPP to 7 years of age in girls (except in patients with rapidly progressing puberty or presence of neurological symptoms), but suggest to continue to perform a routine MRI in all boys who present with CPP.

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5.7. An increasing tendency of precocious puberty among Korean children from the perspective of COVID-19 pandemic effect

Choi KH, Park SC

Front Pediatr. 2022 Aug 25;10:968511.

PMID: 36090560.

https://www.frontiersin.org/articles/10.3389/fped.2022.968511/full

Brief summary: This study investigated the evolution of precocious puberty incidence in Korea between 2016 and 2021. A rapid increase was observed from April 2020, which the authors link to the restrictions due to the COVID-19 pandemic.

The onset of secondary sex characteristics has shown a secular trend towards earlier ages for many decades (1). This has been attributed to socioeconomic and environmental changes. Like many other countries, a steady increase in the incidence of precocious puberty has been well described in Korea and other countries (2-4).

In this study, the numbers of patients diagnosed with precocious puberty in medical facilities in Korea between 2016 and 2021 was analyzed using the Healthcare Big Data Hub, as well as the status of insurance treatment costs. Two new trends were discovered. First, the incidence of precocious puberty increased much more rapidly between 2020 and 2021 (1.2-fold increase between January 2016 and March 2020, versus 1.68-fold increase between January 2016 and March 2021). The monthly analysis pinpoints to a rapid increase specifically from April 2020 until June 2021. This period corresponds to the start of the COVID-19 pandemic. The authors suggest several causes for this phenomenon. The increase in BMI linked to a sedentary lifestyle and the high-calorie food consumption could play a key role in this trend. Excessive use of electronic devices could also interfere with melatonin production, which in turn can affect the hypothalamic-pituitary-gonadal axis (5).

Finally, other factors such as exposure to endocrine disruptors, anxiety, change in sleep patterns and direct stimulation of the central nervous system due to a COVID-19 infection are also cited as potential causes.

The second trend detected here is the increasing proportion of males diagnosed with precocious puberty. This is attributed to greater parental awareness in addition to environmental factors. Indeed, the increase in the proportion of male patients diagnosed before 9 years of age and the increase in the total number of male patients occurred simultaneously, which means, the prevalence rate increased as a result of the growing awareness of precocious puberty in males. In addition, the sudden increase in male patients coincided with the period of COVID-19, likely due to restriction of physical activity and increased BMI in previously active males.

In conclusion, this large study shows a rapid increase in the number of Korean patients diagnosed with precocious puberty since April 2020, as a potential consequence of the COVID-19 pandemic, in accordance with other smaller studies (6–8). Further investigations are required to determine the possible causes of this increasing prevalence of precocious puberty and to monitor whether the rapid rise will reverse.

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5.8. GnRH replacement rescues cognition in Down syndrome

Manfredi-Lozano M, Leysen V, Adamo M, Paiva I, Rovera R, Pignat JM, Timzoura FE, Candlish M, Eddarkaoui S, Malone SA, Silva MSB, Trova S, Imbernon M, Decoster L, Cotellessa L, Tena-Sempere M, Claret M, Paoloni-Giacobino A, Plassard D, Paccou E, Vionnet N, Acierno J, Maceski AM, Lutti A, Pfrieger F, Rasika S, Santoni F, Boehm U, Ciofi P, Buée L, Haddjeri N, Boutillier AL, Kuhle J, Messina A, Draganski B, Giacobini P, Pitteloud N, Prevot V

Science. 2022;377(6610):eabq4515.

PMID: 36048943.

https://www.science.org/doi/10.1126/science.abq4515?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed

Brief summary: This study identified a new role for GnRH in higher brain function using a rodent model of Down Syndrome. It reports for the first time an improvement of cognitive functions in patients with Down Syndrome treated with pulsatile GnRH.

GnRH neurons are classically described as a population of neurons located in the hypothalamus and responsible for the activation and regulation of the hypothalamic-pituitary-gonadal axis. However, the recent description of GnRH and its receptor in extrahypothalamic areas, such as the cortex and the hippocampus, has opened new avenues regarding its potential role in higher brain functions (1–3).

Patients with Down syndrome do not appear to differ from the general population in terms of puberty timing (4, 5). However, affected boys often present with gonadal insufficiency starting from infancy/puberty and being fully apparent in adulthood (4). These authors showed that the olfactory and cognitive deficits observed in a rodent model of Down syndrome (Ts65Dn mice) parallel a post-pubertal decrease in hypothalamic and extra-hypothalamic expression of GnRH. Indeed, conventional immunofluorescence and iDISCO showed a profound loss of both hypothalamic and extra-hypothalamic GnRH somata and fibers after puberty onset in Ts65Dn mice compared to controls. This was associated with an imbalance in a microRNA-gene network known to regulate

GnRH neuron maturation. Hypothalamic GnRH compensation using epigenetic, chemogenetic and pharmacological interventions abolished olfactory and cognitive defects in Ts65Dn mice. Pulsatile administration of GnRH improved cognitive function in Ts65Dn mice. Finally, pulsatile GnRH therapy in adult men with Down syndrome increased cognitive performance in 6 out of 7 cases.

This study identifies a new and unsuspected role for GnRH in cognitive function and opens new possibilities toward the therapeutic use of GnRH in people with Down syndrome. Surprisingly, pulsatile GnRH appears necessary for its role in cognitive function.

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Basic Research

5.9. MKRN3 inhibits puberty onset via interaction with IGF2BP1 and regulation of hypothalamic plasticity

Naulé L, Mancini A, Pereira SA, Gassaway BM, Lydeard JR, Magnotto JC, Kim HK, Liang J, Matos C, Gygi SP, Merkle FT, Carroll RS, Abreu AP, Kaiser UB

JCI Insight. 2023;8(8):e164178.

PMID: 37092553.

Brief summary: Using human induced pluripotent stem cells as well as transgenic mouse models, this paper shows that MKRN3 could initiate pubertal onset by regulating hypothalamic development and plasticity.

The reactivation of GnRH secretion at puberty is thought to result from a loss in inhibitory input together with an increase in transactivation onto GnRH neurons. The main component of this inhibitory tone was incompletely understood until the discovery of Makorin ring finger protein 3 (MKRN3) loss-of-function mutations in patients with familial central precocious puberty (CPP). Such mutations are the most common cause of familial CPP (1, 2). Recent studies have started to decipher the role of MKRN3 in controlling pubertal timing.

MKRN3 is a ubiquitin ligase expressed in the mediobasal hypothalamus and the preoptic area. Its expression is high during embryonic life and sharply decreases before the onset of puberty (3). MKRN3 is expressed specifically in kisspeptin neurons and regulates *KISS1* and *TAC3* promoter activity. Overall, these data suggest that MKRN3 acts upstream of GnRH, at the level of kisspeptin and neurokinin B neurons to inhibit GnRH secretion before puberty.

These authors used human induced pluripotent stem cells differentiated into arcuate nucleus neurons to explore the mechanisms of action of MKRN3 in the hypothalamic control of puberty. MKRN3 was not expressed in iPSCs but became highly induced during hypothalamic differentiation. MKRN3 deletion in hypothalamic neurons resulted in significant changes in the expression of genes involved in axon guidance and synapse formation, suggesting a role in hypothalamic development and plasticity. Consistent with those results, MKRN3 deletion in a mouse model led to an increase in dendritic spines in the arcuate nucleus, but did not affect GnRH neuron morphology. MKRN3 deletion was associated with earlier first estrus in females and a milder phenotype of advanced puberty in males. Overall, the CPP phenotype was more moderate than in the previously published knock-out model obtained using TALEN technology (4). Mkrn3 deletion did not affect *GnRH1*, *kiss1* or *tac3* mRNA expression, but decreased neurokinin B protein expression, suggesting a post-transcriptional

mechanism involved in the activation of GnRH secretion around puberty. The study also identified a potential new player in the control of puberty, as MKRN3 interacted with IGF2BP1 and decreased its protein expression in the arcuate nucleus. IGF2BP1 is known to be involved in mRNA translation.

In conclusion, this article brings significant new data supporting a potential role for MKRN3 in hypothalamic development.

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5.10. Hypothalamic overexpression of makorin ring finger protein 3 results in delayed puberty in female mice

Roberts SA, Naulé L, Chouman S, Johnson T, Johnson M, Carroll RS, Navarro VM, Kaiser UB

Endocrinology 2022 Nov; 163(11): bqac 132.

doi: 10.1210/endocr/bqac132.

PMID: 35674456.

https://academic.oup.com/endo/article-abstract/163/11/bqac132/6668856?redirectedFrom=fulltext

Brief summary: This article shows that intra-cerebroventricular injections of a recombinant adeno-associated virus expressing MKRN3 resulted in delayed puberty in female mice.

MKRN3 loss-of-function mutation is the most frequent cause of familial central precocious puberty (CPP) (1).

These authors investigated the effect of MKRN3 overexpression on pubertal timing, by generating a mouse model of neonatal hypothalamic Mkrn3 overexpression. Furthermore, they collected tissues from the mediobasal hypothalamus (MBH) to measure expression of genes encoding kisspeptin, neurokinin B, dynorphin, which are other known regulators of pubertal initiation (2). Arcuate nucleus neurokinin B and kisspeptin protein levels were assessed in order to explore the potential mechanisms of action of Mkrn3.

Overexpression of Mkrn3 in female mice resulted in delayed vaginal opening and first estrus, compared with controls, but with subsequent normal fertility. Interestingly, there was no change in pubertal timing in male mice, using preputial separation as the marker of pubertal onset. This sexual dimorphism in the effect of MKRN3 deletion has also been observed in humans (3).

Mkrn3 overexpression resulted in reduced hypothalamic kisspeptin and neurokinin B protein levels, but increased Kiss1, Tac2, and Pdyn mRNA levels in the MBH in females at postnatal day 28. These data suggests that MKRN3 may be involved in protein degradation of the KNDy neuron neuropeptides, thereby resulting in a compensatory increase in their mRNA levels. The exact mechanism of this interaction requires further study.

In conclusion, the authors demonstrated that hypothalamic overexpression of Mkrn3 delays pubertal onset in female mice, by a potential interaction between MKRN3 and Kiss1 neurons. *MKRN3* gain of function could therefore be a candidate explanation for delayed puberty.

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5.11. Dicer ablation in Kiss1 neurons impairs puberty and fertility preferentially in female mice

Roa J, Ruiz-Cruz M, Ruiz-Pino F, Onieva R, Vazquez MJ, Sanchez-Tapia MJ, Ruiz-Rodriguez JM, Sobrino V, Barroso A, Heras V, Velasco I, Perdices-Lopez C, Ohlsson C, Avendaño MS, Prevot V, Poutanen M, Pinilla L, Gaytan F, Tena-Sempere M

Nat Commun. 2022;13(1):4663.
PMID: 35945211.
https://www.nature.com/articles/s41467-022-32347-4

Brief summary: A newly developed mouse model of congenital ablation of Dicer in kisspeptin neurons was used to identify a role for miRNAs in kisspeptin neuron activity and control of reproduction.

The last few years have seen a shift in paradigm with the discovery of epigenetic mechanisms regulating GnRH neuron activity and thus puberty and reproduction. In particular, miRNAs appear to play a crucial role in the maturation and function of the hypothalamic-pituitary-gonadal axis. While MKRN3 (1) and GnRH (2) expression are known to be regulated by miRNA, such regulation of Kisspeptin had not been explored.

DICER is an RNAse enzyme, which is necessary for the maturation of all miRNAs and the formation of the RNA-induced silencing complex (3). In order to study miRNA regulatory pathways in kisspeptin neurons, the authors developed a mouse model in which Dicer was selectively ablated in kiss1 neurons (KiDKO mice). Such congenital ablation led to hypogonadotropic hypogonadism in adult male and female mice. Adult KiDKO males and females showed undetectable levels of Kiss1 expression in the arcuate nucleus and KO females also showed no detectable levels in the AVPV. KiDKO mice showed a significant LH response to GnRH and kisspeptin, indicating that the functional defect was in kisspeptin neurons. Interestingly, KiDKO males and females showed normal puberty timing as well as normal gonadotropin and sex steroid levels prepubertally. Males were fertile at 2 months of age but not at 4 months, while females did not start ovulation and were infertile at 2 and 4 months of age. As miRNA are mostly gene silencers, it appears that miRNAs probably decreased the expression of Kiss1 repressors, as hinted by increased expression of *mkrn3*, *eap1* and *Cbx7* in isolated kisspeptin neurons from KiDKO mice. Notably, Kiss1 expression was largely conserved around minipuberty, which suggests that conservation of kisspeptin neuronal populations during minipuberty is sufficient to activate puberty.

In conclusion, the newly developed model of congenital ablation of Dicer illustrates the essential role of miRNA in Kisspeptin neurons for the completion of puberty and maintenance of fertility in both sexes. In contrast, miRNAs in Kisspeptin neurons are dispensable for kisspeptin neuron survival and pubertal onset. These data suggest that abnormal miRNA biogenesis could explain some forms of late onset hypogonadotropic hypogonadism.

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5.12. Defective jagged-1 signaling affects GnRH development and contributes to congenital hypogonadotropic hypogonadism

Cotellessa L, Marelli F, Duminuco P, Adamo M, Papadakis GE, Bartoloni L, Sato N, Lang-Muritano M, Troendle A, Dhillo WS, Morelli A, Guarnieri G, Pitteloud N, Persani L, Bonomi M, Giacobini P, Vezzoli V

JCI Insight. 2023;8(5):e161998.
PMID: 36729644.
https://insight.jci.org/articles/view/161998

Brief summary: Using a combination of expression studies in human embryos as well as functional studies in zebrafish and genetic sequencing of patient with congenital hypogonadotropic hypogonadism, this study identified a novel role for Jag1/Notch signaling in the development of GnRH neurons.

GnRH neurons have a unique characteristic as they start life in the olfactory placode and then migrate into the hypothalamus during embryonic development, thanks to a complex network of factors involved in cell adhesion, cell migration and neurite outgrowth (1). It is now well established that defects in GnRH neuron migration cause congenital hypogonadotropic hypogonadism (2).

Notch1 is a signaling molecule which interacts with Notch ligands, such as Jagged-1 on adjacent cells (3). Notch1 was recently described to have a role in the development of the vomeronasal organ, where GnRH neurons originate (4).

These authors show that genetic invalidation of the zebrafish ortholog of *JAG1* (*jag1b*) resulted in altered GnRH migration and olfactory axonal projections to the olfactory bulb. Pharmacological invalidation of the Notch signaling pathway impaired the motility of a murine immortalized GnRH cell line and of human fetal GnRH-secreting neuroblasts. In addition, the authors identified 9 heterozygous *JAG1* mutations among 467 patients with congenital hypogonadotropic hypogonadism.

In conclusion, this study identified a role of Jag1/Notch signaling in the development of GnRH neurons and as new cause of congenital hypogonadotropic hypogonadism.

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5.13. NOS1 mutations cause hypogonadotropic hypogonadism with sensory and cognitive deficits that can be reversed in infantile mice

Chachlaki K, Messina A, Delli V, Leysen V, Maurnyi C, Huber C, Ternier G, Skrapits K, Papadakis G, Shruti S, Kapanidou M, Cheng X, Acierno J, Rademaker J, Rasika S, Quinton R, Niedziela M, L'Allemand D, Pignatelli D, Dirlewander M, Lang-Muritano M, Kempf P, Catteau-Jonard S, Niederländer NJ, Ciofi P, Tena-Sempere M, Garthwaite J, Storme L, Avan P, Hrabovszky E, Carleton A, Santoni F, Giacobini P, Pitteloud N, Prevot V

Sci Transl Med. 2022;14(665):eabh2369.
PMID: 36197968.
https://www.science.org/doi/10.1126/scitranslmed.abh2369?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed

Brief summary: This study identified nitric oxide synthase 1 (NOS1) heterozygous missense variants in 6 patients with hypogonadotropic hypogonadism. Altered minipuberty and puberty as well as cognitive impairment were observed in NOS1 deficient mice.

Nitric oxide (NO) is produced under the control of NO synthase in hypothalamic neurons. NO plays a crucial role in regulating gonadotropin-releasing hormone (GnRH) secretion, acting as a strong inhibitory signal which integrates both metabolic and gonadal information (1). *Nos1 (NO synthase 1*) deficient mice exhibit infertility, which suggests that *NOS1* loss-of-function mutations could lead to GnRH deficiency and infertility due to congenital hypogonadotropic hypogonadism (CHH).

This study identified 6 ultra-rare *NOS1* missense variants among a cohort of 341 patients with CHH. All affected patients had absent puberty, suggesting severe GnRH deficiency. *In vitro* characterization of the mutants confirmed they resulted in *NOS1* loss-of-function.

Immunohistology showed that NOS1 is expressed in some migrating GnRH neurons in humans. In adults, GnRH neurons interacted morphologically with NOS1 neurons but did not themselves express NOS1. Some kisspeptin neurons expressed NOS1.

NOS1 deficient mice showed increased firing of GnRH neurons around minipuberty together with increased LH and FSH levels and further showed delayed puberty. They also showed impaired hearing, olfaction and cognition, which is consistent with the hearing loss, anosmia and cognitive difficulties reported in patients with *NOS1* mutations. Notably, both reproductive and cognitive phenotypes were corrected by inhaled NO treatment in mice.

In conclusion, this very elegant study shows that Nos1 activity shapes minipuberty and puberty, and also plays a role in cognition. Notably, it also suggests a potential therapeutic application for inhaled NO.

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5.14. New horizons: Gonadotropin-releasing hormone and cognition

Prévot V, Tena-Sempere M, Pitteloud N
J Clin Endocrinol Metab. 2023 Jun 1:dgad319.
doi: 10.1210/clinem/dgad319. Epub ahead of print.
PMID: 37261390.
https://academic.oup.com/jcem/advance-article-abstract/doi/10.1210/clinem/dgad319/7187944?redirectedFrom=fulltext&login=false

Brief summary: This literature review summarized the recent advances in our understanding of the GnRH system throughout life and its implication in the brain development beyond its conventional role in reproductive control.

During embryonic development GnRH neurons migrate from the olfactory placode to the hypothalamus, where they control the hypothalamus–pituitary–gonadal axis (1,2). But many GnRH neurons migrate to other brain regions, such as the cortex and the hippocampus (1), and exert possible non-reproductive functions.

These authors analysis of reported data on rodent studies resulted in 3 theories:

1) Minipuberty is potentially important for brain development and the maintenance of sensory and cognitive function throughout life (3).

2) The pulsatile pattern of GnRH secretion is potentially important for olfactory perception and cognition (4).

3) GnRH regulates postnatal myelination in the neocortex and may influence adult brain structure and function (4).

Taken together, these findings raise questions about potential long-term side effects of (non-pulsatile) depot GnRH agonist therapy for precocious puberty or transgender children and adults, as well as the effects of downstream hormone replacement to treat congenital hypogonadotropic hypogonadism. It also opens new avenues regarding the potential use of pulsatile GnRH administration to prevent aging-related cognitive decline and learning abilities.

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6. Adrenals

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For this year's chapter on 'Adrenals', we have searched the PubMed for articles on 'adrenal' or 'steroidogenesis' published in English between June 1, 2022 and May 31, 2023. Our search yielded more than 6,000 citations. We have examined all citations individually and selected the following collection of basic research and clinical articles. Whenever possible, we have avoided topics that have been discussed in the Yearbook 2022, unless progress in the field has been incremental. Emerging themes for this year's chapter include: i) Loss of SUMO-specific protease 2 causes isolated glucocorticoid deficiency by blocking adrenal cortex zonal transdifferentiation in mice; ii) Circadian regulation of hippocampal function is disrupted with corticosteroid treatment; iii) Leukocyte telomere length is reduced in children with Congenital Adrenal Hyperplasia; iv) Metabotypes of CAH determined by gas chromatography-mass spectrometry in spot urine; v) A polygenic risk score helps discriminate primary adrenal insufficiency of different etiologies; vi) Crinecerfont, a CRF1 Receptor Antagonist, lowers adrenal androgens in adolescents with Congenital Adrenal Hyperplasia.

Mechanism of the Year

6.1. Loss of SUMO-specific protease 2 causes isolated glucocorticoid deficiency by blocking adrenal cortex zonal transdifferentiation in mice

Dufour D, Dumontet T, Sahut-Barnola I, Carusi A, Onzon M, Pussard E, Wilmouth JJ, Olabe J, Lucas C, Levasseur A, Damon-Soubeyrand C, Pointud JC, Roucher-Boulez F, Tauveron I, Bossis G, Yeh ET, Breault DT, Val P, Lefrançois-Martinez AM, Martinez A

Nat Commun. 2022; 13(1):7858.

PMID: 36543805.

https://pubmed.ncbi.nlm.nih.gov/36543805/

Brief summary: This study investigates the role of SUMOylation in adrenal development and function

The murine adrenal cortex is a constantly self-renewed endocrine organ composed of concentric zones, including the outermost zona glomerulosa (zG) producing mineralocorticoids and the innermost zona fasciculata (zF) producing glucocorticoids. According to the centripetal migration model occurring during postnatal development, progenitor cell populations located in the adrenal capsule or within the zG, consecutively differentiate into steroid-producing zG cells, then through a process of zonal transdifferentiation, convert into zF cells, and eventually undergo apoptosis at the corticomedullary junction (**1, 2**). Genetic models in mouse and *in vitro* approaches have identified two important signaling pathways for adrenal cortex homoeostasis. On the one hand, the WNT/Rspondin/β-catenin pathway is necessary for the maintenance of progenitor pools and the acquisition of zG identity (**3, 4**). On the other hand, cAMP/PKA signaling, following

stimulation by the ACTH, triggers the recruitment of progenitors by inducing transdifferentiation of zG cells into zF cells and stimulates glucocorticoid production (**5**). The mechanisms that maintain adrenal cortex zonation and balance between these two pathways have not been elucidated yet.

SUMOylation is a dynamic posttranslational modification, which provides fine-tuning of protein function involved in the cellular response to stress, differentiation, and tissue development. In the adrenal cortex, the SUMOylation gradient is inversely correlated with the gradient of cellular differentiation raising important questions about its role in functional zonation and the response to stress (**6**). Therefore, the adrenal gland could provide a paradigm to study how SUMOylation dynamics can interact with the function and homoeostasis of an organ, which is in charge of constant adaptation to stress.

Considering that SUMO-specific protease 2 (SENP2), a deSUMOylating enzyme, is upregulated by ACTH/cAMP-PKA signaling within the zona fasciculata (**20-21**), the authors generated mouse models of adrenal hyperSUMOylation by conditional ablation of Senp2 in the cortex (Senp2cKO). Their results reveal that Senp2cKO mice show zone-specific adrenal atrophy, isolated glucocorticoid deficiency and blunted response to ACTH. Progressive atrophy of zF evoked by SENP2 deficiency results from a blockade of zonal transdifferentiation, early apoptosis and impaired PKA catalytic activity that cannot be rescued by genetic derepression of the PKA holoenzyme. SENP2-deficient adrenals also show increased β-catenin SUMOylation and activity that may help to antagonize PKA signaling, thus maintaining the suppression of zF identity. As Senp2 expression is itself under the control of ACTH/PKA, these data identify SUMOylation as a feedforward mechanism that readies the adrenal cortex to respond to stress and maintain functional zonation.

These findings highlight the central role of SUMOylation in physiological processes, such as differentiation, tissue maintenance, and stress response. They also suggest that genetic alterations leading to excessive SUMOylation could be associated with isolated glucocorticoid deficiency in patients.

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New Mechanisms

6.2. Circadian regulation of hippocampal function is disrupted with corticosteroid treatment

Birnie MT, Claydon MDB, Troy O, Flynn BP, Yoshimura M, Kershaw YM, Zhao Z, Demski-Allen RCR, Barker GRI, Warburton EC, Bortolotto ZA, Lightman SL, Conway-Campbell BL

Proc Natl Acad Sci U S A. 2023; 120(15): e2211996120.

PMID: 37023133.

https://pubmed.ncbi.nlm.nih.gov/37023133/

Brief summary: This study investigates the effect of long-acting synthetic glucocorticoids on circadian rhythmicity and hippocampal function

Glucocorticoids exert their effects by binding to glucocorticoid receptors (GRs), which regulate up to ∼20% of the genome via both direct (by binding to glucocorticoid responsive elements in promoter regions) and indirect mechanisms (by interacting with bound transcription factors and epigenetic modifiers) (**1, 2**). GRs play a key role in the feedback regulation of the hypothalamic- pituitary-adrenal (HPA) axis. They are highly expressed in the hippocampus and their distribution is heterogeneous depending on the hippocampal subregion both at

baseline and in response to stress (**3, 4**). Circadian oscillations of glucocorticoids are a fundamental characteristic of adrenal hormone secretion in all mammals. A major coordinator of circadian biological systems is adrenal glucocorticoid secretion, which exhibits a pronounced pre-awakening peak that regulates metabolic, immune, and cardiovascular processes, as well as mood and cognitive function. Loss of this circadian rhythm by chronic stress or synthetic glucocorticoid therapy results in adverse effects, such as impaired memory, mood, and sleep. Surprisingly, the mechanisms that underlie this deficit have not been delineated.

In this 'reverse translational' study, the authors demonstrate that 5-day treatment with methylprednisolone induces prolonged activation of GRs, disrupts the rhythmic activity of hippocampal function through GRs, induces *N*-methyl-D-aspartate receptor (NMDAR)-dependent synaptic dysfunction, and subsequently impairs memory. This model is supported by several lines of evidence: 1) Corticosteroids do not influence the master clock (SCN) or light/dark entraining behaviors due to a lack of GR expression in the SCN; 2) the hippocampus is rich in GRs, which in the presence of corticosteroids, bind to GREs on the essential clock gene Per1 to mediate hippocampal activity in competition with central clock-mediated control; 3) synthetic corticosteroid treatment eliminates the circadian variation in circulating corticosteroids, and consequently the circadian variation in the activity of hippocampal GRs to influence clock gene expression and destabilize clock-entrained NMDAR/CamkII complexes; and 4) synthetic corticosteroid treatment blocks long-term potentiation (LTP), a cellular correlate of long-term memory, by disrupting NMDAR-dependent processes to impair long-term, but not short-term, hippocampal-dependent memory.

These findings provide mechanistic insights into how the transcriptional clock machinery within the hippocampus is influenced by corticosteroid exposure, leading to adverse effects on critical hippocampal functions. They also identify a molecular basis for memory deficits in patients treated with long-acting synthetic corticosteroids. Given that a large proportion of patients treated with corticosteroids report cognitive decline and memory impairment (**5**), these data further support the importance of devising improved regimens of glucocorticoid therapy that recapitulate endogenous glucocorticoid rhythmicity.

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Important for Clinical Practice

6.3. Metabotypes of congenital adrenal hyperplasia in infants determined by gas chromatography-mass spectrometry in spot urine

Kamrath C, Friedrich C, Hartmann MF, Wudy SA

J Steroid Biochem Mol Biol. 2023; 231:106304.

PMID: 36990162.

https://pubmed.ncbi.nlm.nih.gov/36990162/

Brief summary: This study investigates metabotyping using steroid profiles, obtained with GC–MS, as a method to monitor the treatment in children with classical congenital adrenal hyperplasia.

The aim of treatment in classic congenital adrenal hyperplasia (CAH) is to provide adequate glucocorticoid substitution to prevent adrenal crises and to suppress the excess adrenal androgen production. However, in clinical practice this is often difficult, and patients may develop in tandem hypercortisolism and/or hyperandrogenism because of over- or undertreatment with glucocorticoids. Long-term, such suboptimal

treatment may result in adverse consequences (**1, 2**). Hence, monitoring of glucocorticoid replacement therapy is important in order to optimize treatment and improve outcome. Metabotyping refers to grouping metabolically similar individuals and helps to monitor treatment of children with CAH using GC-MS urinary steroid metabolome analysis. This method allows classification in adequately-, over-, or undertreated children, as well as identification of patients with treatment failure. (**3**).

In this study, the authors metabotyped patients with classic CAH ($n=60$, <4 years) using GC–MS urinary steroid metabolome analysis in spot urine samples. Urinary steroid metabolome analysis by GC–MS is a non-invasive diagnostic approach that provides qualitative and quantitative data regarding adrenal steroid biogenesis from a single sample (**4**). Three metabotypes were identified in the cohort, each representing a group of patients that were treated adequately, over treated or under treated. Metabotype #1 ($n=15$ (25%)) showed high concentrations of androgen and 17-hydroxyprogesterone (17OHP) precursor steroids, metabotype #2 ($n=28$ (47%)) revealed balanced metabolic control, and metabotype #3 ($n=17$; 28%) demonstrated severe adrenal suppression with low concentrations of androgen and 17OHP precursor steroids. Previously, in a study using 24-hour urinary samples, a fourth metabotype consistent with treatment failure was identified (**3**). The current study failed to identify this group.

In summary, the authors were able to identify three clinically important treatment groups. Identifying patients with indications of over- or undertreatment is extremely important in the management of classic CAH in order to achieve optimal treatment and subsequently avoid negative side effects in the long-term perspective. Therefore, metabotyping may be an additional tool to use when monitoring glucocorticoid replacement therapy in patients with classic CAH.

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6.4. A polygenic risk score to help discriminate primary adrenal insufficiency of different etiologies

Aranda-Guillén M, Røyrvik EC, Fletcher-Sandersjöö S, Artaza H, Botusan IR, Grytaas MA, Hallgren Å, Breivik L, Pettersson M, Jørgensen AP, Lindstrand A, Vogt E, Norwegian Addison Registry Study Group, The Swedish Addison Registry Study Group, Husebye ES, Kämpe O, Wolff ASB, Bensing S, Johansson S, Eriksson D

J Intern Med. 2023; 294(1): 96–109.

PMID: 37151110.

https://pubmed.ncbi.nlm.nih.gov/37151110/

Brief summary: The authors designed a polygenic risk score (PRS) to aid in estimating disease susceptibility in patients with autoimmune Addison's disease (AAD).

Autoimmune Addison's disease (AAD) is the most common cause of primary adrenal insufficiency (PAI) in adults. Despite its exceptionally high heritability, tools to estimate disease susceptibility in individual patients are lacking (**1–3**). The aim of this study was to investigate whether the polygenic risk score (PRS) for AAD could help investigate the pathogenesis of PAI in pediatric patients.

Herein, the authors designed a PRS to aid in estimating disease susceptibility in individual patients with AAD. The PRS was evaluated in 1223 seropositive cases with AAD and 4097 controls. In addition, the score was reevaluated in 18 children with presumed AAD along with whole genome sequencing, and in 120 seronegative patients with idiopathic PAI. The final $PRS14_{AAD}$ consisted of 5 SNPs and 9 HLA alleles.

The odds ratio for AAD per 1 S.D. of PRS was 6.4 and the average PRS in cases was about 1.5 S.D. above the average PRS in controls, meaning that 96% of cases were above the 50th centile of the controls. In addition, 79% of cases and 80% of controls were correctly classified.

In a subset of the cohort cases with childhood onset of AAD ($n = 18$), the genetic susceptibility at the individual level was calculated using $PRS14_{AAD}$ along with whole genome sequencing of the cases and their family members. Most patients with early onset AAD had PRS at or above the median for cases with confirmed AAD. In the two cases with a low PRS, WGS identified pathogenic variants explaining the cause of PAI (CYP11A1 and NROB1). For every S.D. increase of PRS, the average age of disease onset decreased 3 years and the collective burden of known risk alleles explained up to 20 years of difference of average age of onset.

The PRS in seronegative (21OH-Ab) patients with AAD was lower than in seropositive cases. Cases without comorbidity centered between seropositive cases and controls, while those that had type 1 diabetes had a PRS at the level of the seropositive AAD cases. In addition, the authors show that patients that were seronegative already within 5 years after diagnosis had a lower genetic predisposition to AAD compared to those that screened negative for 21OH autoantibodies after 20 years of diagnosis. Seronegative cases are thus not merely a subset of individuals that over time lost their autoantibodies, but whether they have a different disease etiology still remains to be investigated.

In conclusion, estimation of the $PRS14_{AAD}$ at the individual level has a good negative predictive value for AAD. For patients with a negative or borderline titer of 21OH-Ab, a low PRS should lead the clinician to investigate the etiology further, such as whether the PAI has a monogenic cause. The PRS at the individual level remains constant during the life-span.

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6.5. International recommendations for the diagnosis and management of patients with adrenoleukodystrophy: A consensus-based approach

Engelen M, van Ballegoij WJC, Mallack EJ, Van Haren KP, Köhler W, Salsano E, van Trotsenburg ASP, Mochel F, Sevin C, Regelmann MO, Tritos NA, Halper A, Lachmann RH, Davison J, Raymond GV, Lund TC, Orchard PJ, Kuehl JS, Lindemans CA, Caruso P, Turk BR, Moser AB, Vaz FM, Ferdinandusse S, Kemp S, Fatemi A, Eichler FS, Huffnagel IC

Neurology. 2022; 99(21): 940–951.

PMID: 36175155.

https://pubmed.ncbi.nlm.nih.gov/36175155/

Brief summary: This paper describes consensus expert recommendations for the diagnosis and management of patients with Adrenoleukodystrophy.

Adrenoleukodystrophy (ALD), a progressive metabolic disorder with variable and unpredictable clinical course, is caused by pathogenic variants in *ABCD1* gene leading to deficient β-oxidation of saturated very-long-chain fatty acids (VLCFAs) (**1, 2**). Patients are asymptomatic at birth but develop symptoms as the disease progresses. There are 3 core clinical syndromes: a slowly progressive myeloneuropathy (adrenomyeloneuropathy), a rapidly progressive leukodystrophy (cerebral ALD), and primary adrenal insufficiency. Women develop myeloneuropathy, while men can develop all 3 syndromes (**3, 4**). These syndromes are not present in all individuals and are not related to genotype. Cerebral ALD and adrenal insufficiency require early detection and intervention and warrant clinical surveillance because of variable penetrance and age at onset. Newborn screening has increased the number of presymptomatic individuals under observation, but clinical surveillance protocols vary.

This paper describes a consensus-based modified Delphi approach among 28 international ALD experts to develop best-practice recommendations for diagnosis, clinical surveillance, and treatment of patients with ALD.

They identified 39 discrete areas of consensus. Regular monitoring to detect the onset of adrenal failure and conversion to cerebral ALD is recommended in all male patients. Hematopoietic cell transplant (HCT) is the treatment of choice for cerebral ALD. This guideline addresses a clinical need in the ALD community worldwide as the number of overall diagnoses and presymptomatic individuals is increasing because of newborn screening and greater availability of next-generation sequencing. The poor ability to predict the disease course informs current monitoring intervals but remains subject to change as more data emerge. This knowledge gap should direct future research and illustrates once again that international collaboration among physicians, researchers, and patients is essential to improving care.

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Clinical Trials – New Treatments

6.6. Crinecerfont, a CRF1 receptor antagonist, lowers adrenal androgens in adolescents with congenital adrenal hyperplasia

Ron S Newfield, Kyriakie Sarafoglou, Patricia Y Fechner, Natalie J Nokoff, Richard J Auchus, Maria G Vogiatzi, George S Jeha, Nagdeep Giri, Eiry Roberts, Julia Sturgeon, Jean L Chan, Robert H Farber

J Clin Endocrinol Metab. 2023; dgad270. doi: 10.1210/clinem/dgad270.

PMID: 37216921.

https://pubmed.ncbi.nlm.nih.gov/37216921/

Brief summary: This study evaluated the safety, tolerability and efficacy of Crinecerfont, a CRF1R antagonist in adolescents with classic congenital adrenal hyperplasia (CAH).

Classic congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) is characterized by impaired cortisol synthesis and excess adrenal androgen secretion. Sufficient suppression of adrenal andogen production in classic CAH may be difficult with current formulations of glucocorticoid treatment (**1**). Novel therapeutic approaches with corticotropin-releasing factor type 1 receptor (CRF1R) antagonists could be a potential way forward to overcome this difficulty and improve medical care. CRF1R antagonists decrease ACTH concentrations, thereby suppressing adrenal androgen excess and obviating the need for administration of supraphysiologic doses of glucocorticoids (**2, 3**).

This open-label, phase 2 study (NCT04045145) evaluated the safety, tolerability, and efficacy of crinecerfont, a CRF1R antagonist, in adolescents with classic CAH at 4 centers in the United States. Participants were 8 adolescents (3 males, 5 females) aged 14–17 years with classic CAH. Crinecerfont was administered orally (50 mg twice daily) for 14 consecutive days with morning and evening meals. The main outcomes were change from baseline to day 14 in circulating concentrations of ACTH, 17-hydroxyprogesterone (17OHP), androstenedione, and testosterone. After 14 days of crinecerfont, median percent reductions from baseline to day 14 were as follows: ACTH, −57%; 17OHP, −69%; and androstenedione, −58%. In female participants, 60% (3/5) had 50% reduction from baseline in testosterone.These results are consistent with a study of crinecerfont in adults with classic CAH.

In summary, crinecerfont treatment for 14 days decreased ACTH concentrations and afforded clinically meaningful reductions of elevated 17OHP, androstenedione, testosterone (women), or androstenedione/testosterone ratio (men) in adolescents with classic CAH. Longer-term studies are required to evaluate the effects of crinecerfont on clinical end points of disordered steroidogenesis and glucocorticoid exposure in these patients. Studies in children with classic CAH are on-going.

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6.7. [^{11}C]metomidate PET-CT versus adrenal vein sampling for diagnosing surgically curable primary aldosteronism: A prospective, within-patient trial

Wu X, Senanayake R, Goodchild E, Bashari WA, Salsbury J, Cabrera CP, Argentesi G, O'Toole SM, Matson M, Koo B, Parvanta L, Hilliard N, Kosmoliaptsis V, Marker A, Berney DM, Tan W, Foo R, Mein CA, Wozniak E, Savage E, Sahdev A, Bird N, Laycock K, Boros I, Hader S, Warnes V, Gillett D, Dawnay A, Adeyeye E, Prete A, Taylor AE, Arlt W, Bhuva AN, Aigbirhio F, Manisty C, McIntosh A, McConnachie A, Cruickshank JK, Cheow H, Gurnell M, Drake WM, Brown MJ

Nat Med. 2023; 29(1):190–202.

PMID: 36646800.

https://pubmed.ncbi.nlm.nih.gov/36646800/

Brief summary: This study validates dexamethasone-suppressed [^{11}C]metomidate positron emission tomography computed tomography (MTO) scanning as a CYP11B2-selective investigation for lateralizing PA.

Primary aldosteronism (PA) is the most common single cause of hypertension, accounting for 5–14% of all cases and 20–25% of treatment-resistant hypertension (**1, 2**). Traditionally, patients with PA are divided equally into those in whom aldosterone excess is due to a unilateral aldosterone-producing adrenal adenoma (APA), in whom surgical cure is possible, and those with bilateral production (often termed idiopathic hyperaldosteronism (IHA), who require long-term medical therapy. Current consensus is that patients with unilateral APAs should be offered laparoscopic surgical removal of that adrenal gland and those with IHA should be treated with aldosterone antagonist drugs (**3**). The current criterion standard used to distinguish patients with APA from those with IHA is adrenal vein sampling (AVS)—an invasive, technically demanding procedure with restricted availability. Metomidate—a methyl analog of the anesthetic agent etomidate—is a potent inhibitor of CYP11B1 (11β-hydroxylase) and CYP11B2 (aldosterone synthase), the final two enzymes involved in cortisol and aldosterone synthesis, respectively. Metomidate can be 11CH3 labeled and used as a positron emission tomography (PET) radiotracer in combination with high-resolution computed tomography (CT) to detect adrenocortical tumors expressing these enzymes. A previous proof-of-concept study indicated that pre-treatment with dexamethasone for 3 days before [^{11}C]metomidate PET-CT (MTO) scanning suppresses adrenal CYP11B1 (but not CYP11B2) protein expression, thus achieving *in vivo* selectivity (**4**). This selectivity facilitates the detection of focal adrenal lesions with high [^{11}C]metomidate uptake due to CYP11B2 expression and separates them from normal adrenal tissue. International guidelines for the management of PA acknowledge the potential for molecular imaging in the subtyping of PA11, but prospective outcomes-based data are needed to influence clinical practice.

The primary objective of this prospective study of 143 patients with PA (NCT02945904) was to compare the accuracy of a non-invasive test, [^{11}C]metomidate positron emission tomography computed tomography (MTO) scanning, with adrenal vein sampling (AVS) in predicting the biochemical remission of PA and the resolution of hypertension after surgery. A total of 128 patients reached 6- to 9-month follow-up, with 78 (61%) treated surgically and 50 (39%) managed medically. Of the 78 patients receiving surgery, 77 achieved one or more PA surgical outcome criterion for success. The accuracies of MTO at predicting biochemical and clinical success following adrenalectomy were, respectively, 72.7% and 65.4%. For AVS, the accuracies were 63.6 and 61.5%. MTO was not significantly superior, but the differences of 9.1% (95% confidence interval $= -6.5$ to 24.1%) and 3.8% (95% confidence interval $= -11.9$ to 9.4) lay within the pre-specified -17% margin for non-inferiority ($P = 0.00055$ and $P = 0.0077$, respectively). Of 24 serious adverse events, none was considered related to either investigation and 22 were fully resolved.

These findings indicate that MTO following pre-treatment with dexamethasone enables non-invasive detection of unilateral APAs and is at least as accurate in the prediction of outcomes from adrenalectomy as the standard,

invasive investigation, AVS. Therefore, it will now be possible to diagnose unilateral APAs when AVS is either unavailable, unsuccessful or not desired by the patient. Unlike AVS, MTO is quick, safe and reliable.

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New Hope

6.8. A proof of concept of a machine learning algorithm to predict late-onset 21-hydroxylase deficiency in children with premature pubic hair

Agnani H, Bachelot G, Eguether T, Ribault B, Fiet J, Le Bouc Y, Netchine I, Houang M, Lamazière A

J Steroid Biochem Mol Biol. 2022; 220:106085.

PMID: 35292353.

https://pubmed.ncbi.nlm.nih.gov/35292353/

Brief summary: Steroid analysis using LC-MS/MS in association with clinical parameters may be used to develop a diagnostic score that could successfully differentiate premature pubarche (PP) from non-classic congenital adrenal hyperplasia (NCCAH), thereby obviating the need for ACTH stimulation testing.

Late onset, non-classic congenital adrenal hyperplasia (NCCAH) due to 21-hydroxylase deficiency (21-OHD) should be ruled out in children with premature pubarche (PP). To this end, ACTH stimulation testing is currently used in these patients (**1–3**).

In this prospective study, the authors aimed to differentiate children with NCCAH from children with PP using a machine learning based approach to steroid profiles determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS) and clinical parameters obtained from a cohort of children with PP ($n = 97$). A metabolic footprint was assigned for each patient using clinical data, bone age, and adrenal steroid levels recorded by LC–MS/MS. Separated in two sets, one training ($n = 58$) and one validation ($n = 39$), the authors developed a score using a mathematical machine learning model to identify patients with NCCAH in a cohort of children with PP. Based on selected variables, the prediction score was accurate (100%) at differentiating PP from late onset NCCAH patients. The most significant variables were 21-deoxycorticosterone, 17-hydroxyprogesterone and 21-deoxycortisol steroids.

These findings indicate that this new test has excellent sensitivity and specificity for the diagnosis of NCCAH, due to an ML approach. The authors suggest that if implemented as a routine test, it could eliminate the need for ACTH stimulation testing for the purpose of identifying NCCAH patients with PP. The results need further validation but these initial results are encouraging.

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6.9. Leukocyte telomere length in children with congenital adrenal hyperplasia

Raftopoulou C, Abawi O, Sommer G, Binou M, Paltoglou G, Flück CE, van den Akker ELT, Charmandari E
J Clin Endocrinol Metab. 2023; 108(2):443–452.
PMID: 36181470.
https://pubmed.ncbi.nlm.nih.gov/36181470/

Brief summary: This prospective observational cohort study determined leukocyte telomere length (LTL) in 76 patients with genetically confirmed CAH (83% classic CAH). LTL was shorter in patients with classic vs nonclassic CAH, in overtreated than in optimally treated patients, and patients receiving prednisolone compared with hydrocortisone.

Telomeres are tandem repeats of a noncoding hexameric nucleotide sequence (5′-TTAGGG-3′) located at the ends of human chromosomes (**1**). In somatic cells, telomerase activity is very low, almost undetectable, so telomeres shorten progressively with cell division, ultimately leading to loss of telomere protection and a DNA damage response that induces senescence or cell death (**2**). A frequently used assay involves the measurement of telomere length in leukocytes (leukocyte telomere length, LTL), while the shortening of LTL is a robust marker of biological aging and contributes to the rise in mortality rates found in chronic conditions, such as increased body weight, cardiovascular disease and chronic stress (**3**). LTL is influenced by changes in the activity of telomerase (telomere terminal transfer-ase), as well as by the alternative lengthening of telomeres pathway (**2**). Studies have also shown that an individual's exposure to hypercortisolism (e.g. in Cushing's syndrome) is associated with decreased LTL (**1**). Individual risk factors analysis has shown that telomere length variability may be partially explained by lifestyle practices, with healthy lifestyle being associated with longer LTL (**4**).

Interestingly an increase in LTL has been reported when beneficial interventions have been studied, such as the increase in LTL following 12 months of a multidisciplinary, personalized, lifestyle intervention program in children and adolescents with overweight and obesity, indicating the potential of LTL to assess both current health status and also the benefits of an intervention (**3**). This phenomenon has been attributed to increased circulation of younger leukocytes, suggesting that telomere attrition is likely a modifiable factor as there is substantial variability in the rate of telomere shortening probably independent of chronological age (**5**).

In this prospective observational cohort study, conducted at 4 academic pediatric endocrinology outpatient clinics, 76 patients (median age: 12 years, IQR: 6.3–15,1) with genetically confirmed CAH were assessed at 2 follow-up visits (mean 4.1 ± 0.7 months apart). At each visit, LTL was determined by quantitative real-time PCR. LTL was shorter in patients with classic vs nonclassic CAH (-0.29, $P = 0.012$), in overtreated than in optimally treated patients (-0.07, $P = 0.002$), and in patients receiving prednisolone compared with hydrocortisone (-0.34, $P < 0.001$). LTL was not associated with undertreatment or daily hydrocortisone-equivalent dose ($P > 0.05$).

The decreased LTL in these patients may be attributed to chronic exposure to supraphysiologic glucocorticoid concentrations, as has been previously described in Cushing's (**6**). This is supported by the fact that patients with Cushing syndrome have shorter LTL compared with healthy controls, which increases following successful treatment (6). Furthermore, the lack of association in this study between undertreatment and reduced LTL, may indicate that chronically elevated androgen concentrations per se might not be associated with accelerated shortening of telomeres. Indeed, this is also shown in other conditions associated with increased androgen concentrations, such as PCOS, where androgens were not associated with shorter LTL (**7**). A potential mechanism comes from *in vitro* and epidemiologic studies showing that androgens might upregulate telomerase expression and/or activity and enhance telomere length through aromatization to estradiol (**8**). This is an interesting thought and can incite further research studies aiming to elucidate the relation between the chronic androgen burden in CAH due to 21-OHD and changes in LTL. It is worth noting that the greatest effect on LTL shortening was observed in patients treated with the more potent, longer-acting prednisolone compared with hydrocortisone. This finding reinforces the need to avoid prolonged use of prednisolone in children with CAH, as suggested in the current international guidelines, given the less favorable metabolic profile and growth outcomes (**9**).

These data indicate that LTL might have a use as a biomarker for monitoring glucocorticoid treatment, particularly the adverse effects of supraphysiologic dosing or long-acting prednisolone. This is of particular clinical significance given the recognized increased cardiometabolic morbidity even from an early age in overtreated subjects (**10**).

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6.10. Body composition in children and adolescents with non-classic congenital adrenal hyperplasia and the risk for components of metabolic syndrome: An observational study

Ben Simon A, Brener A, Segev-Becker A, Yackobovitch-Gavan M, Uretzky A, Schachter Davidov A, Alaev A, Oren A, Eyal O, Weintrob N, Lebenthal Y

Front Endocrinol (Lausanne). 2022;13:1022752.

PMID: 36353234.

https://pubmed.ncbi.nlm.nih.gov/36353234/

Brief summary: This retrospective case-control observational study assessed body composition of children with non-classic congenital adrenal hyperplasia (NCCAH) using bioelectrical impedance analysis (BIA). It showed that children with NCCAH have an imbalance between muscle and fat tissues compared with control subjects, which may place them at increased risk for early-onset cardiometabolic morbidity.

Non-classic congenital adrenal hyperplasia (NCCAH) is a group of enzymatic disorders characterized by a mild defect in cortisol biosynthesis (**1**). Glucocorticoid therapy is not always indicated but rather reserved for symptomatic cases of hyperandrogenism, in an attempt to alleviate androgen overproduction (**2**). Chronic androgen excess has been reported in association with increased visceral adiposity, insulin resistance and their metabolic consequences (**1, 2**).

In this study, the authors explored the interaction between muscle-to-fat ratio (MFR) and components of metabolic syndrome in pediatric patients with NCCAH. The study group consisted of 75 subjects [12.3 years (interquartile range: 8.9, 15.4), 26 males] with NCCAH [61 hydrocortisone-treated (21 males) and 14 untreated (5 males)] and 134 healthy sex- and age-matched subjects (41 males) with normal puberty served as controls. Body composition was measured by bioelectrical impedance analysis (BIA) and muscle-to-fat ratio (MFR) *z*-scores were calculated. Stepwise linear regression models were applied to evaluate explanatory variables for MFR *z*-scores, blood pressure percentiles, lipid profiles and glucose metabolism.

Children and adolescents with NCCAH had higher mean BMI *z*-scores and lower median MFR z-scores compared with their healthy sex- and age-matched controls. Factors, including lower socioeconomic position and higher birthweight *z*-scores adversely affected their body composition while the duration of hydrocortisone therapy was found to be beneficial.

The novelty of the study comes from the paucity of BIA data on NCCAH. In this study, subjects with NCCAH did not have an increased rate of obesity, but did have higher BMI *z*-scores on average compared to healthy controls. Interestingly, the BIA study of the NCCAH subjects was characterized by a low MFR *z*-score due to higher fat mass, indicating that muscle mass was relatively low compared to their fat mass, thus placing them at risk for sarcopenic obesity (**3**). This unfavorable body composition in NCCAH patients, characterized by an imbalance between muscle and adipose tissue may be attributed to the complex interaction between circulating androgens and body composition parameters (**4, 5**).

In summary, children with NCCAH have a body composition characterized by an imbalance between muscle and fat tissue, placing them at increased risk for early onset cardiometabolic derangements. Implementation of BIA as a part of routine assessment may assist in the identification of cardiometabolic risk factors in NCCAH. It is reassuring that low-dose glucocorticoid therapy in pediatric patients with NCCAH aimed to alleviate androgen overproduction does not appear to adversely affect body composition.

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New Genes

6.11. Identification of risk loci for primary aldosteronism in genome-wide association studies

Le Floch E, Cosentino T, Larsen CK, Beuschlein F, Reincke M, Amar L, Rossi GP, De Sousa K, Baron S, Chantalat S, Saintpierre B, Lenzini L, Frouin A, Giscos-Douriez I, Ferey M, Abdellatif AB, Meatchi T, Empana JP, Jouven X, Gieger C, Waldenberger M, Peters A, Cusi D, Salvi E, Meneton P, Touvier M, Deschasaux M, Druesne-Pecollo N, Boulkroun S, Fernandes-Rosa FL, Deleuze JF, Jeunemaitre X, Zennaro MC

Nat Commun. 2022; 13(1): 5198.

PMID: 36057693.

https://pubmed.ncbi.nlm.nih.gov/36057693/

Brief summary: This genome-wide association study (GWAS) reports the first genetic loci for risk of primary aldosteronism. New candidate genes and their potential mechanisms for the development of aldosterone excess are described.

Primary aldosteronism (PA) is the most frequent form of secondary hypertension (5% of patients with hypertension in primary care and 10–20% of patients with hypertension referred to specialist care). PA is associated with cardiovascular complications that exceed those of patients with essential hypertension matched for age and blood pressure (**1, 2**). In this study, the authors hypothesized that genetic variation may predispose to the development of PA. They sought to identify risk loci for PA by performing a genome-wide association study (GWAS).

Three risk loci were identified on chromosome 1, 13 and X, respectively. The locus on chromosome 13 was specific to males. The authors further investigated their top two significant risk loci, on chromosome 1 and 13, and identified candidate genes in these regions (*CASZ1* and *RXFP2*). *CASZ1* and *RXFP2* are expressed in the adrenal gland and suppress mineralocorticoid biosynthesis in adrenocortical H295R-S2 cells overexpressing either CASZ1 or RXFP2, indicating a novel ability to modify mineralocorticoid output in the adrenal gland. Hence, the authors suggest that risk alleles at chromosome 1 and 13 may increase the susceptibility to develop PA. The identification of new risk loci as well as the new candidate

genes for the development of PA may open up new perspectives for the diagnosis and treatment of arterial hypertension.

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New Paradigms

6.12. Regulatory mechanisms of microRNAs in endocrine disorders and their therapeutic potential

Ledesma-Pacheco SJ, Uriostegui-Pena AG, Rodriguez-Jacinto E, Gomez-Hernandez E, Estrada-Meza C, Banerjee A, Pathak S, Ruiz-Manriquez LM, Duttaroy AK, Paul S

Front Genet. 2023; 14: 1137017.

PMID: 36896239.

https://pubmed.ncbi.nlm.nih.gov/36896239/

Brief summary: This study summarises the involvement of specific miRNAs in diabetes mellitus, thyroid diseases, osteoporosis, pituitary tumours, Cushing's disease, adrenal insufficiency and multiple endocrine neoplasia's. Furthermore, the potential of miRNA as candidates for developing novel diagnostic and therapeutic tools is also discussed.

Endocrine disorders are common worldwide and represent a considerable public health problem due to long term-effects that can be difficult to manage and affect the quality of life of patients. In addition, endocrine disorders may also be a significant cause of death worldwide (**1**). To date, none of the conventional diagnoses or therapies for endocrine disorders are efficient or precise, which has outstood the urge to develop novel and more functional techniques.

MiRNAs are small (21–24 nucleotides long) endogenous non-coding RNA molecules that post-transcriptionally regulate gene expression either by degradation of mRNA or translational inhibition (**2, 3**). Recently, miRNAs have been demonstrated to regulate various processes associated with endocrine disorders, such as high-glucose-induced apoptosis, insulin secretion, and proliferation, as well as glycolipid metabolism in diabetes mellitus; abnormal ciliogenesis in thyroid diseases; osteoclast and osteoblast differentiation in osteoporosis; proliferation and apoptosis in pituitary tumors; proliferation in Cushing's syndrome; steroidogenesis in adrenal insufficiency; and adrenocortical proliferation in multiple endocrine neoplasia; among others. Although the precise roles of several miRNAs in various signaling pathways during the development of endocrine disorders have yet not been fully elucidated, these small molecules have drawn the attention of global researchers to be used for novel therapeutic purposes. Furthermore, miRNAs have shown promising results as disease biomarkers to detect the disease at an early stage, distinguish among different conditions of the patients, as well as to determine the disease's severity.

Altogether, miRNAs have potential as novel biomarkers that allow the precise detection and management of endocrine disorders.

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6.13. Development and function of the fetal adrenal

Pignatti E, du Toit T, Flück CE
Rev Endocr Metab Disord. 2023;24(1):5–21.
PMID: 36255414.
https://pubmed.ncbi.nlm.nih.gov/36255414/

Brief summary: This review covers the recent advances in the understanding of fetal adrenal development and the interplay with the feto-placental unit. A short overview of related adrenal disorders is presented. The effect of other hormone systems is also discussed and the reliability of using rodent models to study adrenal pathophysiology.

Structural development of the fetal adrenal and its transition to the adult organ: There are differences in the development of the human and mouse fetal adrenal. The human fetal adrenal anlage/primordium (AP) is formed in parallel with the genital primordium (GP), the former in the anterior coelomic epithelium at 30 dpc, and the latter in the posterior coelomic epithelium at 33 dpc. In mouse, there is first a common AGP at E9.0 that expresses WT1, GATA4 and CITED2. At E10.5 there is a split to the mouse AP and GP, where WT1 is suppressed in the AP. In human, the adrenal precursors are devoid of GATA4 and have low expression of CITED2, but as in mouse have a strong expression of SF1 (stimulated by WT1). Contrary to WT1, CITED2 and GATA4, whose expressions in steroidogenic cells are confined to embryonic stages, SF1 is expressed in both embryonic and postnatal adrenals in mouse and human. It acts as a master regulator of the transcription of cytochrome P450 steroid hydroxylases involved in steroidogenesis. In mouse, high SF1 expression is needed for proper adrenal development, while sex determination and the gonadal development are not affected by SF1 haploinsufficiency. This suggests that gonadal specification in mouse relies on a threshold of SF1 expression, whereas high SF1 levels are associated with the development of adrenocortical cells. In humans, gonadal development and function is more commonly affected than adrenal function even in SF1 haploinsufficiency.

At around 48-52 dpc in human (E12.5 in the mouse) a subset of neural crest cells invades the AP to form the chromaffin cells and form the adrenal medulla. At this stage the adrenal capsule forms from mesenchymal cells surrounding the AP and the adrenal anlage stratifies into an outer definitive zone and an inner fetal zone. The middle transitional zone develops during mid-gestation. The fetal zone disappears by apoptosis after birth and there is a rapid fall in DHEA and DHEAS. The definitive zone and the transitional zone give rise to the adult adrenal cortex. In mouse, inherent proliferation of adrenocortical cells and recruitment of progenitor cells from the capsule that migrate towards the center of the cortex maintain the adrenocortical cellularity and the progenitors differentiate into cells of the steroidogenic lineage. To conclude, the structural features of the fetal adrenal are outlined by gestational week 8, while the steroidogenic production during fetal stage changes throughout gestation to support the development of the fetus.

Fetal adrenal function and its role in the feto-placental unit: The fetal steroidogenesis is a combined contribution of the fetal adrenal, the 46, XY gonad, the placenta and the shuttling of steroids from the mother. The fetal zone produces DHEAS from cholesterol and is converted in the placenta to androstenedione (A4), estrone (E1) and estradiol (E2). E1 and E2 enter the maternal circulation. Maternal DHEA(S) also contributes to placental estrogen biosynthesis. Estrogens, GC and PROG are metabolized by the feto-placental unit and regulate fetal development. Fetal cortisol is produced from placental PROG by GW8-10 (post conception) together with *de novo* cortisol production from the fetal adrenal by GW8-14. The Leydig cells synthesize testosterone (T) from cholesterol and T and DHT bind to the AR (expressed 8-20 wpc) in the testis and initiate the differentiation of male external genitalia. The C11-oxy androgens (11OHA4, 11KA4, 11KT) are produced in the so called *back-door pathway* via fetal origin of 11OHA4 and its conversion in the placenta to 11KA4 and 11KT. PROG, allopregnanolone and androsterone are produced in the placenta. PROG is the precursor to the *back-door pathway.* DHT is produced from PROG and 17OHP via catalytic pathways in the fetal liver, fetal adrenal, fetal genital skin and the placenta. The feed-back between placental CRH and fetal adrenal cortisol is essential for parturition and fetal organ maturation. Prior to parturition there is an increase in cortisol in amniotic fluid due to an increase of 11bHSD1 activity in placenta, amnion and chorion.

6.14. Congenital adrenal hyperplasia

Auer MK, Nordenström A, Lajic S, Reisch N
Lancet. 2023; 401(10372): 227–244.
PMID: 36502822.
https://pubmed.ncbi.nlm.nih.gov/36502822/

Brief summary: This comprehensive narrative review provides a "Seminar" on congenital adrenal hyperplasia (CAH). The authors performed a thorough comprehensive qualitative summary of articles published mainly in Cochrane Library, MEDLINE, and Embase in English between Jan 1, 2005, and June 30, 2021, while not excluding commonly referenced and highly regarded older publications.

Congenital adrenal hyperplasia (CAH) is an 'umbrella' clinical term comprising a group of autosomal recessive disorders leading to multiple complex hormonal imbalances caused by various enzyme deficiencies in the adrenal steroidogenic pathway (**1**). Research in the past decades has advanced our understanding of disease genetics and pathophysiology (**2**). These complex hormonal imbalances can manifest with potentially life-threatening consequences, while disease-related and treatment-related morbidity and mortality are increased. After the introduction of life-saving hormone replacement therapy in the 1950s and neonatal screening programmes in many countries, nowadays neonatal survival rates in patients with congenital adrenal hyperplasia are high (**3**).

Alternative steroid pathways have been identified and a multitude of novel treatment approaches are being developed (**1**). Optimising diagnostic and treatment strategies, a successful transition process to adult services, patient empowerment, and continuous psychological support throughout the lifespan are key factors for improved outcome and quality of life and reduced long-term sequelae in patients with CAH (**4**).

The most common type of CAH is due to steroid 21-hydroxylase (21OH) deficiency; the classic (severe) form of 21OH deficiency is characterised by life-threatening adrenal crises and is the most common cause of atypical genitalia in neonates with 46,XX karyotype (**5**). Non-classic (mild) forms of CAH caused by 21OH deficiency are more common than the classic ones; they are detected clinically and primarily identified in female patients with hirsutism or impaired fertility (**6**). Finally, disease-related mortality in CAH remains increased and therapeutic management challenging, with multiple long-term complications related to treatment and disease affecting growth and development, metabolic and cardiovascular health, and fertility. Novel treatment approaches are required that they either mimick physiologic circadian cortisol secretion or reduce adrenal hyperandrogenism without the adverse effects of supraphysiologic glucocorticoid treatment (**7**).

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6.15. Primary bilateral macronodular adrenal hyperplasia: Definitely a genetic disease

Cavalcante IP, Berthon A, Fragoso MC, Reincke M, Stratakis CA, Ragazzon B, Bertherat J
Nat Rev Endocrinol. 2022; 18(11): 699–711.
PMID: 35922573.
https://pubmed.ncbi.nlm.nih.gov/35922573/

Brief summary: This narrative review summarizes the important progress made in the past 10 years in our understanding of the genetics of primary bilateral macronodular adrenal hyperplasia (PBMAH).

Primary bilateral macronodular adrenal hyperplasia (PBMAH) is an adrenal cause of Cushing syndrome, attributed to the disrupted integrity of the adrenal cortex zonation that is important for steroidogenesis (**1**). Nowadays, the diagnosis of PBMAH is more frequent following the progress in the diagnostic methods for adrenal incidentalomas (**2**). The histologic milieu of PBMAH consists of adrenal enlargement with multiple bilateral benign adrenocortical nodules (diameter above 1 cm) that can result to cortisol excess independent of ACTH from the pituitary (adrenal Cushing syndrome) (**3**). PBMAH is diagnosed in adults, most often without any other personal or family history that would be suggestive of a genetic origin. However, the development of bilateral multiple benign adrenocortical tumours in the same patient suggests that predisposing genetic factors are important, while rare syndromic forms of PBMAH are known to be of genetic origin (**4**). Thus, in the past 10 years, non- syndromic forms of PBMAH have been recognized as a genetic disease (**4**). Genomics studies have highlighted the molecular heterogeneity of PBMAH and identified molecular subgroups, allowing improved understanding of the clinical heterogeneity of this disease (**5**). Constitutive inactivating variants in ARMC5 (20–25%) and KDM1A (>90%) of food dependent Cushing syndrome have demonstrated that PBMAH, despite mostly being diagnosed in adults aged 45–60 years, is a genetic disorder (**6, 7**).

This review summarizes the important progress made in the past 10 years, particularly the development of omics approaches and integrated genomics in understanding the genetics of PBMAH, which have led to a better understanding of the pathophysiology, opening new clinical perspectives. This important progress has translated into a better understanding of the physiopathology of the condition and has also highlighted factors and signaling pathways essential for the control of adrenocortical homeostasis. Still, the genetic cause (or causes) of the third molecular group of non-syndromic PBMAH that is not caused by ARMC5 or KDM1A remains to be determined. Considering the benefit for the index patient with PBMAH in identifying a genetic cause that could also favour the development of comorbidities, and that it could lead to early diagnosis of adrenal Cushing syndrome in family members, systematic genetic screening of PBMAH should now be considered.

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Food for Thought

6.16. Brain structure in autoimmune Addison's disease

Van't Westeinde A, Padilla N, Siqueiros Sanchez M, Fletcher-Sandersjöö S, Kämpe O, Bensing S, Lajic S
Cereb Cortex. 2023 Apr 4;33(8):4915–4926.
doi: 10.1093/cercor/bhac389. PMID: 36227196.
https://pubmed.ncbi.nlm.nih.gov/36227196/

Brief summary: This study investigated the brain structure in young adult patients with autoimmune Addison's disease (AAD). It found that patients with AAD had a 4.3% smaller total brain volume but no major differences in subcortical structures compared to healthy controls.

Both short- and long-term disruptions in cortisol concentrations and rhythmicity affect brain structure development. Animal studies have shown that both pre- and postnatal disturbances in cortisol affect neuronal growth, dendritic arborization and long-term potentiation, although the effects depend on glucocorticoid (GC) dose, brain region and developmental time-window. In addition to affecting structural development, cortisol is involved in memory formation, selective attention and learning, and further helps to control sleep, motivation, mood and fear. These complex processes require precisely regulated GC concentrations, in which the dynamic hormonal oscillations under natural conditions are crucial. Disruption of the natural cortisol secretion and the chronic effect of hormone replacement therapy may thus affect brain function and brain structure in individuals with primary adrenal insufficiency.

This study investigated for the first time brain structure in young adult patients with autoimmune Addison's disease (AAD) ($n = 52$, age 19–43 years) compared to healthy controls ($n = 70$). T1 and diffusion weighted images were acquired on a 3 Tesla magnetic resonance imaging scanner for estimation of grey matter and white matter microstructure. The association between brain structure and disease related factors, self-reported executive function problems and performance on executive function tests were also explored.

Individuals with AAD had a 4.3% smaller total brain volume but no major differences were identified in subcortical structures compared to controls. A higher GC replacement dose was associated with reduced total brain volume (without ventricles) entailing a reduction of 0.73% TBV for every mg/m^2/day increase in GC dose. A higher dose was also associated with smaller volume of the left rostral anterior cingulate cortex, left lingual gyrus and right supramarginal gyrus. A higher daily GC replacement dose in mg/m^2/day was associated with better performance on one of the visuo-spatial working memory tasks (Span Board forward) ($B = 0.28$, $P = 0.030$).

In contrast to patients with CAH, relatively young patients with AAD do not have major structural brain disturbances in grey matter regions or in white matter microstructure except for a smaller total brain volume (at the same level of magnitude as in CAH). The less substantial structural changes in AAD are consistent with the finding that patients performed within the normal range on a variety of cognitive tasks, although females were previously reported to experience problems with executive function in daily life. The association between GC dose and brain volume requires follow-up studies.

7. Oncology and Chronic Disease

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Introduction

As in previous years, most of the included papers concern the medium and long-term complications of cancer therapy. Issues related to long-term surveillance strategies represent an emerging topic of discussion. These highlighted papers have confirmed and consolidated knowledge in the following areas:

- The hormone deficiencies resulting from cancer treatment are predominantly secondary to radiotherapy.
- Chemotherapy tends to cause a minor long-term endocrine damage, although some agents (particularly alkylating agents) have significant gonadotoxic effects.
- Reduced-intensity conditioning regimens have been proposed and are increasingly used in medically fragile patients or in patients with non-malignant diseases to limit the side-effects of hematopoietic stem cell transplantation. Preliminary data suggest that these new regimens reduce the risk of primary ovarian insufficiency.
- Gonadotoxicity of cancer therapy is widely known, but emerging data also suggest a primary effect of cancer on the gonads. Especially neoplasms of the central nervous system and hematological cancers can have a detrimental effect on the gonads. Germ cell abnormalities can be observed even before the start of cancer treatment.
- Immunotherapy and molecular targeted therapies are evolving and represent standard treatment options for some cancers. Checkpoint inhibitors may cause hypophysitis within weeks or months after treatment onset, and may lead to potentially irreversible hypothalamic–pituitary dysfunction. Both tyrosine kinase inhibitors and immune-checkpoint inhibitors have been reported to cause acute thyroid toxicity. The adverse effects of these new drugs need to be extensively studied to inform surveillance recommendations.

Gonadal Function and Fertility Issues in Childhood Cancer Survivors

7.1. Childhood cancer and hematological disorders negatively affect spermatogonial quantity at diagnosis: a retrospective study of a male fertility preservation cohort

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Hum Reprod. 2023 Mar 1;38(3):359–370.

Brief summary: This multicentre retrospective cohort study evaluated testicular structure in 101 boys aged <14 years with solid tumors, CNS tumors, leukemia/lymphoma, or non-malignant hematological disorders, who were admitted for a fertility preservation programme between 2002 and 2018.

The aim of the study was to clarify a possible role of the disease itself on the risk of infertility, independently of cancer therapies. Clinical data, testicular volume and histological staining of testicular biopsies were obtained at cryopreservation and before treatment, in order to evaluate the number of spermatogonia per tubular cross-

section, tubular fertility index, and the most advanced germ cell type before cancer treatment. Control data were extrapolated by previously published studies reporting testicular characteristics in healthy prepubertal boys.

Histological data showed a reduced number of spermatogonia in prepubertal patients with childhood cancer or hematological disorders, compared to healthy controls (48.5% versus 31.0%). The highest proportion of patients with impaired spermatogonial quantity was found in the CNS tumor and hematological disorder groups (56.7% and 55.6%, respectively), including patients with sickle cell disease pre-treated with hydroxyurea (58.3%) as well as those not exposed to hydroxyurea (50%). The disease had also a detrimental effect on spermatogonial distribution and differentiation. The most relevant spermatogonial quantity reduction was observed in patients <7 years of age, regardless of disease, and probably reflects the effects of cancer onset during a sensitive period of testicular development.

Although treatment effects on germ cells have been extensively investigated, limited data are still available regarding the effects of the disease on the prepubertal male gonad. This study suggests that cancer itself, especially CNS tumors, and severe hematological disorders can affect spermatogonial reserve in prepubertal boys. The mechanisms behind these effects are variable and only partially known. Certain mechanisms can be hypothesised. The function of the hypothalamus–pituitary gonadal axis could be negatively affected by hydrocephalus in patients with CNS tumours. Tumour dissemination in solid tumours, pro-inflammatory cytokines or impaired oxygen and nutrient supply to target organs in blood cancer, vaso-occlusion and hydroxyurea treatment in sickle cell disease could disrupt testicular environment and cause germline mutations.

Regardless of the mechanisms involved, appropriate counselling about side effects of the disease itself and its treatment on the reproductive system is mandatory for patients and parents, even before treatment, when the attention is predominantly focused on cancer diagnosis and staging.

7.2. Towards an individualized management of pubertal induction in girls with hypogonadism: insight into the best replacement outcomes from a large multicentre registry

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Eur J Endocrinol. 2023; 188(6):467–476. doi:10.1093/ejendo/lvad056.
PMID: 37232247.

Brief summary: This longitudinal observational multicentre retrospective study, collected data on 95 young prepubertal or early pubertal girls (age >10.9 years, Tanner stage ≤ 2) with premature ovarian failure (POI) or hypogonadotropic hypogonadism (HH). Their hypogonadism was due to different causes and was treated with transdermal 17β-oestradiol, with a follow-up of at least 1 year. The study aimed to identify the most physiological and effective therapeutic scheme.

The study population was divided according to the cause of puberty failure: cancer treatment (CancerHH or CancerPOI), congenital isolated forms of hypogonadotropic hypogonadism (CHH), multiple pituitary hormone deficiency (MPHD), Turner syndrome spectrum (TS), and secondary POI. Despite some differences in the pubertal induction approach between the 4 participating centres, Tanner stage B5 was reached only in 41% of patients who completed pubertal induction, and it was significantly associated with transdermal 17β-oestradiol dose at progesterone introduction and number of dose changes. Uterine longitudinal diameter (ULD) was suboptimal in $>50\%$ of patients. Mean ULD was shorter in the CancerPOI group, despite no difference in the induction regimens used. In the same group, the uterus had also less frequently an adult shape. Pelvic irradiation, in the context of total body-irradiation or pelvic radiotherapy, was the major determinant of impaired ULD, probably as a result of radiation-induced vascular damage and uterine fibrosis.

Final ULD was not significantly different from the ULD at the time of progesterone introduction and was related to oestrogen levels. The authors suggest to perform a pelvic ultrasound before starting progesterone, rather than introduce this hormone after the first vaginal bleeding, as is usually advised. In girls with small ULD at ultrasound, oestrogen therapy should be extended or increased before progesterone introduction to reach more

appropriate uterine size and shape. However, this approach may be not recommended in poor responders with CancerPOI, whose uterine growth might be restricted by pre-existing damage, and in whom the risk of adverse effects related to high oestrogen doses may be more concerning.

Another aim of pubertal induction is to reach an adult height compatible with mid-parental height. As expected, in this study adult height was lower than mid-parental height in TS and CancerPOI groups.

There is still no agreement on the optimal protocol for pubertal induction, particularly with regard to the combination of oestrogen and progesterone and the timing of progesterone introduction, to ensure adequate breast and uterine development and prevent endometrial hyperplasia. Breast and uterine development are considered crucial both for self-esteem and future opportunity of pregnancy, in patients who already have reduced quality of life due to the disease itself. Despite the limitations due to its retrospective design, the sample size of this study is large, and the results provide innovative messages that will be useful for clinical management of girls requiring pubertal induction.

7.3. Hypothalamic–pituitary–gonadal function, pubertal development and fertility outcomes in male and female medulloblastoma survivors: a single centre experience

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Neuro Oncol. 2023; 25(7):1345–1354. doi: 10.1093/neuonc/noad009.
PMID: 36633935.

Brief summary: This single-centre retrospective study analysed gonadal function in 62 patients (41 males) with medulloblastoma, treated between 1987 and 2021. The aim of the study was to characterize gonadal function and identify risk factors for gonadal failure.

Survival rates of patients with medulloblastoma have significantly improved with the combination of many therapeutic approaches, such as adjuvant chemotherapy, craniospinal irradiation (CSI), surgery, and proton-therapy, together with risk-stratified and molecular-matched targeted treatments. However, a concomitant increase in long-term sequelae of treatment has been observed. Long-term adverse effects on the hypothalamic–pituitary–gonadal axis (HPGA) and gonads may manifest as precocious puberty (PP), hypogonadotrophic hypogonadism (HypoH), hypergonadotrophic hypogonadism (HyperH), or transient impairment of gonadal function.

This study, with a mean follow-up of 9.2 and 12 years for males and females, respectively, reported a significant higher frequency of clinical or biochemical gonadal dysfunction in females than in males (76% vs 34%). A higher mean cyclophosphamide-equivalent dose (CED) was positively correlated with HPGA impairment in females but not in males. Males experienced PP and HypoH, while females showed indirect signs of HypoH, or a combination of HypoH and HyperH, as suggested by inappropriately normal FSH associated with other signs of ovarian insufficiency (low AMH levels and/or menstrual cycle abnormalities). Four successful pregnancies, all achieved by *in vitro*fertilization, were reported in females, while none was reported in males.

It is notable that the evaluation of the extent of fertility impairment cannot be reduced to a pregnancy and/or offspring count, because comorbidities, such as cognitive damage or other endocrinopathies, can affect the desire and opportunity to conceive. Comparison between the current results and previous data is not easy, due to the heterogenous sample characteristics (sex, age at diagnosis and at follow-up, pubertal stage at evaluation), inclusion and exclusion criteria, follow-up duration, treatment protocols, time and modality of endocrine evaluation, and definition of HPGA dysfunction.

Although the sample size of this study is not small, the work has some limitations that might partially explain the discordances with previous studies. Most patients were treated in the 1990s and 2000s, the recruitment period (1987–2021) is very long and reflects the evolution in treatment regimens. Clinical parameters were extracted from medical records up to the last visit and patients >18 years were invited to fill in questionnaires regarding hormone replacement therapy, fertility wishes and attempts to conceive, but only a small number of

questionnaires was collected, probably reflecting a cognitive impairment secondary to the disease and/or its treatment. Finally, males were older than females at the last visit (range 9–40 vs 7–28 years), so the evaluation of gonadal function, fertility, and family planning, could have been limited especially in females.

7.4. Reduced-intensity conditioning mitigates risk for primary ovarian insufficiency but does not decrease risk for infertility in pediatric and young adult survivors of hematopoietic stem cell transplantation

Bender JD, Oquendo-Del Toro H, Benoit J, Howell JC, Badia P, Davies SM, Grimley MS, Jodele S, Phillips C, Burns K, Marsh R, Nelson A, Wallace G, Dandoy CE, Pate A, Strine AC, Frias O, Breech L, Rose SR, Hoefgen H, Khandelwal P, Myers KC
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Transplant Cell Ther. 2023 Feb;29(2):130.e1–130.e8.

Brief summary: This single-center, retrospective, cross-sectional study compared the prevalence of gonadal insufficiency and infertility among 58 pubertal, post pubertal and young adult survivors of hematopoietic stem cell Transplantation (HSCT), treated with conditioning regimens of different intensity.

Gonadal insufficiency or infertility affects almost all HSCT survivors who received a myeloablative conditioning (MAC) regimen. In recent years, reduced-intensity conditioning (RIC) regimens have been proposed and are increasingly used in medically fragile patients or in patients with nonmalignant diseases to limit the toxicities associated with HSCT.

In this study, females who received RIC showed a lower prevalence of primary ovarian insufficiency (0% versus 53%), although most females in the RIC (75%) and MAC (93%) groups had low levels of anti-Mullerian hormone (AMH), indicating an impaired follicular reserve. Male RIC recipients less frequently showed testicular failure (5% vs 25% of MAC recipients) and low Inhibin B levels (31% versus 67%), although these differences did not reach statistical significance. Almost all males who underwent semen analysis had azoospermia or oligospermia: 10/11 RIC recipients and 10/10 MAC recipients.

All patients undergoing HSCT should receive counseling about the high risk of gonadal toxicity, and efforts should be made to preserve their fertility before receiving cancer treatment. This study shows that RIC regimens reduce the risk of primary ovarian insufficiency, although both RIC and MAC conferred a significant risk of infertility in survivors of both sexes. The study reports the largest series of semen analyses in young recipients of RIC regimens – unfortunately, azoospermia or oligospermia was found in nearly all (91%) RIC survivors.

Study limitations include the lack of pre-HSCT data on gonadal function to identify patients with a pre-existing gonadal dysfunction, and LH and FSH measurements irrespective of the menstrual cycle in females. Moreover, the cross-sectional study design does not allow longitudinal assessment of gonadal function, given the possible recovery of both gonadal function and fertility many years after transplantation. To this end, the same working group is conducting a larger longitudinal study with a long follow-up, to draw more definitive conclusions about the different short- and long-term gonadotoxicity associated with MAC and RIC regimens.

7.5. Ovarian function and spontaneous pregnancy after hematopoietic stem cell transplantation for leukemia before puberty: An L.E.A. cohort study

Chabut M, Schneider P, Courbiere B, Saultier P, Bertrand Y, Tabone MD, Pochon C, Ducassou S, Paillard C, Gandemer V, Kanold J, Dalle JH, Poiree M, Plat G, Thouvenin S, Plantaz D, Sirvent N, Weinhard S, Berbis J, Baruchel A, Leverger G, Hamidou Z, Auquier P, Michel G
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Transplant Cell Ther. 2023 Jun;29(6):378.e1–378.e9.

Brief summary: This French retrospective observational study evaluated ovarian function, premature ovarian insufficiency (POI) and spontaneous pregnancy in 178 women who had undergone hematopoietic stem cell

transplantation (HSCT) for leukemia before puberty; 116/178 had received total body irradiation (TBI) and 62 had received a busulfan-based conditioning regimen.

Sixty percent of women needed pubertal induction; only 40% had spontaneous menarche and half of them later developed POI, most within 5 years after HSCT. Older age at HSCT was associated with a higher risk of POI. Only 22/178 patients (12%) had one or more spontaneous pregnancy, with a total number of 37 spontaneous pregnancies leading to 17 live births, 14 miscarriages, 4 legal abortions and 2 therapeutic abortions.

Interestingly, this study reported that spontaneous puberty and spontaneous pregnancies are possible after HSCT, even if an older age at HSCT is confirmed to be a major risk factor for ovarian dysfunction. Surprisingly, more than 10% of spontaneous pregnancies ended in a legal abortion, suggesting the option to use a replacement therapy with contraceptive effect in young adults to avoid undesired pregnancy. Strengths of the study are the large and homogeneous population, who all received HSCT before puberty, and the long follow up to 18 years post-HSCT. A possible limitation is that they probably underestimated the total number of planned pregnancies, because mean age at the last evaluation, 24.9 years, is still much earlier than the mean age that healthy women have their first child, now around 29 years in European countries.

7.6. The impact of vincristine on testicular development and function in childhood cancer

Clark I, Brougham MFH, Spears N, Mitchell RT
Hum Reprod Update. 2023 Mar 1;29(2):233–245.

Brief summary: This is a systematic review on the effects of vincristine-containing regimens, commonly considered to have low gonadotoxicity, on human prepubertal testis, after the reports of testicular tissue abnormalities and severely impaired fertility due to vincristine exposure in prepubertal rodents.

Twenty-four studies were identified that reported testicular development and fertility after treatment with vincristine-containing regimens. Only 9/24 studies included a control group and 4/24 provided a sub-analysis of the relative gonadotoxicity of vincristine-based agents. No study reported a negative association between vincristine and the potential to sire a pregnancy. No significant differences in semen analysis (16 studies) were reported in vincristine-exposed patients versus healthy controls. A single study, using FSH levels and semen analysis, reported no significant impact of vincristine on spermatogenesis.

The evidence presented in this review does not support the hypothesis that vincristine exposure adversely affects future fertility in males. Strengths of the study are the clinical relevance of the topic and its comprehensive search strategy. However, existing studies have significant limitations in establishing the specific contribution of vincristine. In most cases, multiple treatment regimens were used, regimens without vincristine were unavailable for comparison, and the cohorts with adequate assessment of fertility outcomes were very limited.

For an appropriate comparison of interventions, as vincristine is usually part of multi-agent regimens, the specific treatment protocol should be well-defined. It is plausible that vincristine have synergistic adverse effects on fertility when used with other chemotherapy agents.

7.7. The uterine volume is dramatically decreased after hematopoietic stem cell transplantation during childhood regardless of the conditioning regimen

Courbiere B, Drikes B, Grob A, Hamidou Z, Saultier P, Bertrand Y, Gandemer V, Plantaz D, Plat G, Poirée M, Ducassou S, Pochon C, Dalle JH, Thouvenin S, Paillard C, Kanold J, Sirvent A, Rousset-Jablonski C, Duros S, Gueniffey A, Cohade C, Boukaidi S, Frantz S, Agopiantz M, Poirot C, Genod A, Pirrello O, Gremeau AS, Bringer-Deutsch S, Auquier P, Michel G
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Fertil Steril. 2023 Apr;119(4):663–672.

Brief summary: This French multicenter prospective study analyzed uterine volume by pelvic MRI in 88 women (age range 18–40 years), who were survivors of childhood acute leukemia treated with hematopoietic stem cell transplantation (HSCT). They were compared to 88 healthy women matched for age and parity.

Conditioning regimens before HSCT included alkylating agents for 34 women and total body irradiation (TBI) for 54 women. Scans were centralized and read double-blinded by two radiologists, unaware of the type of conditioning regimen. 77/88 survivors were considered as having a 'correct hormonal balance' on hormone replacement therapy (HRT).

The mean uterine volume was 80 mL in the control group, but 45 mL (−43%) in HSCT women who had received alkylating agents and only 20 mL (−75%) in women who had TBI conditioning. Among women treated with alkylating agents, uterine volume was significantly lower in those with POI not on HRT, compared to women with a correct hormonal balance (15 vs. 49 mL). In contrast, among women treated with TBI, uterine volume was unaffected by adequacy of oestrogenisation (16 vs. 20 mL). Among alkylating agent treated women, those who had reached menarche at the time of HSCT had a significantly lower uterine volume than other women (27 vs. 72 mL).

A total of 18 women (20.4%) reported at least 1 pregnancy, with 8 live births, including 14 women with at least 1 spontaneous pregnancy leading to 6 live births. There were many more live births in among alkylating agent treated women than TBI treated (60% vs. 11%), and live births after spontaneous pregnancy were seen only among women treated with alkylating agents.

Until recently, only radiotherapy was known to damage the uterus and adversely impact on obstetric outcomes after cancer. This interesting study shows that women have decreased uterine volume whatever conditioning was used before HSCT, even if the effects of alkylating agent regimens seem less severe. Strengths of the study are the homogeneous population of HSCT survivors with standardized conditioning regimens, and the blinded MRI analysis. The evaluation of the relationships between age at HSCT, conditioning regimen, and uterine volume and the chance of spontaneous pregnancy and life birth was limited. Finally, different modalities of hormone replacement therapy and patients' adherence to therapy were not considered potential determinants of uterine volume. It would be interesting to compare this outcome in other conditions that require hormone replacement therapy for puberty induction (e.g., Turner syndrome and congenital hypogonadotropic hypogonadism).

Bone Health in Childhood Cancer Survivors

7.8. Risk and determinants of low and very low bone mineral density and fractures in a national cohort of Dutch adult childhood cancer survivors (DCCSS-LATER): a cross-sectional study

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Lancet Diabetes Endocrinol. 2023 Jan;11(1):21–32.

Brief summary: This Dutch cross-sectional study aimed to assess risk factors for impaired bone mineral density (BMD) and fractures (particularly vertebral fractures) in adult survivors of childhood cancer. The relationships between low BMD (*Z*-score ≤ -1), very low BMD (*Z*-score ≤ -2), fractures, vertebral fractures, and demographic, treatment-related, endocrine, and lifestyle-related factors was analyzed by logistic regression.

BMD was assessed by dual-energy x-ray absorptiometry (DXA). Fractures that occurred at least 5 years after cancer diagnosis were self-reported and further defined using available medical history. Fracture history was compared to population data from the Swedish national registry.

Low BMD was found in 559/1548 patients (36.1%) and very low BMD in 149/1548 (9.6%). Factors associated with low BMD were: male sex, underweight, high carboplatin dose, any cranial radiotherapy, hypogonadism, hyperthyroidism, low physical activity, and severe vitamin D deficiency. Factors associated with very low BMD were: male sex, underweight, cranial radiotherapy, growth hormone deficiency, and severe vitamin D deficiency. The standardized incidence ratio of any first fracture was 3.53 for men, and 5.35 for women, and 33/249 (13.3%) participants had vertebral fractures. Factors specifically associated with vertebral fractures were: older age at follow-up, previous treatment with platinum compounds, growth hormone deficiency, and low physical activity.

It is well known that childhood cancer survivors are prone to skeletal comorbidities in adulthood. In this study, reduced BMD (particularly very low lumbar spine BMD) was found to be a strong indicator for increased fracture risk. However, the cross-sectional design could only assess associations between current risk factors and history of fractures, and not their effects on future fracture risk. Moreover, fragility fractures resulting from low-energy trauma could not be differentiated from high-energy fractures, and vertebral fracture assessment was performed only in a subgroup of patients.

Interestingly, high-dose carboplatin therapy was identified as a new and independent treatment-related risk factor for low BMD. On the contrary, it is noteworthy that treatment with high doses of glucocorticoids (expressed as prednisone-equivalent dose) was not associated with either low or very low BMD or fracture risk. Adult survivors of childhood cancer are confirmed to be at increased risk of vertebral and non-vertebral fractures compared to the general population. Survivors treated with cranial, craniospinal, or total body irradiation show the highest risk and need intensive long-term follow-up of bone health. Surveillance is also recommended for cancer survivors with endocrine disorders as growth hormone deficiency, hyperthyroidism, and hypogonadism. Lifestyle changes, such as increased physical activity and an adequate vitamin intake, could contribute to improved bone health.

Bone Health in Inflammatory Bowel Disease

7.9. Young adult male patients with childhood-onset IBD have increased risks of compromised cortical and trabecular bone microstructures

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Inflamm Bowel Dis. 2023 29(7):1065–1072. doi: 10.1093/ibd/izac181.
PMID: 35993421.

Brief summary: This prospective longitudinal study of childhood-onset inflammatory bowel disease (IBD) analysed areal bone mineral density (aBMD) and alterations of bone microstructure in 49 young adult male patients with childhood-onset IBD, compared to 249 controls from the same region and matched for age, sex and height.

Dual x-ray-absorptiometry (DXA) is routinely used in clinical practice to evaluate aBMD and the related risk of fracture, but it is unable to give information about bone microstructure, as provided by high-resolution peripheral quantitative computed tomography (HR-pQCT). This study evaluated bone structure in IBD patients, using both DXA and HR-pQCT, and correlated bone parameters with skeletal muscle index and physical exercise.

IBD patients had smaller cortical area, lower median total vBMD, thinner median cortical layer, lower median trabecular volume fraction, reduced trabecular thickness and greater median trabecular separation. Patients affected by ulcerative colitis had mostly cortical impairment, while patients with Crohn's disease more likely had trabecular modifications. Skeletal muscle index was positively and independently associated with total, cortical, and trabecular areas, as well as with total vBMD, in both patients and controls. After adjusting for skeletal muscle index, physical exercise was not independently associated with bone geometry, vBMD, or microstructural measurements.

A strength of this study is the concomitant evaluation using both DXA and HR-pQCT. On the other hand, data were lacking on biochemical parameters of bone metabolism, dietary habits, sun exposure, concomitant calcium and/or vitamin D intake. The message that emerges is that it is essential to prevent poor bone quality in

childhood-onset IBD patients by promoting regular physical activity, to increase skeletal muscle and consequently improve bone architecture and strength.

Growth and Puberty in Chronic Kidney Disease

7.10. Estrogen replacement therapy: effects of starting age on final height of girls with chronic kidney disease and short stature

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BMC Pediatr. 2022 Jun 21;22(1):355.

Brief summary: This Iranian open label, quasi-experimental and matched controlled clinical trial included 59 girls with stage III–IV chronic kidney disease (CKD), short stature and delayed puberty. Patients were treated with GH (mean dose 0.05 mg/kg/day) and Ethinyl Estradiol (EE). Initial EE dose was 5 μg/day orally, doubled every 3–6 months to a maximum dose before growth plate closure 30 μg/day, and then increased gradually up to 500 μg/day. EE therapy was started at age 11 years in Group 1 and at age 13 years in Group 2. Group 3 comprised patients with short stature and pubertal delay who did not accept GH or EE therapy until age 15 years, receiving only dialysis and common renal failure treatments.

Earlier age at EE start was associated with taller final height, despite comparable mid-parental heights (mean 154.5, 151.6 and 146.1 cm in groups 1, 2 and 3, respectively). Moreover, lumbar bone mineral density was significantly higher when EE was started at the age of 11 years.

About 50% of children with end-stage renal disease have delayed puberty and reduced final height. Their pubertal growth spurts are blunted and shorter in duration. This study suggests that a relatively early start of estrogen replacement therapy could improve the growth spurt and result in taller final height. If confirmed, this finding would support the message that it is not advisable to delay the start of oestrogen therapy in order to maximise final height.

Using a quasi-experimental design, this empirical interventional study estimated the impact of an intervention on a target population without random assignment. The small sample size is a clear limitation and the different age at start and duration of GH therapy in group 1 and 2 could have influenced final heights. Furthermore, the use of oral EE to induce puberty has now been almost abandoned and replaced by the use of natural oestrogens administered transdermally. The results need to be confirmed in randomized controlled trials involving larger numbers of short girls with CKD and absent puberty, in order to evaluate the real effect of age oestrogen therapy on final height and peak bone mass accrual.

Adrenal Function and Cancer Treatment

7.11. Cortisol response in children with cancer and fever during chemotherapy: A prospective, observational study using random serum cortisol levels

Boekstegers A, Schmidt H, Kurzay M, Vallée T, Jung E, Dubinski I, Maxwell R, Schmid I
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Cancer Med. 2023 Apr;12(8):9247–9259.

Brief summary: This single-centre retrospective study evaluated cortisol responses during febrile episodes in children with cancer. Low cortisol responses were common and were unrelated to current steroid therapy.

Random serum cortisol and ACTH were measured in 75 children and adolescents with cancer while admitted for fever during chemotherapy; 47/75 patients received glucocorticoids as part of their treatment (steroid group). A low cortisol response (LCR) was defined as cortisol <14.6 mcg/dL during fever. In total, 52/75 (69%) patients had LCR

without significant difference between patients receiving steroid therapy or not. In the steroid group, most patients with low cortisol (30/32) had low or normal ACTH; only 2/32 patients had low cortisol and high ACTH.

The high doses of glucocorticoids used in cancer treatment can suppress the hypothalamic pituitary adrenal (HPA) axis for several months, causing impaired stress response to infections. Many authors recommend steroid replacement therapy during periods of serious stress. In this study, the equivalent cumulative dexamethasone (DXM) dose did not correlate with cortisol and ACTH levels during fever. Large inter-individual variability in stress responses was observed, especially when comparing patients who received the same equivalent cumulative steroid dose and had the same interval from the last dose. Patients with LCR did not show a more severe clinical course, with no difference in clinical presentation, duration of fever or hospital stay. In the steroid naive group, all 20 patients with LCR had low or normal ACTH levels and 4/20 patients were treated with posaconazole; none had LCR and high ACTH levels.

Few studies have assessed adrenal function in children undergoing treatment for cancer and none have assessed adrenal function during stress (e.g. fever). The strength of the study is its focused attention on this relevant clinical problem. Unfortunately, the results did not definitively answer the question, as there was no difference in cortisol response even between patients treated with steroids or not. Methodological limitations include the random sampling of cortisol and ACTH, regardless of time of the day and fasting and without contextual evaluation of blood sugar. Likewise, ACTH stimulation testing was not performed to assess adrenal reserve, with the questionable assumption that fever represents a sufficient stimulus for a cortisol response. Similarly, the cut-off values used to define LCR could also be questioned. Finally, no stratification was made by severity of infection (viral or bacterial, localized or generalized).

New prospective studies are desirable in children with cancer during stressful situations to better define the adrenal response to stress, taking into consideration ongoing or prior steroid therapy and the concomitant use of drugs (such as posaconazole) that interfere with adrenal function.

7.12. The assessment of the hypothalamic–pituitary–adrenal axis after oncological treatment in pediatric patients with acute lymphoblastic leukemia

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J Clin Res Pediatr Endocrinol. 2022 Dec 1;14(4):393–401.

Brief summary: Chemotherapy, radiotherapy and corticosteroids used to treat acute lymphoblastic leukemia (ALL) can have endocrine side-effects, such as adrenal insufficiency (AI). This cross-sectional single-centre study aimed to assess AI frequency after completion of ALL therapy, by comparison to healthy controls matched for age and sex, and to identify biomarkers of adrenal function and reserve.

Study patients were at least 16 months post-completion of steroid therapy. Patients and controls had a fasting blood sample collected for plasma cortisol, aldosterone, plasma renin activity (PRA), dehydroepiandrosterone-sulfate (DHEAS), adrenocorticotropic hormone (ACTH), anti-adrenal antibodies, fasting blood glucose, sodium and potassium. The diurnal profile of cortisol and 24-hour urinary excretion of free cortisol was also assessed. Additionally, patients with ALL underwent adrenal imaging and low-dose ACTH testing to assess their adrenal reserve. The study population was divided into 3 groups: 1) up to 2 years remission; 2) 2–5 years remission; 3) >5 years remission.

ACTH testing revealed impaired cortisol responses in 4/43 (9%) ALL survivors, two of whom needed hydrocortisone replacement therapy, and showed also low levels of free urinary cortisol, midnight cortisol and DHEAS. Antibodies against the adrenal cortex were negative and adrenal ultrasound was normal in the entire study population. Urinary free cortisol positively correlated with evening and midnight cortisol levels, DHEAS, systolic and diastolic blood pressure, without differences between groups with different remission time.

Of the treatment-related endocrinopathies, AI is certainly the most important to be diagnosed, due to the risk of adrenal crisis, a life-threatening event. Therefore, it would be very useful to identify a simple and inexpensive clinical screening modality for AI. It is well known that long-duration glucocorticoid therapy causes central AI,

which is usually transient, even if the different impacts of dexamethasone and prednisolone on the severity of adrenal suppression and recovery time have not been clearly demonstrated.

These authors concluded that cortisol levels after ACTH testing, DHEAS, urinary free cortisol and midnight plasma cortisol were the essential indicators of HPA function. Of these, DHEAS levels seem the most practical marker, as it has been shown that DHEAS levels return to normal 2 weeks before complete HPA recovery, and they correlate with urinary free cortisol. The half-life of DHEAS is longer than that of cortisol and diurnal fluctuation is minimal, so that a single sample is sufficient, regardless time of the day or food intake.

Long-Term Surveillance for Endocrine Complications in Childhood Cancer Survivors

7.13. Management of childhood cancer survivors at risk for thyroid function abnormalities: A Delphi study

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Pediatr Blood Cancer. 2022 Dec;69(12):e29942.

Brief summary: This study used a Delphi approach to generate consensus guidelines for screening of asymptomatic childhood cancer survivors for thyroid dysfunction and recommendations for the management of abnormal thyroid screening results.

A Delphi panel of 40 clinical experts (oncologists, endocrinologists, and primary care physicians) participated in three rounds of anonymous questionnaires, formatted as clinical scenarios. Consensus was defined by agreement by at least 90% of the panelists. Disagreement was defined as $<70\%$ panelist agreement.

Panelists reached consensus that childhood cancer survivors treated with radiation, including neck, total body, whole brain or hypothalamic–pituitary axis (HPA), and therapeutic meta-iodobenzylguanidine (MIBG), should undergo lifelong screening by annual TSH and free T4 measurements, starting within one year after the completion of treatment (98% agreement). Panelists disagreed on long-term screening for thyroid dysfunction in childhood cancer survivors with acute thyroid injury after immunotherapy (31%–50%). There was also disagreement on indications for brain (17%–43%) or thyroid (50%–65%) imaging, laboratory tests to assess HPA function (29%–75%), and the appropriate TSH cut-off to start treatment for subclinical hypothyroidism. Lack of evidence was the most frequent reason for not recommending additional testing or medication. Panelists' opinions did not vary by geographic area, specialty, or specific clinical experience.

Thyroid function abnormalities, such as primary or central hypothyroidism and thyroid nodules, are common sequelae of childhood cancer treatment. Evidence is still scarce for the management of thyroid dysfunction among asymptomatic childhood cancer survivors. As expected, panelists agreed that thyroid dysfunction is most associated with the different modalities of radiotherapy involving irradiation of the neck and/or HPA. Conversely, the use of traditional cytotoxic chemotherapy only should not prompt screening for thyroid dysfunction, even if alkylating agents and bleomycin have been associated with hypothyroidism. As this study demonstrates, thyroid ultrasound is still controversial as a screening measure in asymptomatic childhood cancer survivors without suggestive clinical findings (e.g. a thyroid lump on neck palpation). On the other hand, ultrasound is a noninvasive diagnostic tool commonly used to evaluate survivors with biochemical thyroid abnormalities or those with a high risk of thyroid nodules (i.e. patients treated with neck or total body irradiation).

Interestingly, this study, which assessed expert physicians, found that consensus was lacking for the screening of individuals treated with immunomodulatory agents. Growing evidence links immunomodulatory agents to acute thyroiditis during therapy, which can clinically manifest as hyper or hypothyroidism (1). Both tyrosine kinase inhibitors (TKI) and immune-checkpoint inhibitors have been reported to cause acute thyroid toxicity. TKI cause thyroid disruption related to vascular damage and disruption of the transport and metabolism of thyroid hormones. Early autoantibody positivity and elevated serum cytokines are associated with immune-related thyroid dysfunction during treatment with immune-checkpoint inhibitors. There is recent data that TKI-induced

hypothyroidism persists after treatment. However, there is little evidence on the clinical course of thyroiditis induced by immune-checkpoint inhibitors.

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7.14. Hypothalamic–pituitary and other endocrine surveillance among childhood cancer survivors

van Iersel L, Mulder RL, Denzer C, Cohen LE, Spoudeas HA, Meacham LR, Sugden E, Schouten-van Meeteren AYN, Hoving EW, Packer RJ, Armstrong GT, Mostoufi-Moab S, Stades AM, van Vuurden D, Janssens GO, Thomas-Teinturier C, Murray RD, Di Iorgi N, Neggers SJCMM, Thompson J, Toogood AA, Gleeson H, Follin C, Bardi E, Torno L, Patterson B, Morsellino V, Sommer G, Clement SC, Srivastava D, Kiserud CE, Fernandez A, Scheinemann K, Raman S, Yuen KCJ, Wallace WH, Constine LS, Skinner R, Hudson MM, Kremer LCM, Chemaitilly W, van Santen HM

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Endocr Rev. 2022 Sep 26;43(5):794–823.

Brief summary: In this extensive and updated review, the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) report expert consensus-based guidelines that harmonize recommendations for surveillance of endocrine disorders in childhood cancer survivors.

This interdisciplinary panel of 42 international experts formulated new surveillance recommendations for hypothalamic–pituitary (HP) dysfunction. Existing IGHG surveillance recommendations for premature ovarian insufficiency, gonadotoxicity in males, fertility preservation, and thyroid cancer were summarized. The essential points of the review are:

- Whenever possible, young survivors with a history of CNS tumor or surgery near or within the HP area, or treated with radiation therapy to the HP region, should be referred to an endocrinologist or followed by a multidisciplinary team including an endocrinologist due to the high risk of developing HP dysfunction.
- The impact of chemotherapy on endocrine organs is controversial and evidence is scarce. There is low quality evidence that chemotherapy in addition to radiotherapy does not increase the risk of growth hormone deficiency, ACTH deficiency or central precocious puberty in patients with CNS tumors. No studies have robustly analyzed the risk of central hypothyroidism or hypogonadotropic hypogonadism after chemotherapy only.
- Screening should start 1 year after the completion of radiotherapy or from diagnosis in survivors with CNS tumors treated with surgery near or within the HP region.
- Screening should be scheduled every 6 months in prepubertal and pubertal survivors, and every year in post-pubertal and adult survivors.
- Hormonal tests should always include FT4, TSH and morning cortisol. Morning testosterone and LH should be added in adult males; estradiol, FSH and LH should be added in adult females.
- Height velocity and pubertal development are the most important clinical outcomes to evaluate in children.
- Although IGF-I and IGFBP-3 have high specificity to detect growth hormone deficiency in children, their sensitivity in cancer survivors is low, potentially leading to underdiagnosis. This especially happens when early or precocious puberty is present and height velocity is apparently normal, but is inappropriately low for pubertal stage. Pubertal stage and other confounders (i.e., impaired spinal growth due to previous irradiation) should be correctly evaluated when interpreting height velocity in cancer survivors.

Interestingly, the use of provocative tests to diagnose growth hormone deficiency in prepubertal and pubertal cancer survivors is not mentioned in this review. The attention is focused exclusively on height velocity, as the essential parameter to correctly diagnose growth hormone deficiency.

No recommendations are formulated for HP dysfunction following newer treatment modalities, including specific types of radiotherapy (i.e. proton vs photon therapy) or targeted biologicals and immune modulators.

Immunotherapy and molecular targeted therapies are rapidly evolving and becoming standard treatment options for different types of cancer. Checkpoint inhibitors can cause hypophysitis within weeks or months after the initiation of therapy, and the resulting HP dysfunction is potentially irreversible. The adverse effects of these new drugs need to be extensively investigated to inform surveillance recommendations.

7.15. Frailty and sarcopenia within the earliest national Dutch childhood cancer survivor cohort (DCCSS-LATER): a cross-sectional study

van Atteveld JE, de Winter DTC, Pluimakers VG, Fiocco M, Nievelstein RAJ, Hobbelink MGG, Kremer LCM, Grootenhuis MA, Maurice-Stam H, Tissing WJE, de Vries ACH, Loonen JJ, van Dulmen-den Broeder E, van der Pal HJH, Pluijm SMF, van der Heiden-van der Loo M, Versluijs AB, Louwerens M, Bresters D, van Santen HM, Hoefer I, van den Berg SAA, den Hartogh J, Hoeijmakers JHJ, Neggers SJCMM, van den Heuvel-Eibrink MM, Dutch LATER study group
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Lancet Healthy Longev. 2023 Apr;4(4): e155–e165.

Brief summary: This cross-sectional study explored the risk factors for pre-frailty, frailty, and sarcopenia in a cohort of Dutch childhood cancer survivors diagnosed between 1963 and 2001.

Frailty was defined as the presence of at least two (pre-frailty) or three (frailty) of the following criteria: low appendicular lean mass (low percentage of lean tissue in the limbs assessed by dual-energy X-ray absorptiometry, DXA), low muscle strength (measured with a hand-held dynamometer), exhaustion, slowness, or low physical activity measured by a specific scale already validated in patients with chronic diseases. Sarcopenia was defined as the presence of both low appendicular lean mass and low muscle strength (according to the definition of the European Working Group on Sarcopenia in Older People). Associations between these conditions and demographic, treatment-related, endocrine and lifestyle factors were analyzed.

In survivors with complete assessment, the prevalence of pre-frailty was 20.3%, frailty was 7.4%, and sarcopenia was 4.4%. Pre-frailty were associated with: underweight and obesity, cranial irradiation, total body irradiation, cisplatin dose ≥ 600 mg/m^2, growth hormone deficiency, hyperthyroidism, low bone mineral density, and folic acid deficiency. Frailty was associated with: age at diagnosis between 10–18 years, underweight, cranial irradiation, total body irradiation, cisplatin ≥ 600 mg/m^2, higher carboplatin doses, cyclophosphamide equivalent dose ≥ 20 g/m^2, hyperthyroidism, low bone mineral density and folic acid deficiency. Sarcopenia was associated with: male sex, underweight, cranial irradiation, total body irradiation, hypogonadism, growth hormone deficiency, and vitamin B12 deficiency.

This study identifies childhood cancer survivors as individuals at high-risk of pre-frailty, frailty, and sarcopenia, and provide insights into opportunities to decrease the detrimental impact of these conditions on adult life. As expected, cranial and total body irradiation have the greatest adverse impact on body composition and muscle strength. This impact is likely to be even more severe when irradiation occurs during the rapid pubertal growth spurt. Interestingly, body weight seems to be related to body strength in different ways. Obese individuals have a greater risk of disability and impaired physical performance, and obesity is associated with a chronic inflammatory state, which might contribute to frailty. Conversely, underweight and body composition changes characterized by reduced lean mass (sarcopenia) and increased fat mass may be the result of premature systemic ageing.

Most antineoplastic treatments are genotoxic and their association with pre-frailty/frailty is consistent with the increasing evidence that DNA damage is at the basis of the process of ageing and multimorbidity (1). Genome instability, epigenetic changes, impaired mitochondrial function, proteostatic stress, and telomere dysfunction are the biological changes already identified as implicated in the ageing process (2). Early identification of modifiable risk factors as vitamin deficiencies and adequate replacement of hormonal deficiencies, may represent relatively simple interventions to decrease the risk of frailty and sarcopenia. Future studies are needed to evaluate the clinical effectiveness of these measures.

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8. Type 1 Diabetes

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Clinical Trials – New Treatments

8.1. Effect of Verapamil on pancreatic beta cell function in newly diagnosed pediatric type 1 diabetes: A randomized clinical trial

Forlenza GP, McVean J, Beck RW, Bauza C, Bailey R, Buckingham B, DiMeglio LA, Sherr JL, Clements M, Neyman A, Evans-Molina C, Sims EK, Messer LH, Ekhlaspour L, McDonough R, Van Name M, Rojas D, Beasley S, DuBose S, Kollman C, Moran A, CLVer Study Group
JAMA 2023;329(12):990–999.
PMID: 36826844

Brief summary: In this double-blind, randomized clinical trial conducted in 6 US centres, 88 children and adolescents (aged 7–17 years) with newly diagnosed type 1 diabetes (T1D) were randomized to either once-daily oral verapamil or placebo, within 1 month from diagnosis. Treatment with verapamil led to better stimulated C-peptide secretion at 52 weeks post-diagnosis, with levels 30% higher compared to placebo.

Verapamil is a calcium channel blocker, already in use in adults as an anti-hypertensive drug. Of interest, *in vitro* studies showed that verapamil reduces the expression of thioredoxin-interacting protein, a cellular redox regulator contributing to β-cell apoptosis and death induced by glucotoxicity (1). A previous small study in adults with T1D showed a beneficial effect of verapamil in preserving β-cell function (2).

In this clinical trial, verapamil was tested in a small group ($n = 88$) of children and adolescents with newly diagnosed T1D, with the aim of preserving residual β-cell function. Levels of C-peptide remained stable over time in the verapamil group, whereas declined in the placebo group, leading to 30% higher C-peptide level at 52 weeks in the former group. Treatment with verapamil was safe with similar rates of adverse events than placebo.

These findings could have important clinical implications. There is an ongoing focus on preserving β-cell function by intervening soon after diagnosis of T1D. This is based on the association between residual endogenous insulin production and better long-term glycemic outcomes and less complications (3). Verapamil is a marketed low-cost drug, which can be taken orally, once daily, and with a relatively safe profile. These characteristics make it an ideal treatment, less burdensome than other immunotherapies tested in previous or ongoing clinical trials. However, this will likely be a drug to be used as part of a 'combination approach' with other immunomodulatory and immunosuppressive agents, acting directly on the immune system (4). It remains to be clarified whether the effect of verapamil persists and for how long after stopping treatment.

Limitations of the current study are the small sample size, full recruitment was not achieved due to the COVID-19 pandemic, and only children with a weight of at least 30 Kg were included (due to the available verapamil dosing options). Further studies are needed to confirm these findings, expand them to a wider age range, and better define verapamil's safety profile. In this context, the ongoing 'Verapamil SR in Adults with Type 1 Diabetes (Ver-A-T1D)' trial (ClinicalTrial.gov: NCT04545151), assessing the effect of verapamil in adults newly diagnosed with T1D, will provide additional information in future.

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8.2. Closed-loop therapy and preservation of C-peptide secretion in type 1 diabetes

Boughton CK, Allen JM, Ware J, Wilinska ME, Hartnell S, Thankamony A, Randell T, Ghatak A, Besser REJ, Elleri D, Trevelyan N, Campbell FM, Sibayan J, Calhoun P, Bailey R, Dunseath G, Hovorka R, CLOuD Consortium

N Engl J Med 2022;387(10):882–893.
PMID: 36069870

Brief summary: In this multicenter, open-label, parallel-group, randomized trial, 97 adolescents (aged 10–16.9 years) were randomized within 21 days after the diagnosis of type 1 diabetes (T1D) to receive either hybrid closed-loop therapy or standard insulin therapy (control) for 24 months. Although closed-loop therapy was associated with better glycemic outcomes, there were no differences in C-peptide between the two groups.

The Closed Loop from Onset in Type 1 Diabetes (CLOuD) trial addressed the important question whether sustained best-possible glucose control with hybrid closed-loop therapy can preserve residual β-cell function in young people with new-onset T1D. Although the efficacy and safety of several closed-loop insulin delivery systems has been widely proven both in adult and pediatric populations, including very young children [1,2], there are no data on the effect on β-cell function and glycemic outcomes following an early implementation soon after diagnosis.

This trial enrolled adolescents soon after diagnosis and followed up to 24 months to assess residual β-cell function, as measured by stimulated C-peptide during a mixed meal tolerance test. It could not show any benefit of an early implementation of the closed-loop system on C-peptide levels. Indeed, the area under the curve for the meal-stimulated C-peptide level at 12 months was similar in the two groups, as was the decline in C-peptide secretion over the 24-month trial period. Glycemic control was superior in the closed-loop than control group, as indicated by lower HbA1c and a higher proportion of time in the target glucose. It is important to note that the mean HbA1c at 12 months was 52 mmol/mol (6.9%), which is above the UK recommended target (48 mmol/mol, 6.5%), raising the question whether achieving even better glycemic targets is needed to slow the decline in residual β-cell function. This trial was performed in several centres in the UK and Europe. Of interest, similar results were reported by a more recent trial performed in the USA in 113 youth (7–17 years old) newly diagnosed with T1D using a different closed loop system (3). Again, there was no difference in C-peptide between the closed-loop system vs. standard therapy, but closed-loop system led to better glycemic targets.

Overall, these findings indicate the value of early implementation of closed loop systems to achieve good glycemic targets, which are linked to better long-term outcomes. However, the findings do not support the so-called 'glucotoxicity hypothesis', which posits that hyperglycemia plays a key role in the loss of residual β-cell function post-T1D diagnosis.

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Important for Clinical Practice

8.3. What does the licensing of teplizumab mean for diabetes care?

Quinn LM, Swaby R, Tatovic D, Narendran P, Besser REJ, Dayan CM

Diabetes Obes Metab 2023;25(8):2051–2057.
PMID: 36999237

Brief summary: This commentary discusses the implications of the recent licensing of Teplizumab by the Food and Drug Administration (FDA) as the first immunosuppressant for individuals at risk for type 1 diabetes (T1D).

The approval of Teplizumab by the FDA in November 2022, as an intervention to delay the onset of stage 3 T1D (clinical T1D) in adults and children aged 8 or older who have stage 2 T1D (two or more islets autoantibodies and dysglycemia but no symptoms), marks a milestone and new era for the management of T1D. Remarkably, this approval happened just a century after the discovery of insulin.

Teplizumab (brand name Tzield) is a humanised anti-CD3 monoclonal antibody, which was approved based on data from a randomized clinical trial, where 77 first-degree relatives of T1D patients received either teplizumab or placebo, in the form of a single 14-day course of intravenous infusions, to delay progression from stage 2 to stage 3 T1D (2). 45% of people who received teplizumab were later diagnosed with stage 3 T1D compared with 72% of those on placebo. Treatment with teplizumab delayed by 24-32 months the onset of clinical T1D and therefore the need for insulin therapy.

Although the approval of teplizumab is undoubtedly a milestone in T1D, it comes with issues and barriers to overcome. Firstly, teplizumab is an immunosuppressive agent associated with side effects during the treatment phase, including, among others, transient lymphopenia, cytokine release syndrome, risk of infections and hypersensitivity reactions. In addition, Teplizumab treatment consists in a 14-day infusion, and the estimated price is around $200 000. With the availability of a ‘prevention agent’, the focus is now on the need for screening programs to identify people at risk who could receive treatments to modify the early disease course during the asymptomatic phase that precedes symptom onset (1). Population screening for T1D was already reviewed in Yearbook 2022 (3), and has gained even further interest in the last year, with several screening programs being tested in different countries, as well as the planning of pre-T1D registries, and the development of consensus guidelines on screening and subsequent monitoring of individuals identified with autoantibodies (4).

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8.4. Two-age islet-autoantibody screening for childhood type 1 diabetes: a prospective cohort study

Ghalwash M, Dunne JL, Lundgren M, Rewers M, Ziegler AG, Anand V, Toppari J, Veijola R, Hagopian W, Type 1 Diabetes Intelligence Study Group

Lancet Diabetes Endocrinol 2022;10(8):589–596.
PMID: 35803296

Brief summary: Using data from the Type 1 Diabetes Intelligence (T1DI) cohort ($n = 24\,662$), this prospective study aimed to identify optimal ages for initial islet autoantibody (IAb) screening to predict the development of clinical type 1 diabetes (T1D). The identified optimal screening ages were 2 years and 6 years, with sensitivity of 82% and positive predictive value of 79% for T1D by age 15 years.

Screening for T1D is a growing research topic and, although for many years the focus has been on family members of individuals with T1D, it is now extending to general population screening (1). As for any potential new screening program, several aspects should be considered and the classical Wilson & Jungner criteria should be fulfilled (2). One important question is the optimal age for IAb testing.

In this large study combining data from 5 prospective birth cohorts from Finland (DIPP), Germany (BABYDIAB), Sweden (DiPiS), and the USA (DAISY and DEW-IT), 6722 out of the total 24 662 children

were followed up to age 15 years or until onset of T1D, with 672 developing T1D. The main finding was that screening at only two ages (2 years and 6 years) can identify most children (4 out of 5) who will develop T1D by age 15 years. This is important because fewer tests means lower screening costs and greater accessibility. Indeed, for wide implementation of population screening several aspects need to be considered: costs, global access, acceptability, and monitoring and support/education programs for people identified at-risk.

Previous epidemiological data showed that seroconversion occurs during infancy and early childhood with a peak around the age of 1–1.5 years (3). The proposed two-age approach aims to catch as many cases as possible. However, one limitation of first screening at age 2 is that it would miss the small number of very young children who develop T1D before age 2 and who often have the highest rates of DKA at diagnosis.

The study also confirmed that, after the detection of islet autoantibodies, the rate of progression to clinical T1D varies depending on genetics, autoantibody characteristics (number, type, and titre), metabolic parameters and demographic factors (age, race and ethnicity, and sex). Some differences in prediction performance were found among cohorts, likely due to geographical diversity in the genetic and environmental factors, and these factors need to be further investigated in future studies.

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8.5. Impact of the COVID-19 pandemic on long-term trends in the prevalence of diabetic ketoacidosis at diagnosis of paediatric type 1 diabetes: an international multicentre study based on data from 13 national diabetes registries

Birkebaek NH, Kamrath C, Grimsmann JM, Aakesson K, Cherubini V, Dovc K, de Beaufort C, Alonso GT, Gregory JW, White M, Skrivarhaug T, Sumnik Z, Jefferies C, Hörtenhuber T, Haynes A, De Bock M, Svensson J, Warner JT, Gani O, Gesuita R, Schiaffini R, Hanas R, Rewers A, Eckert AJ, Holl RW, Cinek O

Lancet Diabetes Endocrinol 2022;10(11):786–794.

PMID: 36202118

Brief summary: This international multicentre study collected data from 104 290 children and adolescents (6 months-18 years-old), to compare prevalence of diabetic ketoacidosis (DKA) at diagnosis of type 1 diabetes (T1D) before and during the COVID-19 pandemic. Prevalence of DKA at T1D diagnosis increased from 27.3% pre-pandemic to 39.4% during the pandemic, and the increased trends were associated with the pandemic containment measures.

DKA is a life-threatening condition, which often occurs at the onset of T1D in children and adolescents (1). This multinational study provides convincing data on trends in DKA at T1D diagnosis before and during the COVID-19 pandemic, based on data from over 100 000 young people from 13 national diabetes registries (Australia, Austria, Czechia, Denmark, Germany, Italy, Luxembourg, New Zealand, Norway, Slovenia, Sweden, USA [Colorado], and Wales). The pre-COVID prevalence (2009-2016) of DKA at the onset of T1D was 27.3%, with a mean annual increase of 1.6%. Remarkably, it rose to 39.4% in 2020 and 38.9% in 2021. Of note, the prevalence of DKA was associated with the pandemic containment measures, whereas there was no association with the severity of COVID-19 infection in that setting.

These findings highlight how the pandemic exacerbated an already increasing trend in the prevalence in DKA, and this was likely due to delays in seeking medical attention, due to restrictions in place, and fear of contracting COVID-19 infection. However, additional factors, such as the roles of a viral infection in triggering clinically manifested diabetes in susceptible individuals, and/or infection-related insulin resistance/inflammation in precipitating disease onset should also be considered (2). The increasing trends in DKA already pre-pandemic highlight the need of further efforts to improve recognition of the presenting signs/symptoms by individuals, caregivers and healthcare professional, particularly through awareness campaigns (1). These data also support

the ongoing discussion on the value of population screening for T1D and associated monitoring and education programs, as an effecting way to prevent rates of DKA and lead to better outcomes (3).

Of note, there is an ongoing debate on whether SARS-CoV2 itself might directly attack pancreatic β-cells, particularly after a very recent systematic review and meta-analysis reported an alarmingly increase (by 16-28%) in the incidence of T1D in the two years following the COVID-19 pandemic (4).

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8.6. Continuous glucose monitoring versus blood glucose monitoring for risk of severe hypoglycaemia and diabetic ketoacidosis in children, adolescents, and young adults with type 1 diabetes: a population-based study

Karges B, Tittel SR, Bey A, Freiberg C, Klinkert C, Kordonouri O, Thiele-Schmitz S, Schröder C, Steigleder-Schweiger C, Holl RW
Lancet Diabetes Endocrinol 2023;11(5):314–323.
PMID: 37004710

Brief summary: In this large registry-based study, including 32 117 children and young people (aged 1.5–25 years) with type 1 diabetes (T1D), the use of continuous glucose monitoring (CGM) was associated with decreased rates of diabetic ketoacidosis (DKA) and severe hypoglycemia. Of interest, some CGM metrics predicted risk for these complications.

CGM systems are now widely used by children with T1D, and there is evidence both from clinical trials and real-word data that their use is associated with better glycemic outcomes (1). Indeed, international guidelines strongly recommend CGM use in all children, adolescents, and young adults with T1D (2).

This study investigated whether rates of acute complications of T1D, such as DKA and severe hypoglycemia, are lower in young people using CGM. The study reports real-world data collected from the large Diabetes Prospective Follow-up initiative (DPV), with available data from 511 paediatric diabetes centres across Austria, Germany, Luxembourg, and Switzerland. The study compared 10 883 participants using CGM to 21 234 using fingerstick blood glucose monitoring and clearly showed that the use of CGM was associated with lower rates of both severe hypoglycemia and DKA. More importantly, those using CGM had nearly half the incidence of hypoglycemic coma and severe DKA. Another key finding was that CGM metrics can help predict risk of these complications. Specifically, $\geq$4% of time below target glucose range ($<$3.9 mmol/l) and high glycemic variability (coefficient of variation $\geq$36%) were associated with higher risk of severe hypoglycemia. Mean sensor glucose $\geqq$10.0 mmol/L or percentage of time in target glucose range (3.9–10 mmol/l) $<$50%, and above glucose range $\geq$50% were identified as robust risk markers of DKA.

Overall, this large study provides further support for the use of CGM in young people with T1D and highlights the value of CGM metrics to identify individuals at particular risk of acute severe complications who would benefit from timely interventions. However, although technology has definitely revolutionised the management of T1D, there are still barriers, such as costs and inequalities primarily associated with deprivation and ethnicity (3), which although not considered in the current study, need to be overcome to allow a wider access to the best available technology for all young people with T1D.

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8.7. Diabetes stigma and clinical outcomes in adolescents and young adults: The SEARCH for diabetes in youth study

Eitel KB, Roberts AJ, D'Agostino R, Barrett CE, Bell RA, Bellatorre A, Cristello A, Dabelea D, Dolan LM, Jensen ET, Liese AD, Mayer-Davis EJ, Reynolds K, Marcovina SM, Pihoker C

Diabetes Care 2023;46(4):811–818.
PMID: 36883290

Brief summary: Using data collected from 1608 adolescents and young adults (aged 10–24.9 years) with type 1 or type 2 diabetes recruited in the SEARCH for Diabetes in Youth study, this study assessed the cross-sectional association between diabetes stigma and diabetes outcomes. Diabetes stigma was more common among females and was associated with suboptimal glycemic outcomes and acute and chronic complications.

Stigma, defined as a negative social judgment that leads to unwarranted rejection or exclusion, is common in people with chronic diseases (1). Diabetes-related stigma refers to negative social attitudes towards diabetes that can lead people experiencing blame, stereotyping, rejection, exclusion, and/or discrimination (2). Previous studies have reported a high frequency of stigma among adults with diabetes, but there are limited data on this topic for younger populations (2).

In this study of the large SEARCH for Diabetes in Youth cohort of young people with type 1 and type 2 diabetes, a five-question survey, specifically designed for this study, was used to assess the frequency of perceived diabetes-related stigma, with three questions assessing perception or experience of diabetes stigma and the other two questions exploring consequences of diabetes stigma. One key finding was that diabetes stigma was more common among females. This is in line with the higher burden of other psychosocial comorbidities, such as depression, diabetes distress, and disordered eating behaviors in females. The study also showed a strong association between diabetes-related stigma and elevated HbA1c, which is a common finding in adolescents and young adults. In contrast, no association was found with education, employment status, health insurance, or use of technology. In participants with T1D, diabetes-related stigma was also independently associated with higher rates of recent episodes of hypoglycemia and ketoacidosis, as well as with retinopathy and nephropathy.

This study highlights the importance of addressing diabetes stigma in comprehensive diabetes care in adolescents and young adults with diabetes, who are particularly vulnerable due to age-specific characteristics, such as the focus on peer relationships, personal identity, and establishing autonomy (3). Stigma not only impact on the psychological well-being of young people with diabetes but represents an additional risk factor for suboptimal diabetes outcomes.

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New Paradigms

8.8. Type 1 diabetes as a prototypical condition challenging what we know about sleep

Gregory AM, Rutter MK, Ware J, Madrid-Valero JJ, Hovorka R, Buysse DJ

Sleep 2022;45(11):zsac194.
PMID: 36130325

Brief summary: This 'Letter to the Editor' provides a critical review on how behavioural and physiological aspects of type 1 diabetes (T1D) and its management can interfere with standard principles of good quality and duration of sleep.

Quality of sleep is important for general human functioning, cognitive performance, emotional well-being, as well as immune function and cardiovascular health (1). The benefits of optimal sleep apply to everyone and even more to people with T1D, given the known associations with glucose regulation and cardiovascular health (1). However, individuals with T1D do not meet the recommended duration of sleep for age (2).

Sleep and T1D can be seen as 'jarring bedpartners' due to several reasons. T1D is a complex medical condition requiring frequent glucose monitoring and insulin administration. Episodes of acute hyperglycemia or hypoglycaemia can occur at any time of the day. In young children, risk of hypoglycemia is particularly high at night, and this often causes fear and lack of sleep in their caregivers. Of note, optimal sleep occurs when we feel safe, and this can be difficult for people with T1D or their caregivers. When treatment decisions need to be made at nighttime this might be suboptimal due to sleep deprivation of sleep inertia. Use of diabetes technology, such as continuous glucose monitoring, can interfere with sleep. Although technology has undoubtedly improved diabetes management, nighttime alarms, signalling abnormal glucose levels, or system malfunctioning, can lead to sleep disruption (3). In addition, standard approaches used to improve sleep, such as the advice to parents to let babies to 'self soothe' can be problematic for caregivers of children with T1D, who need to provide support overnight to their children. Techniques such as behavioural therapy including promotion of sleep restriction to produce more consolidated sleep could be unsafe for people with T1D.

More research into sleep and T1D is advocated by the authors, including qualitative studies to gain more insight into causes and consequences of sleep disturbances, and attitude and sleep priorities for people and caregivers. Longitudinal studies looking at associations between sleep and diabetes outcomes are also needed, as well as intervention studies aiming at improving sleep quality.

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8.9. Elevations in blood glucose before and after the appearance of islet autoantibodies in children

Warncke K, Weiss A, Achenbach P, von dem Berge T, Berner R, Casteels K, Groele L, Hatzikotoulas K, Hommel A, Kordonouri O, Elding Larsson H, Lundgren M, Marcus BA, Snape MD, Szypowska A, Todd JA, Bonifacio E, Ziegler AG, GPPAD POInT Study Groups

J Clin Invest 2022;132(20):e162123.

PMID: 36250461

Brief summary: In this longitudinal study, blood glucose trajectories and their relationship with autoantibodies appearance were assessed in 1050 children with high genetic risk of type 1 diabetes (T1D) between 4 months and 3.6 years. Post-prandial blood glucose levels increased around 2 months prior to autoantibody seroconversion, with further increases thereafter, suggesting that islet autoimmunity co-occurs or follows insults on the β-cells.

This recent study proposes a re-assessment of the classical cascade of events in the natural history of T1D, which states that genetically susceptible individuals develop islet autoimmunity, followed after a variable time by the appearance of glucose abnormalities (1). The authors measured pre- and postprandial blood glucose concentrations in a large number of children at increased genetic risk of developing T1D, recruited in the Primary Oral Insulin Trial (POInT) (2). The findings reveal a dynamic process of glucose regulation during infancy and early childhood. Of interest, pre-prandial glucose levels showed a U-shaped curve, with the highest levels in infancy, a nadir at 12-18 months of age, and a subsequent increase up to 3.5 years. The finding of decreasing glucose levels was novel, and the

reported patterns likely reflect changes in the pancreatic islet cells and suggest the need to study physiological maturation of glucose metabolism and β-cell function earlier in life. Glucose trajectories were influenced by sex, BMI, and genetic factors, including the T1D susceptibility genotype at the insulin gene, *INS*.

Increased blood glucose concentrations were observed in children who developed islet autoantibodies, with the 30-minute post-prandial blood glucose levels being 0.3 mmol/l (6 mg/dl) higher already 2 months before autoantibody seroconversion. After seroconversion there was a further increase in post-prandial and subsequent rises in pre-prandial values. These findings suggest that the onset of early islet autoimmunity is associated with or preceded by insults or changes to the pancreatic β-cells that deregulate glucose metabolism.

Overall, the study highlights that impaired glucose homeostasis is present earlier in the T1D disease process than previously thought. There is a need for further research to understand the molecular basis of the early deterioration of glucose homeostasis and β-cells. Future studies should assess fasting glucose and consider a better standardization for post-prandial glucose measurements, as well as included children seroconverting at a later age, to assess if glucose changes occur independently of age.

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8.10. Functional and metabolic alterations of gut microbiota in children with new-onset type 1 diabetes

Yuan X, Wang R, Han B, Sun C, Chen R, Wei H, Chen L, Du H, Li G, Yang Y, Chen X, Cui L, Xu Z, Fu J, Wu J, Gu W, Chen Z, Fang X, Yang H, Su Z, Wu J, Li Q, Zhang M, Zhou Y, Zhang L, Ji G, Luo F

Nat Commun 2022;13(1):6356.
PMID: 36289225

Brief summary: Using in-depth multi-omics analyses of human type 1 diabetes (T1D) samples, the authors profiled gut microbial functional and metabolic alterations. The T1D microbiota showed decreased butyrate production and bile acid metabolism and increased lipopolysaccharide (LPS) biosynthesis. Fecal microbiota transplantation in animal models proved that T1D gut microflora is a causative factor in the regulation of glucose metabolism.

The etiology of T1D is known to be multifactorial, consisting of genetic susceptibility and environmental factors (1). Among the environmental factors, there is an increasing interest in the role of gut microbiota alterations. Intestinal commensal bacteria play a crucial role in host physiology in health and disease by regulating endocrine and immune functions. An aberrant gut microbiome structure and function have been documented prior and during T1D onset (2), but an extensive profiling of functional and metabolic abnormalities of gut microbiota in T1D is lacking.

This study reveals a unique T1D-associated gut dysbiosis, characterized by increased LPS biosynthesis and decreased butyrate production and bile acid metabolism. It also identified a cluster of nine bacterial species and nine fecal metabolites yielding excellent discriminatory power for new-onset T1D. It also showed that human T1D gut microbiota induced elevated fasting glucose levels and reduced insulin sensitivity when transplanted to antibiotic-treated mice; and butyrate and LPS had protective and destructive effects, respectively, on glucose metabolism and islet structure and function in a mouse model of streptozotocin-induced diabetes.

Overall, these data combined with previous findings support a role for specific gut bacteria and metabolites as novel diagnostic and therapeutic targets for T1D (3). One potential therapeutic option could be blocking LPS production and entrance into the blood.

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New Mechanisms

8.11. Soluble RAGE prevents type 1 diabetes expanding functional regulatory T cells

Leung SS, Borg DJ, McCarthy DA, Boursalian TE, Cracraft J, Zhuang A, Fotheringham AK, Flemming N, Watkins T, Miles JJ, Groop PH, Scheijen JL, Schalkwijk CG, Steptoe RJ, Radford KJ, Knip M, Forbes JM

Diabetes 2022;71(9):1994–2008.
PMID: 35713929

Brief summary: using a murine model of diabetes and *ex vivo* experiments in human T-cell cultures, this study showed that short-term administration of an antagonist to the receptor for advanced glycation end products (sRAGE) modulates functional T regulatory cells (Treg) expansion and thus prevents diabetes.

This study provides further support for a role of the advanced glycation end-product (AGEs)-AGE receptor (RAGE) pathways in the pathogenesis of type 1 diabetes (T1D) (1). RAGE, a member of the immunoglobulin superfamily, is considered a proinflammatory receptor expressed in various cells, including T cells involved in the pathogenesis of T1D (2). Greater RAGE expression, leading to enhanced T-cell cytokine production and survival, has been reported on T cells from at risk individuals who progress to clinical T1D (3). Circulating RAGE isoforms, which lack the transmembrane domain, collectively termed soluble RAGE (sRAGE), are thought to have anti-inflammatory properties by competing with membrane bound RAGE for ligand binding (2). Low sRAGE concentrations are associated with higher risk for developing T1D (1), leading to proposals that sRAGE may be a potential novel therapeutic target to prevent T1D.

In this study, reduced circulating sRAGE concentrations in a murine model were targeted by short-term (2 weeks) administration of recombinant sRAGE. Treatment increased Tregs within the islets, pancreatic lymph nodes, and spleen, along with islet insulin expression and function. These beneficial effects were abrogated by Treg depletion and were shown to be dependent on antagonizing RAGE in a RAGE knockout mouse model. *Ex vivo*, human Tregs treated with a RAGE ligand downregulated several genes implicated in suppression, migration, and Treg homeostasis and these effects were reverted by sRAGE.

Although further confirmatory studies are needed, these results pinpoint a novel immunomodulatory role of sRAGE and suggest that treatment with sRAGE may be a promising T1D preventative therapy.

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8.12. ZnT8 loss-of-function accelerates functional maturation of hESC-derived beta cells and resists metabolic stress in diabetes

Ma Q, Xiao Y, Xu W, Wang M, Li S, Yang Z, Xu M, Zhang T, Zhang ZN, Hu R, Su Q, Yuan F, Xiao T, Wang X, He Q, Zhao J, Chen ZJ, Sheng Z, Chai M, Wang H, Shi W, Deng Q, Cheng X, Li W

Nat Commun 2022;13(1):4142.
PMID: 35842441

Brief summary: In this experimental study, genome editing and *in vitro* pancreatic differentiation of human pluripotent stem cells (SC) were used to generate *ZNT8* loss-of-function (LOF) SC-β-cells. These cells showed

accelerated functional maturation, increased insulin secretion and improved resistance to metabolic stress. Transplantation of *ZnT8* LOF SC-β-cells into mice with preexisting diabetes significantly improved their glucose levels.

Use of human embryonic SC-β-cells is a potential therapy that holds great promise for the treatment of type 1 diabetes (T1D) (1). The unlimited source provided by SC-β-cells could overcome the scarcity in cadaveric donor tissues for islet transplantation. However, the widespread use of SC-β cells is still limited by their immature function and fragility in a hostile environment. In this respect, the use of gene editing has been proposed as a tool to enhance engraftment, function, and survival of SC-β cells (1).

In this study, gene editing was applied and directed to the *ZnT8* gene. This target was chosen based on genetic findings that LOF mutations in *ZNT8* decrease the risk of diabetes and improve insulin secretion (2). ZnT8 is a zinc transporter, mainly expressed in pancreatic β cells, which regulates zinc influx into insulin granules and facilitates insulin hexamer formation (3). There is evidence suggesting that excessive zinc disrupts the homeostasis of the endoplasmic reticulum (ER) and mitochondrial function, resulting in impaired cell function and viability. Therefore, acting on zinc levels, through *ZNT8* gene editing, could represent a way of improving SC-β cells function and survival. Indeed, the generated *ZnT8* LOF SC-β cells showed greater insulin secretion capacity, as well as resistance to glucotoxicity/lipotoxicity-triggered cell death, by alleviating ER stress through modulation of zinc levels. *ZnT8* LOF also led to improved glycemic control in a mouse model of diabetes.

Overall, these findings highlight the beneficial effects of *ZnT8* LOF on the functional maturation and survival of SC-β cells, which are a potential source for cell replacement therapies. Several aspects remain to be clarified through further research, such as how decreased intracellular zinc alleviates ER and mitochondrial dysfunction, and also the effect of different variants of the *ZnT8* gene, which previously showed discordant associations with zinc levels and protection from diabetes.

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8.13. Low-dose IL-2 reduces IL-21(+) T cell frequency and induces anti-inflammatory gene expression in type 1 diabetes

Zhang JY, Hamey F, Trzupek D, Mickunas M, Lee M, Godfrey L, Yang JHM, Pekalski ML, Kennet J, Waldron-Lynch F, Evans ML, Tree TIM, Wicker LS, Todd JA, Ferreira RC

Nat Commun 2022;13(1):7324.
PMID: 36443294

Brief summary: This study used high-resolution single-cell multiomics and flow cytometry on blood samples from patients with type 1 diabetes (T1D) to examine the effects of low-dose recombinant IL-2 (iLD-IL-2). Administration of iLD-IL-2 expanded thymic-derived FOXP3$^+$HELIOS$^+$ Tregs and CD56^{bright} NK cells, reduced frequency of IL-21-producing CD4$^+$T cells, and induced long-lived anti-inflammatory transcriptional changes in all T and NK cell subsets.

iLD-IL2 has been used in several inflammatory and autoimmune conditions based on the predominant stimulation of CD4$^+$FOXP3$^+$T regulatory cells (Tregs) that promote self-tolerance and prevent autoimmunity (1). Non-clinical, preclinical and clinical data suggest that iLD-IL2 therapy could prevent pancreatic β-cells destruction by increasing the number of functional Tregs (1). Small studies in adults with T1D have determined minimal effective doses and optimal frequency of iLD-IL2 administration [2,3].

This experimental study confirmed the safety of iLD-IL2, as indicated by a preferential expansion of HELIOS$^+$Tregs and CD56^{br}NK cells and absence of cytotoxic gene expression signature in any T cell subset. Of particular interest was the effect of iLD-IL-2 in reducing IL-21-producing T cells. IL-21 has been implicated in the

pathogenesis of several autoimmune diseases, such T1D, systemic lupus erythematosus, rheumatoid arthritis, and psoriasis, and therefore the study findings further support the use of iLD-IL-2 use in these conditions. Furthermore, iLD-IL-2 induced prolonged transcriptional changes with anti-inflammatory properties in both T and NK cell subsets that were found one month after cessation of treatment. This suggests a longer treatment effect, which might be due to a binding of IL-2 to the extracellular matrix, leading to prolonged release even after the end of treatment.

Although the mechanisms underlining the long-lived gene expression signatures need to be clarified, this study provides further support for the safety and efficacy of iLD-IL-2. ILD-IL2 could be used as a combination strategy, along with other immunomodulatory/immunosuppressant agents, in early stages of clinical T1D or even to prevent diabetes in at risk individuals. Further support for the role of iLD-IL2 will hopefully come from the incoming results of a recently completed randomised controlled trial of iLD-IL2, administered every 3-4 days, to preserve β-cell function in children and adolescents with newly diagnosed T1D (4).

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New Hopes

8.14. Glucose-responsive microneedle patch for closed-loop dual-hormone delivery in mice and pigs

Yang C, Sheng T, Hou W, Zhang J, Cheng L, Wang H, Liu W, Wang S, Yu X, Zhang Y, Yu J, Gu Z

Sci Adv. 2022;8(48):eadd3197.

PMID: 36449622

Brief summary: In this experimental study, a transdermal polymeric microneedle (MN) patch for glucose-responsive closed-loop insulin and glucagon delivery was developed. In chemically-induced type 1 diabetes (T1D) mouse and minipig models, this glucose-responsive dual-hormone MN patch demonstrated tight regulation in blood glucose.

The interplay between insulin and glucagon is essential for blood glucose regulation in individuals with or without diabetes. Over the last decades several glucose-responsive insulin delivery systems have been developed to improve glucose management in people with diabetes. The available systems are mainly single hormone and based on insulin delivery modulated by sensor glucose levels (1). There is growing interest in the development of dual-hormone (insulin and glucagon) closed loop systems in order to minimize the risk of hypoglycemia, which is still a barrier against the achievement of optimal glycemic targets (1).

In this study, a unique glucose-responsive MN patch, loaded with both insulin and glucagon, was developed and tested in mouse and pig models of diabetes. A key component of the MN patch was the glucose-responsive phenylboronic acid unit, which binds to glucose to reversibly shift the net charge of the entire polymeric matrix within the MN. This allows a modulated release ratio of the negatively charged insulin or the positively charged glucagon from the patch in response to glucose levels. On exposure to hyperglycemia, the increased negative charge density due to the formation of the glucose-borate complex enhances electrostatic repulsion between the negatively charged insulin and the polymeric matrix, leading to insulin release, whereas the positively charged glucagon remains in the MN. Conversely, on exposure to hypoglycemia, the net charge of the polymeric matrix switches from negative to positive due to the disassociation of the glucose-borate complex, thus inhibiting insulin release and facilitating glucagon release. The system was validated in streptozotocin-induced diabetes mouse and minipig models, confirming that the dual hormone MN patches could mimic the function of pancreatic islets to achieve ideal glycemic targets and reduce the risk of hypoglycemia.

This study is an important step towards the development of a dual glucose responsive closed loop system, to be potentially used in people with T1D to allow a tighter regulation of glucose levels and particularly to reduce episodes of hypoglycemia.

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8.15. Exocrine pancreas regeneration modifies original pancreas to alleviate diabetes in mouse models

Kou X, Liu J, Wang D, Yu M, Li C, Lu L, Chen C, Liu D, Yu W, Yu T, Liu Y, Mao X, Naji A, Cai T, Sun L, Shi S

Sci Transl Med 2022;14(656):eabg9170.
PMID: 35921475

Brief summary: In this experimental study, pancreas-derived mesenchymal stem cells (PMSCs) were implanted into the kidney capsule of mice with streptozotocin (STZ)-induced diabetes. PMSCs led to increased levels of IL-6 in T-helper 1 and T-helper 17 cells, which transiently activated tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), which in turn decreased levels of interleukin-17. This was associated with exocrine pancreas regeneration and rescue of β-cells from immune-mediated damage.

Mesenchymal stem cells (MSC) have been used as a therapeutic approach to treat various autoimmune diseases, including diabetes (1). Through a series of elegant studies in mouse models of diabetes and *in vitro* cell cultures, this study showed the importance of pancreas-specific MSC (PMSC) to regenerate the exocrine pancreas, which in turn was able to protect β-cells, through a network of cytokines. PMSCs were implanted in the mouse kidney capsula and were able to primarily regenerate the exocrine pancreas. In addition, in the STZ-treated mouse model, ectopic pancreas regeneration rescued islet size and insulin producing cells, and corrected hyperglycemia. These effects persisted even after removal of the PMSC implants.

The effects of PMSCs on the endocrine pancreas appeared to be mediated through transient changes in a number of cytokines. In particular there was an increased production of IL-6 in the implants, which transiently activated TNF-α and IFN-γ. The latter had a major role in decreasing levels of IL-17, whose levels were high in this diabetes model, and were believed to drive β-cell apoptosis. The key roles of the changes in IL-6, TNF-α and IFN-γ were confirmed by studying knockout mice, in which PMSC implantation failed to rescue insulin production and hyperglycemia.

These findings are fascinating as they pinpoint the role of specific cytokines in maintaining pancreatic health and highlight their potential role in cell-based therapy. However, there are several aspects to be clarified, including the proposed mechanism through which exocrine pancreas regeneration protects the endocrine pancreas, and the specific actions of changes in cytokines. In addition, it remains to be clarified if the same model and mechanisms found in mice can be translated in humans, as there are differences in our immune systems.

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8.16. Engineering the lymph node environment promotes antigen-specific efficacy in type 1 diabetes and islet transplantation

Gammon JM, Carey ST, Saxena V, Eppler HB, Tsai SJ, Paluskievicz C, Xiong Y, Li L, Ackun-Farmmer M, Tostanoski LH, Gosselin EA, Yanes AA, Zeng X, Oakes RS, Bromberg JS, Jewell CM

Nat Commun 2023;14(1):681.
PMID: 36755035

Brief summary: In this experimental study, immunomodulatory microparticles, consisting of encapsulating self-antigens with rapamycin, were injected into mouse lymph nodes to protect against type 1 diabetes (T1D) and islet graft rejection. Antigens and rapamycin were both required for maximal efficacy and they induced durable tolerance, accompanied by expansion of antigen-specific regulatory T cells (Treg) in both treated and untreated lymph nodes.

Antigen-specific tolerance is a promising approach to prevent autoimmune diseases and allograft rejections, and aims to overcome the complications of extensive immunosuppression (1). This approach involves vaccine-like treatments that deliver autoantigens together with regulatory immune components to redirect autoantigen-specific T cells toward populations with regulatory immune roles (2). Given the key role of lymph nodes in immune responses, targeting this immune tissue with antigen-specific therapy appears an ideal approach.

These authors performed a series of experiments using pre-clinical models of CD4- and CD8-mediated T1D, and an allogenic islet transplant model in which full MHC mismatched donor islets were transferred to recipients after islet depletion. Direct lymph node injection of microparticles containing rapamycin (an immunomodulator) and islet autoantigens or allo-antigens were able to prevent T1D and islet graft rejection. Key results of this approach were that the microparticles induced a systemic antigen-tolerance with expansion in Treg in both treated and untreated lymph nodes. It also led to the development of tolerogenic structural microdomains in lymph nodes promoting memory markers among antigen-specific Tregs. Of note, there were no systemic immunosuppressive effects.

Altogether these data suggest a new model to promote long-lasting and selective tolerance in the context of T1D, with reduced or even absent side effects since it does not require any systemic or peripheral delivery. This model could also be translated to other autoimmune diseases and to prevent allograft rejection.

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New Genetic Approaches

8.17. Genome-wide aggregated trans-effects on risk of type 1 diabetes: A test of the "omnigenic" sparse effector hypothesis of complex trait genetics

Iakovliev A, McGurnaghan SJ, Hayward C, Colombo M, Lipschutz D, Spiliopoulou A, Colhoun HM, McKeigue PM

Am J Hum Genet 2023;110(6):913-926.
PMID: 37164005

Brief summary: Using data on 4964 type 1 diabetes (T1D) cases and 7497 controls, this study assessed whether the effect of common genetic variants (SNPs) on risk of T1D is mediated through *trans*-effects on the expression of core genes. Nine putative core genes were identified, all implicated in immune system regulation. In addition, four T1D-associated genomic regions were identified as master regulators that have trans-effects on gene expression.

The 'omnigenic' model has been proposed as a framework to understand the polygenic architecture of complex traits revealed by genome-wide association studies (GWAS) (1).

This hypothesis postulates that the polygenic effects of common SNPs trait are mediated through weak *trans*-effects (i.e. effects on other distant genomic locations) on the expression of a relatively sparse set of effector ('core') genes, which in turn have a direct effect on the outcome complex disease or trait. In the context of T1D, previous genetic studies have mainly focused on the impact of common variants (SNPs) on genes located nearby, known as *cis*-effects.

These authors tested whether the 'omnigenic' hypothesis applies to T1D, using a large case-control dataset including people with T1D from the Scottish Diabetes Research Network Type 1 Bioresource and individuals

without diabetes from the Generation Scotland study. Published T1D GWAS summary statistics were used to calculate aggregated (excluding the HLA region) *trans*-scores for gene expression in blood. This identified nine candidate genes as putative core genes for T1D: seven of them are involved in the induction and activity of T regulatory cells (*FOXP3, CTLA4, STAT1, CD247, IL10RA, MEOX1, CD5)* and two (*CD1E, LGALS3BP*) are involved in the innate and acquired immune response to lipids. Of note, most of these genes were not detected by the conventional T1D GWAS analysis.

These findings support a new genetic analytical approach to identify the genetic drivers of T1D susceptibility and it could be applied also to other diseases. As the authors discuss, replication of the results is needed and a wider application of this approach will require availability of more comprehensive summary-level results from large GWAS of gene expression in various tissues as well as measurements of genetic effects on splicing variants and post-transcriptional modifications.

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9. Obesity and Weight Regulation

Martin Wabitsch, Stephanie Brandt-Heunemann, Christian Denzer, Melanie Schirmer, Julia von Schnurbein, Daniel Tews, Stefanie Zorn

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P1. Preface

In analogy to previous years, in this year's chapter we can only present 1.5% of the acquired publications (1384) according to our search criteria in Pubmed for the Yearbook 2023. The last year has again been extremely exciting for the field of obesity and weight regulation and it was a significant step into the future in terms of scientific output.

Of particular importance are the new pharmacological therapeutic approaches for adolescents with obesity (see Weghuber et al., N Engl J Med 2022, 387(24):2245–2257. doi: 10.1056/NEJMoa2208601).

The Yearbook chapter 2023 on obesity and weight regulation comprises further exciting articles covering a broad research area.

An Old Question: How to Define Obesity in Children

9.1. Longitudinal changes in various BMI metrics and adiposity in 3- to 7-year-olds

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Pediatrics. 2022 Dec 1;150(6):e2022058302.
doi: 10.1542/peds.2022-058302.
https://pubmed.ncbi.nlm.nih.gov/36443241/.

Brief summary: Freedman *et al*. studied a cohort of n = 343 preschool children (age 3-7 years) with a wide range of BMI to test the ability of 3 BMI metrics (BMI z-scores, percentage of the 50th and 95th percentile in CDC growth charts) to assess adiposity change (change in body fatness) over 4 years. They showed that changes in adiposity among young children are better captured by expressing changes in BMI as percentage of the 50th or 95th percentiles instead of as BMI z-score change.

It is essential for clinical management and for the interpretation of results of weight prevention programs, clinical trials, and monitoring of children, to use the best BMI metric to assess changes in body fatness. This paper clearly shows, for the age group of 3- to 7-years old children, that changes in %50th and %95th percentile are more strongly associated with changes in body fatness over 4 years than changes in BMI z-score. This work joins observations in older children and adolescents where the use of the BMI metrics %50th percentile and 95%th percentile better represented change in body fat mass than change in BMI z-score (1-3). Rather than focusing on change in BMI z-score, it would be preferable to use change in %50th or %95th percentile for clinical management and for monitoring of children over time.

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New Findings in Adipose Tissue

9.2. A single-cell atlas of human and mouse white adipose tissue

Emont M, Jacobs C, Essene AL, Pant D, Tenen D, Colleluori G, Di Vincenzo A, Jorgensen AM, Dashti H, Stefek A, McGonagle E, Strobel S, Laber S, Agrawal S, Westcott GP, Kar A, Veregge ML, Gulko A, Srinivasan H, Kramer Z, De Felippis E, Merkel E, Ducie J, Boyd CG, Gourash W, Courcoulas A, Lin SJ, Lee BT, Morris D, Tobias A, Khera AV, Claussnitzer M, Pers TH, Giodano A, Askenberg O, Regev A, Tsai LT, Rosen ED
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Nature 2022; 603 (7903): 926–933.
Doi: 10.1038/s41586-022-04518-2.
https://pubmed.ncbi.nlm.nih.gov/35296864/.

Brief summary: Emont *et al*. created a cell atlas of human and mouse white adipose tissue, which goes a long way in informing our understanding of the complexity of adipose tissue.

Although adipocytes make up the largest proportion of adipose tissue volume, the cellular structure of adipose tissue is far more complex and includes adipose tissue progenitor cells (APCs), fibroblasts, vascular cells and immune cells. We are also beginning to understand that within this tissue there are subtypes of adipocytes that have specific functional properties. Due to their size and fragility, mature fat cells are inaccessible using current single cell sequencing methods, so information about fat cells is lost when the whole tissue is analyzed at the single cell level. These authors applied single-nucleus sequencing, a method that has been recently applied to murine (1,2) and human adipose tissue (3).

By doing this, they provide a rich dataset of the adipose tissue cellularity of mice and humans. Importantly, they also identify distinct subsets of adipocytes within the tissue. Notably, these subpopulations varied by depots. This opens up a wide field to understand differences in body fat distribution seen in both obesity and partial lipodystrophy. As this technique is now available, it may be easily applied to such cohorts.

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9.3. Subcutaneous adipose tissue expansion mechanisms are similar in early and late onset overweight/obesity

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Int J Obes (Lond) 2022, 46(6):1196–1203.
Doi: 10.1038/s41366-022-01102-6.
https://pubmed.ncbi.nlm.nih.gov/35228658/.

Brief summary: This study investigated adipose tissue cellularity and functionality in more than 400 individuals with early-onset obesity (before the age of 18 years) versus late-onset of obesity (after the age of 18 years)

compared to never-obese subjects. Individuals with obesity had increased subcutaneous white adipose tissue amounts resulting from a combination of increased size and numbers of fat cells.

Interestingly, individuals with early-onset obesity had a higher BMI and a 40% larger adipose fat mass than individuals with late-onset obesity without differences in visceral adipose tissue, adipose tissue cellularity, morphology and fat cell lipolysis. The increase in BMI per year was also higher in subjects with early-onset obesity compared to late-onset obesity.

These data suggest on the one side that there are similar mechanisms of white adipose tissue growth and expansion between early and late-onset of obesity. Irrespective of an early or late start, fat mass expands by a combination of increase in number and size of fat cells. However, on the other side, the dynamics of adipose tissue expansion seems faster in early-onset obesity compared to late-onset obesity. The most important difference between early and late-onset obesity is the more extensive white adipose tissue accumulation in early-onset obesity. Visceral adipose tissue expansion appears not to be influenced by the age of onset of obesity.

The more excessive subcutaneous white adipose tissue accumulation in early-onset obesity might be due to genetic factors, e.g. gene variants influencing energy homeostasis. Such factors, unfortunately have not been investigated in this study.

9.4. SUCNR1 signaling in adipocytes controls energy metabolism by modulating circadian clock and leptin expression

Villanueva-Carmona T, Cedó L, Madeira A, Ceperuelo-Mallafré V, Rodriguez-Pena M, Nunez-Roa C, Maymo-Masip E, Repolles-de-Dalman M, Badia J, Keiran N, Mirasierra M, Pimenta-Lopes C, Sabadell-Basallote J, Bosch R, Caubet L, Escola-Gil JC, Fernandez-Real JM, Vilarrasa N, Verntura F, Vallejo M, Vendrell J, Fernandez-Veledo S
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Cell Metabolism 2023 Apr 4;35(4):601–619.e10.
Doi: 10.1016/j.cmet.2023.03.004.
https://pubmed.ncbi.nlm.nih.gov/36977414/.

Brief summary: Villanueva-Carmona *et al*. identified succinate as a mediating metabolite in leptin secretion. Its action is mediated by the succinate receptor SUCNR in adipocytes via the circadian clock in an AMPK/JNK-C/EBPa-dependent manner.

Although the regulatory circuits of leptin action on hunger and satiety are well understood, the pathways that mediate acute leptin production in the adipose tissue are less investigated. To investigate how adipocytes react to changes in food supply, regulation of leptin expression and secretion by circulating nutrients have been investigated in the past, but without deep understanding of cellular mechanisms. Succinate as an intermediate from the Krebs cycle is a plausible candidate, as its circulating levels transiently increase in response to physiological stimuli including exercise (1), cold exposure (2), or food ingestion (3). It is an interesting finding that circadian mediators are affected by succinate, as leptin itself has a clear diurnal secretion pattern. Further research is needed to provide a complete understanding of succinate as a signal transducer of physiological function. It would be especially interesting to know whether succinate contributes to the high inter-individual differences in leptin levels independently of body mass.

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9.5. A synaptic amplifier of hunger for regaining body weight in the hypothalamus

Grzelka K, Wilhelms H, Dodt S, Dreisow ML, Madara JC, Walker SJ, Wu C, Wang D, Lowell BB, Fenselau H
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Cell Metabolism 2023 May 2;35(5):770–785.e5.
Doi: 10.1016/j.cmet.2023.03.002.
https://pubmed.ncbi.nlm.nih.gov/36965483/.

Brief summary: Grzelka *et al*. identified a hypothalamic circuit that regulates regain of body weight after weight loss.

Body weight is one of the most regulated variables of our body, which is most likely due to the evolutionary history of times when access to food was scarce and volatile. Weight loss triggers a strong counterregulatory response. It increases the hunger drive and greatly increases the reward value of food, making it more difficult to adhere to a diet. Furthermore, energy metabolism is slowed down to reduce energy expenditure. Therefore, a weight loss diet is rarely successful in the long term (1). Caloric deprivation results in downregulation of nutrients and hormones, such as leptin, PYY and CCK, which usually suppress hunger, while the appetite-promoting hormone ghrelin becomes upregulated. By acting on orexigenic agouti-related peptide expressing (AgRP) neurons or anorexigenic pro-opio-melanocortin-expressing (POMC) neurons, these peripheral factors are thought to act on the brain to promote diet relapse and weight regain, but the precise mechanism of adaptations to dieting is not understood.

The authors investigated mice after acute overnight fasting causing substantial ($>10\%$) weight loss accompanied by an increased excitatory drive on AgRP neurons. They found that weight loss promotes a connection between AgRP neurons with another upstream neuron population, paraventricular hypothalamic thyrotropin releasing hormone neurons (PVH^{TRH}) and that activity of this PVH^{TRH} / AgRP circuit is necessary and sufficient to drive weight (re)gain. Most interesting, activation of this pathway induced persistent weight gain, while blocking the PVH^{TRH} / AgRP prevented weight gain after fasting.

Although these results need validation in a clinical setting, Gzelka *et al*. provide evidence of a neuronal pathway consistent with the suggested set-point theory of body weight regulation. Since these experiments were performed in lean mice, it would be interesting to investigate if the identified pathway is also relevant in an obese setting. Furthermore, it would be of particular interest to investigate if this novel pathway interacts with leptin-melanocortin signalling.

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9.6. Human loss-of-function variants in the serotonin 2C receptor associated with obesity and maladaptive behavior

He Y, Brouwers B, Liu H, Lawler K, de Oliveira EM, Lee DK, Yang Y, Cox AR, Keogh JM, Henning E, Bounds R, Perdikari A, Ayinampudi V, Wang C, Yu M, Tu L, Zhang N, Yin N, Han J, Scarcelli NA, Yan Z, Conde KM, Potts C, Bean JC, Wang M, Hartig SM, Liao L, Xu J, Barroso I, Mokrosinski J, Xu Y, Farooqi IS
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Nat Med 28, 2537–2546 (2022).
Doi: 10.1038/s41591-022-02106-5.
https://pubmed.ncbi.nlm.nih.gov/36536256/.

Brief summary: This collaborative study identified 13 monoallelic rare loss-of-function (LoF) variants in the *serotonin 2C receptor* (*HTR2C*) gene in 19 unrelated individuals with hyperphagia, severe early-onset obesity, and some degree of maladaptive behaviour. The authors used exome sequencing in 2548 individuals with severe obesity and 1117 control individuals without obesity. They found that *HTR2C* variants cause monogenic obesity by demonstrating the role of serotonin 2C receptors in the regulation of appetite, weight and behaviour through functional analysis of the identified variants in HEK293 cells and knock-in mice carrying a human LoF *HTR2C* variant.

Serotonin receptors are known to regulate body weight, mood and behaviour. Serotonin reuptake inhibitors and receptor agonists are used to treat obesity, anxiety, and depression (1,2). Previous studies in mice provided evidence that the appetite-suppressing effects are mediated specifically by serotonin receptor 2C, which is expressed in hypothalamic proopiomelanocortin (POMC) neurons (3). POMC neurons play an important role in appetite and weight regulation via the leptin-melanocortin pathway, and cause hyperphagia and severe early-childhood obesity in the complete absence of POMC due to biallelic variants.

This study is the first to identify monoallelic variants in the *HTR2C* gene in humans and provides evidence for the *HTR2C* gene as a novel cause of monogenic obesity. In addition, the study suggests a shared mechanism for obesity and anxiety, mood disorders, and maladaptive behaviour and highlights the need for a more careful assessment of anxiety and maladaptive behaviour in clinical practice in the future. As LoF *HTR2C* variants are likely to affect POMC signalling, affected patients may be amenable to treatment with the melanocortin 4 receptor agonist, setmelanotide, which has been approved in the UK, USA, and Europe for chronic weight management in patients with certain biallelic variants in the leptin-melanocortin pathway (4).

This well-designed study demonstrates the relevance for inclusion of the *HTR2C* gene to existing diagnostic gene panels for individuals with severe obesity due to its functional role in the development of obesity.

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Genetic Obesity and Genetic Risk Score

9.7. Rare antagonistic leptin variants and severe, early-onset obesity

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N Engl J Med 2023, 388(24):2253–2261.

Doi: 10.1056/NEJMoa2204041.

https://pubmed.ncbi.nlm.nih.gov/37314706/.

Brief summary: The authors provide the first description of antagonistic hormone mutations as the cause of congenital disease in humans. The paper describes detailed characterizations of two novel, antagonistic leptin mutations underlying a formerly unrecognized form of congenital leptin dysfunction and delineate the challenges these mutations pose to the diagnosis and therapy of the disease.

After the initial description of biologically inactive leptin variants in 2015 (1), it has been expected that sooner or later patients would be identified who had an antagonizing leptin variant. Based on the knowledge of the molecular ligand-receptor interaction of leptin and its receptor, the existence of antagonizing variants of leptin in humans was likely.

Here, the authors show that specific rare human gene variants in the leptin gene result in leptin molecules with impaired interaction with site III of the leptin receptor responsible for receptor activation, but have high affinity to interaction site II which is responsible for receptor binding. These leptin variants block the leptin receptor when metreleptin is administered and behave as antagonizing ligands at the receptor level, leading to resistance to metreleptin.

Elaborate *in vitro* experiments paved the way for a successful therapy. Based on dose-response experiments applying heterologous cell systems and the expression of the gene variants *in vitro*, the authors were able to calculate the metreleptin dose needed to overcome leptin receptor antagonism *in vivo*. This was the basis for successful clinical treatment with relatively high dose metreleptin without side effects.

The collaborative work of scientists in experimental and clinical medicine in this center for genetic obesity was the basis for a personalized, mechanism-based treatment of the patients.

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9.8. A National Multicenter Study of Leptin (LEP) and Leptin Receptor (LEPR) deficiency and systematic review

Besci Ö, Fırat SN, Özen S, Çetinkaya S, Akın L, Kör Y, Pekkolay Z, Özalkak S, Özsu E, Erdeve SS, Poyrazoglu S, Berberglu M, Aydin M, Omma T, Akinici B, Demir K, Oral EA

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J Clin Endocrinol Metab. 2023 Feb 24;dgad099.
doi: 10.1210/clinem/dgad099. Online ahead of print.
https://pubmed.ncbi.nlm.nih.gov/36825860/.

Brief summary: This paper describes 18 patients with bi-allelic leptin deficiency (*LEP*, $n=11$) or leptin receptor deficiency (*LEPR*, $n=7$), including 10 new cases and two novel variants. In addition, in a review of the literature (until July 2022), the authors identified $n=75$ patients living with *LEP* deficiency and $n=90$ with *LEPR* deficiency ($n=152$ included for comparison between groups).

This report is one of the most comprehensive compilations of available data on these two rare forms of monogenic obesity. Interestingly, they authors found that patients with *LEP* deficiency were diagnosed at a younger age, had a higher median BMI SD score at diagnosis, and were more likely to have hyperinsulinemia.

Unfortunately, this highly interesting article has some limitations. The authors failed to identify or include all publications on patients with *LEP*/*LEPR* deficiency. For example, the largest publication on patients with *LEP*/*LEPR* deficiency (1) ($n=52$ *LEP*, $n=17$ *LEPR*) is not included, even though the authors had identified the article. In this respective publication, clinical data is grouped according to variants, thus data for 45 patients cannot be used for detailed analysis, however data for single patients is provided for $n=24$ patients. Another limitation is they did not consider the fact that BMI SD scores correct for increasingly skewed distributions with age, with the result that BMI SD scores tend to have higher values in younger patients. Thus, if patients with *LEP* deficiency are diagnosed at a younger age, their BMI SDS scores may be artifactually higher than older children with *LEPR* mutations. On the other hand, if *LEP* deficiency does indeed lead to higher BMI SD scores, it might explain why these patients are diagnosed at an earlier age. Here the 69 additional patients from Saeed *et al*. (1) might have been helpful (BMI SDS values are provided for all). Saeed *et al*. reported much higher BMI SD score values without any difference between the groups (1).

Concerning co-morbidities, reported case series might be incomplete and not well defined. The finding of a higher risk of hyperinsulinemia in patients living with *LEP* deficiency compared to patients with *LEPR* deficiency is nevertheless very interesting. Leptin is known to influence glucose metabolism (2) and while some of its effects are modulated via the *LEPR* (2) these findings strengthen the idea that leptin might have a direct influence independent of that receptor.

In summary, while the data collection is not complete, the authors provide interesting insights into the typical phenotypes and differences between these two rare conditions. Future research is needed, including more such patients, to confirm the here found associations.

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9.9. Genetic risk score enhances the risk prediction of severe obesity in adult survivors of childhood cancer

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doi: 10.1038/s41591-022-01902-3.
https://pubmed.ncbi.nlm.nih.gov/35879615/.

Brief summary: This study evaluated the ability of genetic risk scores to predict severe obesity in adult survivors of childhood cancer. Data from 2548 survivors of European ancestry from the St. Jude Lifetime Cohort Study was analyzed and findings were validated in 6064 survivors from the Childhood Cancer Survivor Study. Survivors with higher genetic risk scores had significantly higher odds of severe obesity. Adding genetic risk scores to prediction models that included cancer treatment exposures and lifestyle factors improved prediction accuracy.

Overweight, obesity, and increased risk for diabetes mellitus and cardiovascular disease are common, but often overlooked and undertreated, sequelae of cancer treatment in children, adolescents, and adults (1,2). Cardiovascular morbidity is a major burden for this growing patient population, resulting in impaired quality of life and excess mortality (3,4).

This study of adult survivors of childhood cancer provides important clinical and scientific insights into the risk factors and prediction of severe obesity in this population. The findings highlight the considerably high prevalence of obesity among survivors, emphasizing the need for early identification and targeted interventions. Factors contributing to obesity include treatment exposures (cranial radiation, total body irradiation, corticosteroids), lifestyle behaviours (reduced physical activity, low energy expenditure), and genetic susceptibility (5). Importantly, the study introduces the concept of genetic risk scores as a valuable tool to improve the prediction of severe obesity in childhood cancer survivors. The inclusion of genetic risk scores based on common and rare genetic variants associated with obesity significantly improved the prediction models, resulting in improved identification of high-risk survivors. Incorporating genetic risk scores into risk prediction models has therefore the potential to improve patient management and facilitate targeted interventions, ultimately leading to better long-term health outcomes for this vulnerable population.

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9.10. Excess BMI in early adolescence adversely impacts maturating functional circuits supporting high-level cognition and their structural correlates

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Int J Obes (Lond) 2023. Published online 03 April 2023.
doi: 10.1038/s41366-023-01303-7.
https://pubmed.ncbi.nlm.nih.gov/37012426/.

Brief summary: This study analyzed cross-sectional data on almost 5,000 adolescents from the Adolescent Brain Cognitive Development (ABCD) cohort data (*https://abcdstudy.org/*), including resting state functional MRI, structural MRI and neurocognitive task scores. Excess BMI in young adolescents was associated with profound aberrant topological alterations in maturating functional circuits, as well as with underdeveloped brain structures that may adversely impact cognitive function.

The effects of obesity on the developing brain and associated mental and cognitive health remain incompletely understood (1-3). Impaired cognitive function in obesity has been reported already in children at preschool ages. Lower academic performance and executive function, as well as higher impulsivity, have been reported in adolescents with obesity (4–6).

This study significantly contributes to the incomplete understanding of the relationship between excess body fat and the developing circuits in the brain. Topological network properties were estimated at the scale of individual regions, large scale networks including the reward and social networks, as well as the whole brain connectome as described in detail (7,8). The results suggest that obesity may have widespread detrimental effects on developing neuro-circuits, the morphology of the underlaying brain structure, and the connective processes they support. These alterations may disrupt normal neural maturation and cognitive health. Interestingly, adolescents with obesity performed worse in a task measuring fluid reasoning, which is related to high-level cognitive processes. A strength of this study is the very large cohort resulting in significant and likely generalizable findings.

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9.11. Habitual daily intake of a sweet and fatty snack modulates reward processing in humans

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Cell Metab 2023;35(4):571–584.e6.
Doi: 10.1016/j.cmet.2023.02.015.
https://pubmed.ncbi.nlm.nih.gov/36958330/.

Brief summary: This randomized, controlled study in healthy-weight individuals examined the effect of a daily high-fat/high-sugar (HF/HS) intervention over 8 weeks on fat and sugar preference, alterations of brain response to food and sensory associative learning. It addressed the question, whether the association between obesity and altered brain function is pre-existing, is secondary to obesity or is attributed to western diet.

Current models of obesity consider genetic predisposition to be the cause for obesity in an obesogenic environment (1–3). However, preclinical studies provide evidence that frequent consumption of modern unhealthy processed foods, rich in fat and sugar, rewires brain circuits and shifts our preferences away from low-fat and healthy foods (4–6). Prior neuroimaging studies showed that sweet beverages as well as saturated fat alters striatal response (7,8).

These authors found that a subtle exposure to high-fat/high-sugar (HF/HS) foods (2 snacks per day) in addition to regular diet reduces preferences for low-fat foods (liking and wanting) in healthy-weight participants. Moreover, they showed by fMRI that HF/HS snacks alter brain reward circuits to palatable food and upregulate neural computations that support adaptive associative learning. The authors claim that these findings were independent of a change in BMI or metabolic parameters, although there was a slight gain in weight. In addition, it should be mentioned that there was also a significant reduction in preference for high-fat food in the HF/HS group, as well as a reduced wanting for high-fat food also in the control group, who received low-fat/low-sugar (LF/LS) snacks, and a reduced wanting and liking for low-sugar food in both groups.

Current models of obesity should be expanded based on the results of this well-designed study, which results suggest that the cycle of overeating may begin with environmental exposure rather than, or in addition to, a predisposition for obesity.

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9.12. Impaired brain satiety responses after weight loss in children with obesity

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J Clin Endocrinol Metab. 2022 Jul 14;107(8):2254-2266.

doi: 10.1210/clinem/dgac299.

https://pubmed.ncbi.nlm.nih.gov/35544121/.

Brief summary: Roth *et al*. studied the relationship between obesity outcomes and meal-induced changes in neural activation to high- versus low-calorie food cues before and after 24-week family-based behavioral treatment (FBT) in $n = 28$ children (9–11 years) with obesity and in $n = 17$ children of healthy weight without intervention (9–11 years). Among children with obesity who underwent FBT, unfavorable changes were observed in peripheral hormone secretion after weight loss and also a weaker satiety response.

It is still poorly understood why there are rates up to 50% of unresponsiveness, and why many children regain weight shortly after ending familial-based behavioral treatment (FBT). This study is one of the first to assess the interplay between neuroendocrine factors with obesity treatment in children. It studied the effect of an obesity

intervention on changes in central and peripheral satiety signaling in response to a meal. One of the main findings was that in children with obesity who underwent FBT, a greater reduction in BMI *z*-scores was associated with developing a weaker central satiety response to a meal from before to after FBT. This could predispose children to overeating and weight regain after FBT. These observations support the hypothesis that in the presence of weight loss the brain defends elevated body weight (1), even in young children.

This study and others have also shown that an FBT intervention and weight loss lead to meaningful change of peripheral satiety-regulating hormones (2,3), but Roth *et al*. observed at the same time a weaker central response to a meal. This could mean that even when peripheral satiety responses by gut hormones are intact among children with obesity who are successful in FBT, their central regulation of satiety is disturbed. Long-term lifestyle and behavioral interventions or pharmacological approaches are required to intervene on the obesity-related set point that strongly defends higher body weight.

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Obesity and Insulin/Glucose Metabolism

9.13. Lack of evidence for a causal role of hyperinsulinemia in the progression of obesity in children and adolescents: a longitudinal study

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Diabetes Care 2022;45:1400–1407.

https://doi.org/10.2337/dc21-2210.

Brief summary: This longitudinal study aimed to determine if hyperinsulinemia and postprandial glucose levels could predict obesity progression in n = 591 children and adolescents with obesity. The authors found no association between hyperinsulinemia, insulin secretion, low postprandial glucose levels, and an increase in obesity over a 2–4-year period.

According to the carbohydrate-insulin model (1), diets with a high glycemic load (GL) promote weight gain by increasing the ratio of insulin-to-glucagon in the blood, resulting in an anabolic state that promotes fat deposition and reduces available calories for energy expenditure. As a result, there is an increase in hunger while the body may reduce its energy expenditure in an effort to maintain energy balance. Studies in adults have shown a correlation between insulin secretion and weight gain (2), and a genetic study using a Mendelian-randomization approach suggested a potentially causal relationship between genetically determined insulin secretion and higher BMI (3).

However, limited data exist for the carbohydrate-insulin model in children. The results of the present study indicate that numerous indices of hyperinsulinemia (including area under the curve of insulin, peak insulin, fasting insulin, insulinogenic index, and insulin at 30 minutes) were not associated with greater increases in the degree of obesity over a follow-up period of 2–4 years. Additionally, low postprandial glucose levels were not linked to increased obesity progression. These findings suggest that, in children and adolescents with obesity, exposure to hyperinsulinemia or greater insulin secretion is not a significant driver of obesity progression over the short to medium term, therefore challenging the assumptions of the carbohydrate-insulin model. The study has several noteworthy strengths, including the inclusion of a relatively large and diverse sample, the incorporation of a high-risk group, and the investigation of multiple physiologically relevant aspects of insulin dynamics. However, there are also some limitations to consider. These include potential issues with the validity of insulin assays used, the lack of dietary data to assess the effect of glycemic load on insulin dynamics, and the relatively short duration of follow-up compared to other studies.

In conclusion, Halloun *et al*. contribute to our understanding of nutrition and the role of insulin secretion in weight gain among children and adolescents. However, more research is needed to provide a comprehensive assessment of the relationship between insulin dynamics, dietary factors, and long-term weight outcomes in children.

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9.14. The bad rainbow of COVID-19 time: effects on glucose metabolism in children and adolescents with obesity and overweight

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International Journal of Obesity (2022) 46:1694–1702.

https://doi.org/10.1038/s41366-022-01164-6.

Brief summary: Giannini *et al*. report a timely study on the impact of COVID-19 restrictions on glucose metabolism in children and adolescents with obesity and overweight. They compared data from 741 children over a 13-year period, before and during the COVID-19 pandemic. They found that children in the COVID-19 restriction group did not have significantly increased values of Body Mass Index (BMI), but they did exhibit higher values of Waist Circumference (WC), Waist/Height ratio (WHtR), and body fat mass compared to pre-pandemic children. Fasting glycaemia, glucose, and insulin excursions were also significantly higher in the COVID-19 restriction group. Insulin resistance was higher and insulin secretion was lower in these children, leading to a higher percentage of impaired glucose tolerance. Additionally, High-Density Lipoprotein (HDL) cholesterol levels were significantly lower, and systolic and diastolic blood pressure values were higher in the COVID-19 restriction group.

The COVID-19 restrictions have not only exacerbated the obesity status in children (1) but have also led to significant alterations in glucose and insulin metabolism. In fact, the effects of the COVID-19 pandemic seem to have had a particularly unfavorable impact on the population of vulnerable children at high risk of diabetes, which is underscored by numerous reports of steeply rising numbers of type 2 diabetes cases observed at pediatric diabetes centres (2–4), and epidemiological data demonstrating a significantly increasing incidence of youth-onset type 2 diabetes in 2021, the second year of the pandemic, in Germany (5).

These findings emphasize the urgent need for comprehensive public health strategies to address the short-term and long-term consequences of COVID-19 restrictions on children's health. Such strategies should focus on promoting healthy lifestyle behaviours, including regular physical activity, balanced nutrition, and appropriate weight management. Additionally, targeted interventions should be implemented to provide support and resources to families and individuals at higher risk, particularly those with a predisposition to type 2 diabetes.

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9.15. Insulin and body mass index decrease serum soluble leptin receptor levels in humans

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doi: 10.1210/clinem/dgac699.
https://pubmed.ncbi.nlm.nih.gov/36459457/.

Brief summary: This pooled study, including five cross-sectional or intervention studies ($n = 24–823$) and using publicly available data from genome-wide association studies (GWAS) to perform Mendelian randomization, investigated the influence of glucose, insulin, body fat, body mass index (BMI), food intake, and physical activity on serum soluble leptin receptor (sOb-R) levels. The authors showed that insulin and BMI were associated with decreased serum sOb-R levels, whereas physical activity and food intake acutely increased sOb-R levels, suggesting an involvement in the short-term regulation of leptin signalling.

Membrane-bound leptin receptors (Ob-R) are important to mediate the effects of leptin on hunger and satiety. The human Ob-R can be spliced into six different isoforms with identical extracellular domains but distinct intracellular domains that differ in length and mode of signalling (1). sOb-R is produced by shedding of the extracellular domain of membrane-bound Ob-R (2). It is therefore suggested that sOb-R levels reflect the amount of membrane-bound Ob-Rs, act as a soluble receptor, and prolong the half-life of circulating leptin (3). The regulation and function of sOb-R is largely unknown, but previous studies have found a strong inverse association between sOb-R levels and insulin sensitivity, suggesting a protective effect against the development of type 2 diabetes (4,5).

The strength of this study lies in the use of pooled data from several independent studies. The results strongly support an inverse association between sOb-R and insulin, which may contribute to a lower risk of type 2 diabetes. However, the results should be interpreted with caution, as the exact role and mechanism by which the sOb-R might mediate these effects is still unknown. The study highlights the great interest in understanding the mechanism and clinical implication of sOb-R in humans, which remains to be elucidated in future studies.

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Patient Care: Bariatric Surgery, New Drugs, and Appropriate Language

9.16. Metabolic and bariatric surgery versus intensive non-surgical treatment for adolescents with severe obesity (AMOS2): a multicentre, randomised, controlled trial in Sweden

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Lancet Child Adolesc Health. 2023 Apr;7(4):249–260.
doi: 10.1016/S2352-4642(22)00373-X.
https://pubmed.ncbi.nlm.nih.gov/36848922/.

Brief summary: The AMOS2 study is a randomized, open label, multicentre trial. It reports 2-year BMI changes in $n=25$ adolescents (age 13–16 years) after metabolic and bariatric surgery (MBS) (Roux-en-Y gastric bypass $n=23$, sleeve gastrectomy $n=2$) compared to $n=23$ adolescents who received intensive non-surgical treatment. After 2 years, BMI change was -12.6 kg/m^2 in the MBS group compared to only -0.2 kg/m^2 in the non-surgical treatment group.

In 2015, a Cochrane review highlighted the lack of randomized trials comparing MBS to other treatment modalities in adolescents with obesity (1). That review identified only one randomized controlled trial of adjustable gastric banding. That RCT among adolescent with obesity reported that gastric banding, compared with lifestyle intervention, resulted in a greater percentage achieving 50% reduction in excess weight, corrected for age (2). However, gastric banding is in limited use in adolescents due to associated high long-term re-operation rates.

The AMOS2 study is the only study that compared currently preferred MBS techniques with intensive non-surgical treatment. MBS was superior to intensive non-surgical treatment over 2 years in achieving weight loss among adolescents with severe obesity. This RCT and other observational studies (3–5) have shown that MBS is associated with substantial weight loss, improvement of cardiometabolic risk factors and quality of life in the short and medium term. But there is still the question of the long-term effects of an MBS.

In another recent paper, Beamish *et al*. reviewed published data on the results of MBS in adolescents with a focus on long-term outcomes. They highlighted that further studies are warranted to investigate long-term safety and efficacy and the role of adjunctive pharmacological treatment (6).

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9.17. Once-weekly semaglutide in adolescents with obesity

Weghuber D, Barrett T, Barrientos-Pérez M, Gies I, Hesse D, Jeppesen OK, Kelly AS, Mastrandrea LD, Sorrig R, Arslanian S, STEP TEENS Investigators

N Engl J Med 2022, 387(24):2245–2257.

doi: 10.1056/NEJMoa2208601.

https://pubmed.ncbi.nlm.nih.gov/36322838/.

Brief summary: This phase 3 double-blind, parallel-group, randomized, placebo-controlled trial over 68 weeks randomised (2:1) 201 adolescents with obesity to receive semaglutide 2.4 mg once weekly or placebo. Both groups also received lifestyle intervention. The primary endpoint was the percentage change in BMI. Treatment with semaglutide produced clinically relevant reductions in BMI and body weight, and improvements in cardiovascular risk factors, which were all significantly greater than in the control group..

Based on these data, semaglutide was approved by the FDA and EMA in 2023 for the treatment of obesity in adolescents aged 12 years and older.

Interestingly, the placebo-subtracted change (difference between the two groups) was greater than that achieved in the STEP1 study in adults with obesity using the same drug at the same dose (1). It is unclear why this significantly

better outcome was achieved in adolescents compared to adults and gives us hope that intervention earlier in life will lead to more significant weight loss. There is also a more pronounced effect of semaglutide compared to the results of the pivotal trial of liraglutide for adolescents with obesity (2). The BMI reduction was 16.7 percentage points after 68 weeks with semaglutide once weekly compared to 4.6 percentage points after 56 weeks with liraglutide injected once daily. This corresponded to a mean weight loss of 15.3 kg on semaglutide compared to a weight gain of 2.4 kg on placebo. Although these data suggest a strong advantage of semaglutide compared to liraglutide, caution must be exercised in interpreting them, as there was no direct comparison of the two treatments in a clinical trial.

The safety profile of semaglutide was comparable to that found in adult studies (1) and was consistent with that typical of GLP-1 receptor agonists (3). Gastrointestinal side effects were the most common (62%), but did not lead to any significant study discontinuations.

The study results presented here can certainly not be generalised. The high adherence in the semaglutide group as well as in the placebo group are exceptional for obesity studies. Furthermore, significantly more female than male patients were included and in the distribution of the ethnic groups was not representative for the reference populations. Further follow-up data on the efficacy and safety of semaglutide in adolescents under real-life conditions are needed.

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9.18. Say what you mean, mean what you say: The importance of language in the treatment of obesity

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Obesity (Silver Spring) 2022, 30(6):1189–1196.
Doi: 10.1002/oby.23446.
https://pubmed.ncbi.nlm.nih.gov/35674695/.

Brief summary: This study investigated how frequently negative terminology such as 'fail' and 'morbid obesity' was used in scientific publications dealing with bariatric surgery in peer reviewed journals. 2.4% of the publications analyzed included the term 'fail' and 16.8% contained the term 'morbid' in conjunction with obesity. This study showed that negative language, blaming the patient for the lack of weight loss or weight regain, was present within scientific publications.

In the powerful qualitative part of the study, patients' perspectives on these two terms was obtained. There was a near universal consensus that the expression 'failure' to lose weight implies a personal failure rather than a failure of the intervention. In addition, patients felt that the use of the word 'fail' by medical practitioners served to reinforce their own firmly held beliefs that a failure to maintain weight loss is the result of their lack of will power, rather than a physiological response. With regard to 'morbid obesity', patients who understood this phrase as not directly correlated to a medical definition felt highly stigmatized and that this would mean a scientific assessment of the patient who was likely to soon die as a result of obesity since no effective treatment could be offered.

We all are aware that obesity is a highly stigmatized, chronic disease. Nevertheless, it is deplorable to see that healthcare professionals show the same stigmatizing language and behaviours as the general public. This frequently influences the quality of care provided to the patients (1,2). In many cases the ongoing use of negative language reinforces outdate views that obesity is the result of a personal choice.

It is critical to recognize the importance that language plays in shaping relationships with patients and transmitting stigmatizing beliefs, reinforcing deeply ingrained inherent biases and affecting patient care. Although most journals have developed editorial policies that encourage the use of person-first language,

negative language continues to be used, which perpetuates both overt and implied stigma related to obesity. Therefore, editorial policies need to be adopted and widely implemented in order to discourage the use of ambiguous, pejorative words such as 'fail' and 'morbid obesity'.

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10. Type 2 Diabetes, Metabolic Syndrome and Lipid Metabolism

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Preface

The rise of type 2 diabetes (T2D) worldwide in children is seriously alarming. The adjusted incidence of T2D among children and adolescents nearly doubled, from 9.0 to 17.9 cases per 100 000 persons per year, from 2002-03 to 2017-18. For the first time, the incidence of T2D has surpassed that of type 1 diabetes (T1D). Further to this increase in incidence, a 77% increase in the incidence of T2D in youth was observed in 2021, during the COVID-19 pandemic. Fortunately, the arsenal of new drugs to treat children and adolescents is growing: the results of two new drugs were published this year. Identifying those in need of medications is important. This was highlighted by the evidence, from more than 500 youth followed for up to 9 years, that baseline HbA1c and the change in HbA1c level in the first 6 months after diagnosis can predict rapid deterioration. The long-term effects of parental diabetes on disease progression and complications in youth-onset T2D were described.

This year we included articles on gestational diabetes. Specifically, one study provided new evidence of a relation between gestational glucose intolerance, even below the threshold for gestational diabetes, and the risk of offspring overweight/obesity at late adolescence.

The European Atherosclerosis Society (EAS) released an update to its 2014 consensus on homozygous familial hypercholesterolemia. A new drug, bempedoic acid, was found to reduce the incidence of a composite cardiovascular outcome in people eligible for a statin but not able or willing to take it. A metanalysis revealed increased prevalence of autism among persons with hyperlipidemia. Moderate improvement was demonstrated among individuals with hyperlipidemia who could not tolerate statins compared to a placebo.

New Data on the Epidemiology of T2D in Children

10.1. Trends in incidence of youth-onset type 1 and type 2 diabetes in the USA, 2002-18: results from the population-based SEARCH for Diabetes in Youth study

Wagenknecht LE, Lawrence JM, Isom S, Jensen ET, Dabelea D, Liese AD, Dolan LM, Shah AS, Bellatorre A, Sauder K, Marcovina S, Reynolds K, Pihoker C, Imperatore G, Divers J, SEARCH for Diabetes in Youth Study

Lancet Diabetes Endocrinol. 2023 Apr;11(4):242–250.

DOI: 10.1016/S2213-8587(23)00025-6

Brief summary: The incidences of T1D and T2D in children and young people increased in the USA over the last 2 decades. For the first time, the incidence of T2D has surpassed that of T1D.

Comment: The SEARCH study has served as a national resource to explore the epidemiology and consequences of diabetes in the US. Published over the years, the findings contribute to understanding future trends in other parts of the world. Overall, the current report shows almost doubling of the adjusted incidence of T2DM among children and adolescents, from 9.0 to 17.9 cases per 100 000 persons per year, from 2002-03 to 2017-18. For the specific age group of 15–19 years, the incidence of T2D in 2017-2018 exceeded that of T1D (19.7 per 100 000 vs. 14.6 per 100 000). This is the first time that among youth the incidence of T2D surpassed that of T1D, and is

a substantial and alarming warning that effective prevention plans are needed. Also, for the first time, this study showed a significant seasonal variation in the onset of T2D in children and young people. Specifically, diagnoses were increased in August. Possible explanations are childhood weight gain during the summer vacation from school, and an uptick in physical exams for school athletic programs, which may detect asymptomatic hyperglycemia. This is consistent with the greater proportion of diagnoses at routine health visits rather than following symptoms. Benefits are likely to accrue from early diagnosis of T2D,[1] which allows for prompt multifactorial treatment and attention to cardiovascular risk factors. The findings from the current study emphasize the importance of routine evaluations.

Of note, the increase in the incidence of T2D among youth is not limited to the US. In Germany, a three-fold increase in the prevalence of T2D was reported for 10-19-year-olds during 2002-2020 (from 3.4 to 10.8 per 100 000 persons). [2] The estimated standardized prevalence of T2D was 1.4 times higher among girls (12.8) than boys (9.0). Data from the UK reveals that the number of children registered as having T2D and being treated in paediatric diabetes units in England and Wales has risen by more than 50% in the past five years.

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10.2. The Coronavirus Disease 2019 pandemic is associated with a substantial rise in frequency and severity of presentation of youth-onset type 2 diabetes

Magge SN, Wolf RM, Pyle L, Brown EA, Benavides VC, Bianco ME, Chao LC, Cymbaluk A, Balikcioglu PG, Halpin K, Hsia DS, Huerta-Saenz L, Kim JJ, Kumar S, Levitt–-Katz LE, Marks BE, Neyman A, O'Sullivan KL, Pillai SS, Shah AS, Shoemaker AH, Siddiqui JAW, Srinivasan S, Thomas IH, Tryggestad JB, Yousif MF, Kelsey MM, COVID-19 and Type 2 Diabetes Consortium

J Pediatr. 2022 Dec;251:51–59.e2.

doi: 10.1016/j.jpeds.2022.08.010

Brief summary: A significant increase in the incidence T2D occurred in the USA during the COVID-19 pandemic, particularly among children with obesity and males.

Comment: This is the first multicenter report on the incidence of T2D during the first year of the COVID-19 pandemic in the USA. The average number of new diagnoses per year in the two pre-pandemic years was 825, compared with 1463 during the first pandemic year. This increase of 77% is significantly higher than the 5% expected annual increase in incidence observed in the two previous years.

The authors suggest several explanations for the increase in T2D incidence. First is the increased obesity among young people during the COVID-19 pandemic. While substantial weight gain was observed across all weight and age groups during the pandemic, it was substantially greater for children who were already with overweight or obesity.[1] Weight gain was accompanied by increased consumption of processed foods and decreased physical activity, both of which contribute to the risk of developing T2D. These may have been the 'straws that broke the camel's back'. Another suggested contributor to the pandemic-related increase in T2D is increased psychosocial stress. Finally, non-autoimmune destruction of beta cells due to SARS-CoV-2, results in reduced beta-cell function in adolescents who were predisposed to develop diabetes, was suggested

An additional interesting observation is the increase in the relative proportion of male adolescents with new-onset T2D. This corroborates a publication from Alabama, which showed increased prevalence among males.[2] Data from the current study also demonstrated increased severity of the presentation of youth-onset T2D during the pandemic. HbA1c tended to be higher at presentation and a greater proportion presented with metabolic decompensation. This probably reflects delays in seeking care, due to concerns of being exposed to COVID-19.

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10.3. The obesity paradox: Retinopathy, obesity, and circulating risk markers in youth with type 2 diabetes in the TODAY study

Levitsky LL, Drews KL, Haymond M, Glubitosi-Klug RA, Levitt Katz LE, Mititelu M, Tamborlane W, Tryggestad JB, Weinstock RS, TODAY Study Group

J Diabetes Complications. 2022 Nov;36(11):108259.
doi: 10.1016/j.jdiacomp.2022.108259

Brief summary: This study aimed to decipher the pathophysiology of the lower prevalence of diabetic retinopathy observed among youth with T2D who also have severe obesity. None of the several biomarkers assessed was found to be associated with the obesity paradox. The biomarkers included: inflammatory factors, acute phase reactants or cytokines (VEGF, hsCRP, ICAM-1, VCAM-1, homocysteine, adiponectin, E-selectin, MCP-1, TNF-α, IL-6, fibrinogen, and ApoB).

Comment: High rates of diabetes complications have been reported in youth with T2D. Data from the Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study demonstrated that 13.7% of youth developed retinopathy after a mean 4.9 years of disease. Diabetic retinopathy increases with diabetes duration. The estimated pooled global prevalence of diabetic retinopathy in paediatric T2D increases with disease duration, from 1.1% at less than 2.5 years after diagnosis, to 9.0% at 2.5 to 5.0 years after T2D diagnosis, and 28.1% at more than 5 years after T2D diagnosis.[1]

The 'obesity paradox' describes the association of overweight or mild obesity, compared to normal weight or underweight, with a lower risk of mortality in such conditions as heart disease, diabetes, chronic kidney disease and heart failure. It is called a 'paradox' because it contradicts the generally accepted notion that obesity is a risk factor for various health problems and increased mortality. Using body mass index (BMI) as a surrogate for obesity, the TODAY study found that among 524 youth with T2D who were followed 2–6.5 years, and who underwent retinal examinations with digital fundus photographs, those with greater obesity were less likely to have retinopathy than were those with less obesity. The prevalence of non-proliferative diabetes retinopathy was 16.3% for the lowest BMI tertile (21.6–31.5 kg/m^2), 15.0% for the middle BMI tertile (31.5–37.9 kg/m2) and 9.8% for the highest BMI tertile (37.9–68.7 kg/m^2). Comparing the highest to the lowest BMI tertile, the OR was =0.565 95% CI (0.387, 0.824).

In an attempt to elucidate factors associated with the 'obesity paradox', the TODAY study assessed serum levels of circulating factors that have been identified as associated etiopathologically in the development of diabetic retinopathy. Inflammatory factors, acute phase reactants, cytokines and other factors implicated in the pathogenesis of diabetic retinopathy were obtained (VEGF, hsCRP, ICAM-1, VCAM-1, homocysteine, adiponectin, E-selectin, MCP-1, TNF-α, IL-6, fibrinogen, and ApoB, levels). None of these parameters could explain the lower rate of retinopathy in the highest BMI tertile. Presently, however, the well-known epidemiologic risk factors of hyperglycemia, diabetes duration and hypertension remain the most important markers for an increased risk of diabetic retinopathy in youth with T2D.

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10.4. Once-weekly dulaglutide for the treatment of youths with type 2 diabetes

Arslanian SA, Hannon T, Zeitler P, Chao LC, Boucher-Berry C, Barrientos-Pérez M, Bismuth E, Dib S, Cho JI, Cox D, AWARD-PEDS Investigators

N Engl J Med. 2022 Aug 4;387(5):433–443.
doi: 10.1056/NEJMoa2204601

Brief summary: This randomized, double-blind, phase 3 trial of youths with inadequately controlled T2D, dulaglutide treatment was superior to placebo in reducing the glycated hemoglobin level at 26 weeks.

Comment: T2D in children and adolescents is an aggressive disease with early onset of complications, leading to significant morbidity and mortality. After three decades with no efficient treatment, there is finally light at the end of the tunnel.

Glucagon-like peptide-1 (GLP-1) is a peptide hormone secreted in the fasting state, by intestinal enteroendocrine cells at low basal levels, and that rises briskly within minutes of food consumption.[1] GLP-1 controls meal-related glycemic excursions through augmentation of insulin secretion and inhibition of glucagon secretion. GLP-1 also inhibits gastric emptying and food intake, leading to weight loss. GLP-1 has a short half-life of approximately 2 minutes, as it is rapidly degraded by dipeptidyl peptidase-4 (DPP-4). To increase GLP-1 activity, DPP-4 inhibitors and GLP-1 receptor agonists have been developed.

Dulaglutide (Trulicity), is a GLP-1 receptor agonist that is administered subcutaneously once weekly. Dulaglutide has been approved since 2014 for the treatment of adults with T2DM. This study tested its efficacy and safety in adolescents. A clear benefit is its once-weekly administration. Indeed, this study reported 99% adherence to treatment.

55% of participants identified as Hispanic/Latino and 15% as Black/African American. Their mean age was 14.5 years, mean (BMI 34.1 kg/m^2, mean duration of T2D 2.0 years and mean HbA1C 8.1%). Treatment resulted in significant reductions in HbA1c levels relative to placebo treatment, at week 26, according to the intention-to-treat estimate. HbA1c decreased from baseline by 0.8% but increased by 0.6% in the placebo group. Gastrointestinal symptoms were among the most common adverse events, but they were primarily mild and were most likely to occur soon after the initiation of therapy. There were no clinically meaningful differences in the incidence or annual rate of hypoglycemia between the dulaglutide groups and the placebo group. However, body weight did not decrease significantly following the treatment.

The EU approved dulaglutide as an adjunct to diet and exercise, to improve glycemic control in youth with T2D aged 10 years and older. In November 2022, the FDA approved its use as well.

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1. Drucker DJ. Mechanisms of action and therapeutic application of glucagon-like peptide-1. *Cell Metab*. 2018 Apr 3;27(4):740–756. doi: 10.1016/j.cmet.2018.03.001.

10.5. Once-weekly exenatide in youth with type 2 diabetes

Tamborlane WV, Bishai R, Geller D, Shehadeh N, Al-Abdulrazzaq D, Vazquez EM, Karoly E, Troja T, Doehring O, Carter D, Monyak J, Sjöström CD

Diabetes Care. 2022 Aug 1;45(8):1833–1840.
doi: 10.2337/dc21-2275

Brief summary: This study examined glycemic control following treatment with once-weekly exenatide 2 mg (Bydureon, AstraZeneca) in youth with T2D which was not optimally controlled. At 24 weeks, exenatide was superior to placebo in lowering HbA1c (least squares mean change, −0.36% with exenatide vs. +0.49% with placebo), with a between-group difference of −0.85%.

Comment: The story of the development of exenatide is fascinating. It starts with a venomous lizard named the Gila monster (Heloderma suspectum), native to New Mexico and Arizona. H. suspectum, a long-lived and

reclusive species, spends a significant portion of its life underground. Its venomous bite causes pain and weakness, but is rarely fatal to adult humans.

The discovery of exendin-4 dates back to 1992, when endocrinologist Dr. John Eng, uncovered this peptide while using chemical assays to identify new hormones. Analysing the venom, she identified a peptide named exendin, which triggers the synthesis and release of insulin from beta cells in the pancreas. Dr. Eng, discovered that exendin-4 closely resembled GLP-1, a hormone that stimulates insulin production in the pancreas, when glucose levels are high. However, while GLP-1 remains active for about two minutes, the prolonged effect of exendin-4 lasts several hours. Preclinical studies revealed that in diabetic mice, a single daily injection of exendin-4 normalized blood glucose concentrations. Furthermore, the effects of exendin-4 lasted for several hours. Following extensive clinical testing, exendin-4 was deemed safe and effective, and received FDA approval in 2005. Exenatide is believed to facilitate glucose control in at least five ways. It increases insulin secretion in response to eating meals; suppresses pancreatic release of glucagon in response to eating, helps slow down gastric emptying, and thus decreases the rate at which meal-derived glucose appears in the bloodstream. Exenatide has a subtle yet prolonged effect on reducing appetite, and promotes satiety via hypothalamic receptors.

In the current study, a mean decrease in HbA1c of 0.36% in the once-weekly exenatide group was demonstrated after 24 weeks, compared with a 0.49% increase in the placebo group (a between-group difference of -0.85%). There were no events of major hypoglycaemia, and the most frequently reported adverse events were gastrointestinal (nausea, diarrhoea, vomiting) and injection site reactions (pruritus, erythema, nodules). Across the trial groups, clinically relevant differences were not observed in changes from baseline, for the exploratory weight variables of the BMI *Z*-score, body weight or waist circumference.

Of note, spontaneous cases of acute pancreatitis have been reported among adults on prolonged-release exenatide. Also, a 2-year rat carcinogenicity study found that prolonged-release exenatide (at doses $\geq$2-fold the human systemic exposure) increased incidences of adenomas and C-cell carcinomas. The clinical relevance of these adverse findings is unknown. Nevertheless, individuals with a personal or family history of medullary thyroid carcinoma should not use the therapy.

The prolonged-release suspension for exenatide injection in a pre-filled pen has been approved both by the European Union and the FDA for the treatment of T2DM in children and adolescents aged 10 years and older.

10.6. Deterioration of glycemic control in youth-onset type 2 diabetes: What are the early and late predictors?

Zeitler P, El Ghormli L, Arslanian S, Caprio S, Isganaitis E, Kelsey MK, Weinstock RS, White NH, Drews K
J Clin Endocrinol Metab. 2022 Jul 14;107(8):e3384–e3394.
doi: 10.1210/clinem/dgac254.
PMID: 35486388

Brief summary: In this study of 699 youth 10 to $<$ 18 years old with $<$2 years duration of type 2 diabetes (T2D) at 15 centers across the USA, baseline HbA1c level, and the change in HbA1c level in the first 6 months are predictors of rapid glycemic deterioration. In addition, subsequent loss of control can be predicted based on both baseline and ongoing clinical characteristics.

Comment: The population of individuals with youth-onset T2D is heterogeneous. Two large subsets are characterized as those at risk of losing glycemic control rapidly, and those who maintain glycemic control, defined as HbA1c $<$8%, for a more prolonged period.

The incidence of complications is high among children and teenagers with T2D. Currently good drug treatment options are available. On the other hand, 50% of youth can maintain good glycemic control without the use of medication. Therefore, early identification of the parameters that characterize high-risk individuals, before deterioration, is important. This can prompt initiation of early treatment and differentiate individuals who do not require medications.

These authors assessed baseline anthropometric and metabolic parameters from participants of the TODAY study, aged 10 to $<$18 years old at baseline with $<$2 years T2D duration. Their baseline data were assessed as

predictors of glycemic deterioration at two time points: after 4 years and at the end of follow-up, at 6.8 years. The predicting parameters at 4 years were baseline HbA1c at study entry, the change in HbA1c after the first 6 months, measures of insulin secretion (C peptide and oral disposition index), measures of insulin processing (proinsulin to insulin ratio) and a maternal history of diabetes. One-third of those who had good glycemic control after 4 years subsequently lost it. Reduced baseline insulin secretion (C-peptide index) also predicted late glycemic deterioration. Additional parameters assessed at the 4th year that predicted late deterioration were: an elevated HbA1c level, impaired insulin processing (proinsulin/insulin ratio) and a rise in proinsulin between baseline and 48 months. Of importance, the changes in HbA1c over the first 6 months of treatment were a significant predictor even after 9 years.

Based on these findings, the authors suggest that dysfunctional insulin processing (represented by elevated proinsulin) might be an initial step in the decline of beta-cell function. This dysfunction could be followed by a decrease in insulin secretory capacity, which subsequently contributes to worsening blood sugar control. In summary, clinicians can distinguish youth with T2DM who do not need additional drug therapy from those who are high-risk and need early and more aggressive intervention.

10.7. Maternal diabetes in youth-onset type 2 diabetes is associated with progressive dysglycemia and risk of complications

Shah RD, Chernausek SD, El Ghormli L, Geffner ME, Keady J, Kelsey MM, Farrell R, Tesfaldet B, Tryggestad JB, Van Name M, Isganaitis E

J Clin Endocrinol Metab. 2023 Apr 13;108(5):1120–1131.
doi: 10.1210/clinem/dgac663

Brief summary: This analysis of data from the TODAY study examined the impact of parental diabetes on outcomes in young individuals with T2D during over 12 years of follow-up. This novel observation demonstrates that parental diabetes affects not only earlier T2D onset, but also more rapid long-term progression and more complications.

Comment: This study showed that T2D diagnosis in either parent was associated with younger age at T2D diagnosis in their children, higher HbA1c, and greater risks of impaired β-cell function at diagnosis, and of loss of glycemic control in the first 4 years after diagnosis. However, data are limited regarding the long-term effects of parental diabetes on disease progression and complications in youth-onset T2D.

Among persons with complete data ($n = 486$), T2D in both parents was reported in 16%; T2DM in mothers only, of 36%; and T2DM in fathers only, of 15%. Of the 621 individuals with complete data of maternal diabetes, 35% reported maternal diabetes diagnosed before or during pregnancy, and 16% reported maternal diabetes diagnosed after pregnancy. The 12-year analysis revealed an association of a parent's history of diabetes, with poorer glycemic control and indicators of autonomic neuropathy, such as heart rate variability in offspring.

In addition, maternal diabetes, whether diagnosed before or after pregnancy, was associated with poor diabetes control, reduced β-cell function, glomerular hyperfiltration and an increased risk of autonomic dysfunction in offspring with youth-onset of T2D. The stronger association between maternal diabetes and the metabolic phenotype may reflect the effect of the in-utero environment, or the mitochondrial genome, which is maternally inherited.

10.8. Outcomes in children of women with type 2 diabetes exposed to metformin versus placebo during pregnancy (MiTy Kids): a 24-month follow-up of the MiTy randomised controlled trial

Feig DS, Sanchez JJ, Murphy KE, Asztalos E, Zinman B, Simmons D, Haqq AM, Fantus IG, Lipscombe L, Armson A, Barrett J, Donovan L, Karanicolas P, Tobin S, Mangoff K, Klein G, Jiang Y, Tomlinson G, Hamilton J, MiTy Kids Collaborative Group

Lancet Diabetes Endocrinol. 2023 Mar;11(3):191–202.
doi: 10.1016/S2213-8587(23)00004-9

Brief summary: MiTy Kids is a prospective cohort study of children who were exposed to metformin during pregnancy. At 24 months, no difference was observed between those exposed to metformin and a placebo group, in BMI trajectories, mean BMI *Z*-score, or mean sum of skinfolds. Among those exposed to metformin, the BMI was higher among males than females.

Comment: Metformin is increasingly used during pregnancy in women with T2D, but data on its long-term effects on the children of these women are limited. The MiTy (Metformin in Women with T2D in Pregnancy Trial)[1] is a multicenter, randomized control trial of women with T2DM in pregnancy (sample size $n = 500$). It assessed the effect of the addition of metformin to a standard regimen of insulin, on perinatal morbidity and mortality. Metformin-treated women compared to a placebo group achieved better glycemic control, required less insulin, gained less weight and had fewer caesarean births. Indeed, among newborns of metformin-exposed women with T2D, gestational age at birth and adiposity measures were greatly reduced. However, a higher proportion of infants in the metformin group were small for gestational age (13% vs. 7%).

As a follow-up to the MiTy trial, MiTy Kids assessed whether metformin treatment of pregnant women with T2D might reduce adiposity and improve insulin resistance in their offspring. At 24 months, the mean weight of all children of women with T2D was about 1 standard deviation heavier than the World Health Organisation reference. The groups did not differ in BMI trajectories, mean BMI *Z*-score, or the mean sum of skinfolds.

Interestingly, in males, BMI trajectories differed significantly by treatment. Specifically, mean BMI was higher for males in the metformin group than in placebo group from 6 months to 24 months, however mean BMI for both sexes combined were similar to those of the placebo group. The authors hypothesize that males are more insulin sensitive, which renders them more sensitive to maternal hyperglycemia, and in turn might make them more sensitive to the effects of metformin. Several observational studies have reported an association of early accelerated weight gain with an increased long-term risk of obesity. Therefore, further follow-up is needed to understand the impact of this sexual disparity in treatment effect on obesity in later years.

The Italian association of medical Diabetologists has published an excellent evidence-based position statement[1] on the use of metformin therapy in pregnancy complicated by obesity, gestational diabetes (GDM), type 2 diabetes mellitus (T2DM), polycystic ovary syndrome (PCOS) and in women undergoing assisted reproductive technology (ART).

Reference

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Metabolic Syndrome

10.9. The association of metabolic syndrome status with sensorineural hearing loss in pediatric obese patients

Ozdemir O, Ucar A, Cakir AD, Misir E, Yigit O
Int J Pediatr Otorhinolaryngol. 2023 Feb;165:111454.
doi: 10.1016/j.ijporl.2023.111454

Brief summary: This study assessed the relation between the metabolic syndrome (MetS) and sensorineural hearing loss in children with obesity. Children with MetS were shown to have subclinical hearing loss compared to those without MetS.

Comment: Associations between obesity and hearing loss have been reported in several adult studies.[1] However, similar studies in adolescents have yielded conflicting results. The present study aimed to address this uncertainty by distinguishing among children with obesity, those with and without the MetS.

They studied 252 treatment-naive children (median age 12.5 years), living with obesity. One-third of them had MetS. All underwent otorhinolaryngological examinations, as well as tympanometry and audiometry tests.

None experienced sensorineural hearing loss. However, a significantly higher rate of subclinical hearing loss (< 15 dB) was detected among children with obesity plus MetS compared with those obesity without MetS. A considerable proportion showed one or more component of MetS, evidenced by clinical insulin resistance, hypertension, fasting hyperglycemia, and dyslipidemia in 77%, 43%, 34% and 22% of the children, respectively. However, subclinical hearing loss was not associated with any specific MetS component, suggesting the influence of a combination of factors. The absence of clinical hearing loss was explained by their young age, so possibly an association between MetS and hearing loss may become apparent with age.

Unfortunately, this is yet another less known complication of obesity and the metabolic syndrome which may further have impact on quality of life of obese people. The pathophysiological mechanism behind this association is unclear. A key hypothesis is vasoconstriction in the inner ear. Accordingly, strain on the capillary walls due to excess adipose tissue causes damage to the delicate inner ear system. Several studies have reported associations of hypertension, dyslipidemia and fasting hyperglycemia with pathological changes in the ear, and with increased rates of hearing loss.

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10.10. Iron metabolism and ferroptosis in type 2 diabetes mellitus and complications: mechanisms and therapeutic opportunities

Miao R, Fang X, Zhang Y, Wei J, Zhang Y, Tian J

Cell Death Dis 14, 186 (2023).
https://doi.org/10.1038/s41419-023-05708-0

Brief summary: This paper reviews the associations between cellular iron, ferroptosis and diabetes. It also explores the therapeutic potential of ferroptosis inhibitors in the treatment of diabetic complications.

Comment: Ferroptosis is a form of regulated cell death that is characterized by the decreased capacity of antioxidants and the accumulation of lipid reactive oxygen species (ROS).[1] This, in turn, causes oxidative damage to cell membranes and results in cell death. Ferroptosis is distinct from other forms of cell death, such as apoptosis or necrosis, and its molecular mechanisms are unique. Iron has a critical role in driving lipid peroxidation, and regulation of iron abundance in cells dictates sensitivity to ferroptosis. Increased levels of intracellular labile iron can amplify ROS production and exacerbate lipid peroxidation, promoting ferroptotic cell death. Ferroptosis has been implicated in various pathological conditions, including neurodegenerative diseases and cancer.[2]

This comprehensive review elucidates the regulatory mechanisms underlying ferroptosis in diabetes and diabetes complications. The authors describe the potential of ferroptosis inhibitors as therapeutic interventions for diabetic complications. They reviewed the association between iron metabolism and glucose homeostasis. A potential association was demonstrated between iron storage and T2D. You will remember that 'bronze diabetes' is a known manifestation of the rare iron-overload disease, hereditary hemochromatosis, caused by inherited mutations in iron regulatory genes. It is now clear that even among the general population there is an association of higher plasma serum ferritin levels with an increased risk of T2D. Reducing iron storage *in vivo* results in improved insulin secretion and improved peripheral tissue insulin sensitivity.

Ferroptosis is also associated with microangiopathy, as iron overload is associated with early development and an accelerated course of diabetic nephropathy. An example is in patients with thalassemia, another disorder characterised by iron overload due to repeated blood transfusions.

As ferroptosis is a cause of T2D and its complications, it is a promising therapeutic target for the treatment and prevention of T2D and its complications. It has been suggested that the effectiveness of metformin in T2D is related to its inhibition of ferroptosis. Furthermore, quercetin, a flavonoid, is a natural inhibitor of iron metabolism and is beneficial in improving conditions caused by iron overload. Quercetin treatment has been shown to have a potential beneficial effect on T2DM, by inhibiting pancreas β cell ferroptosis.

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Dyslipidemia

10.11. 2023 Update on European Atherosclerosis Society Consensus Statement on Homozygous Familial Hypercholesterolaemia: new treatments and clinical guidance

Cuchel M, Raal FJ, Hegele RA, Al-Rasadi K, Arca M, Averna M, Bruckert E, Freiberger T, Gaudet D, Harada-Shiba M, Hudgins LC, Kayikcioglu M, Masana L, Parhofer KG, Roeters van Lennep JE, Santos RD, Stroes ESG, Watts GF, Wiegman A, Stock JK, Tokgözoğlu LS, Catapano AL, Ray KK

Eur Heart J. 2023 May 2:ehad197.
doi: 10.1093/eurheartj/ehad197

Brief summary: In May 2023, the European Atherosclerosis Society (EAS) released an update to its 2014 consensus on homozygous familial hypercholesterolemia (HoFH). The 2023 statement provides updated diagnostic criteria, screening recommendations, treatment algorithms, guidance about family planning, and new insights into the genetics of the disease.

Comment: Here is a brief summary of the new findings and recommendations of the updated consensus:

Diagnosis, the updated criteria for HoFH are a LDL-C level >13 mmol/L while untreated, or >8 mmol/L on standard medication. Additional criteria are cutaneous or tendon xanthomas before age 10 years and LDL-C levels consistent with heterozygous FH in both parents. Physicians should exclude other conditions that might elevate LDL-C.

Prevalence: While historically thought to affect one in a million, new research indicates that HoFH prevalence is likely higher, as many as one in 160 000 to 300 000.

Screening: The current guidelines recommend cholesterol screening in children at age ≤ 2 years who have a positive family history of premature atherosclerotic vascular disease or hypercholesterolemia; and universal screening before puberty, at age 5 to 11 years.

Treatment: Lifestyle and maximal dose statin therapy are the mainstays of treatment, and should be started in the first year of life or at initial diagnosis. Patients should start on a high-intensity statin plus ezetimibe, rather than statin monotherapy. Within 8 weeks, proprotein convertase subtilisin/kexin type 9 (PCSK9)-directed therapy should be added. LDL-C should be assessed after 1–2 doses. If there is no response, other treatments should be considered.

Lomitapide is an oral inhibitor of the microsomal triglyceride transfer protein, which affects the production of very low-density lipoproteins, the precursor of LDL-C. In real-world practice, lomitapide added to standard of care treatment reduced plasma LDL-C levels by 60%, albeit with a moderate increase in hepatic fat and hepatic steatosis. *Evinacumab* is a monoclonal antibody targeting angiopoietin-like protein 3 (ANGPTL3), which is licensed for patients with HoFH aged 12 years or older. The ELIPSE HoFH trial reported that evinacumab (15 mg/kg IV every 4 weeks) reduced LDL-C by $\sim$50%, when given in addition to maximally tolerated standard therapy

Follow-up: Given the extremely high risk of early onset severe atherosclerotic vascular disease and its rapid progression, regular screening for subclinical aortic and coronary heart disease is essential. Echocardiography and low-dose computed tomography (CT) angiography are recommended to identify disease burden, with particular emphasis on high-risk lesions in the coronary ostia. Low-dose CT is preferable to detect subclinical coronary and aortic root atherosclerosis.

10.12. Bempedoic acid and cardiovascular outcomes in statin-intolerant patients

Nissen SE, Lincoff AM, Brennan D, Ray KK, Mason D, Kastelein JJP, Thompson PD, Libby P, Cho L, Plutzky J, Bays HE, Moriarty PM, Menon V, Grobbee DE, Louie MJ, Chen CF, Li N, Bloedon L, Robinson P, Horner M, Sasiela WJ, McCluskey J, Davey D, Fajardo-Campos P, Petrovic P, Fedacko J, Zmuda W, Lukyanov Y, Nicholls SJ, CLEAR Outcomes Investigators
N Engl J Med. 2023 Apr 13;388(15):1353-1364.
doi: 10.1056/NEJMoa2215024

Brief summary: In this double-blind, randomized, placebo-controlled trial, bempedoic acid (Nexletol) reduced the incidence of a composite cardiovascular.

Comment: Bempedoic acid is an ATP citrate lyase inhibitor. ATP citrate lyase catalyses the conversion of citrate to acetyl-CoA, the fundamental substrate required for the synthesis of cholesterol and fatty acids. By inhibiting the cholesterol synthesis pathway, bempedoic acid reduces intra-hepatic LDL-C.[1] Bempedoic acid is a pro-drug and is converted to its active metabolite by very-long-chain acyl-CoA synthetase 1 activity. This enzyme is present mostly in the liver and is absent in skeletal muscle, which limits the theoretical possibility of myotoxicity, a common adverse effect of statin therapy.

This double-blind, randomized, placebo-controlled trial was conducted in 13 970 individuals with, or at high risk of, cardiovascular disease, and who were 'statin-intolerant' due to unacceptable adverse effects. The participants were assigned to oral bempedoic acid (180 mg daily) or a placebo. The primary endpoint was a four-component composite of major adverse cardiovascular events (MACE), defined as death from cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke or coronary revascularization, as assessed in a time-to-first-event analysis. The secondary endpoints, also assessed in a time-to-first-event, included a three-component composite of death from MACE, nonfatal stroke or nonfatal myocardial infarction; fatal or nonfatal myocardial infarction; coronary revascularization; fatal or nonfatal stroke; death from cardiovascular causes; and death from any cause.

About one third of the participants in both groups discontinued the drug. In the bempedoic acid group, compared to placebo, the risk of the primary endpoint MACE was 13% lower; and the risk of death from cardiovascular causes, nonfatal stroke or nonfatal myocardial infarction (the first key secondary endpoint) was 15% lower. The risks of fatal or nonfatal myocardial infarction and coronary revascularization were 23% lower and 19% lower, respectively. The relative reduction in MACE was slightly larger among patients without preexisting atherosclerotic vascular disease than among those with secondary-prevention.

Bempedoic acid was associated with a higher incidence of gout and cholelithiasis; and of elevated blood levels of creatinine, uric acid and hepatic enzyme. Concerns about the risks of tendinitis or tendon rupture with bempedoic acid have been reported. The FDA label advises patients to stop treatment if they experience joint pain, swelling or inflammation.

10.13. Association between autism spectrum disorders and cardiometabolic diseases: a systematic review and meta-analysis

Dhanasekara CS, Ancona D, Cortes L, Hu A, Rimu AH, Robohm-Leavitt C, Payne D, Wakefield SM, Mastergeorge AM, Kahathuduwa CN
JAMA Pediatr. 2023;177(3):248–257.
doi: 10.1001/jamapediatrics.2022.5629

Brief summary: This meta-analysis examined the association of autism spectrum disorders (ASD) with cardiometabolic diseases. The study included 276 173 individuals with autism and found a 69.4% higher relative risk of dyslipidemia, indicating higher risks of heart disease and stroke. The risk of type 2 diabetes mellitus (T2D) was 247% higher in individuals with autism, and the risk of type 1 diabetes mellitus (T1D) was 64% higher. Genetic factors, obesity, and autoimmune diseases contribute to these increased risks.

Comment: People with autism are far more likely to experience cardiovascular and metabolic conditions than their peers without autism. In particular, among children with autism compared to age-matched children without, risks of diabetes and hypertension were 2.8 and 2.5 times greater, respectively.

About 1:36 children has been identified with ASD, according to estimates from the CDC's Autism and Developmental Disabilities Monitoring Network.[1] ASD is nearly four times more common among boys than girls. Autism is associated with multiple medical, neurologic and psychiatric comorbidities. Obesity has emerged as an important comorbidity of autism. However, evidence regarding the risk of these obesity-associated comorbidities remains ambiguous due to inconsistencies in recent observational studies.

The aim of this meta-analysis was to examine associations of ASD with cardiometabolic disease (i.e., diabetes mellitus, hypertension and dyslipidemia) and with atherosclerotic macrovascular disease (i.e., cardiovascular, cerebrovascular and peripheral vascular diseases). Data were retrieved from 34 studies, comprising 276 173 individuals with autism, and 7 733 306 individuals without autism (mean [range] age, 31.2 [3.8–72.8] years). The relative risk of dyslipidemia was 69.4% higher among individuals with than without autism. Specifically, triglyceride levels were significantly higher (by 26 mg/dL) and HDL levels were significantly lower (by 9.35 mg/dL). These patterns are consistent with increased risks of both heart disease and stroke. LDL levels did not differ between the groups.

A few risk factors have been identified as contributing to dyslipidemia in autism. These include variations in the genes *NPC1* and *DHCR24*, which are associated with cholesterol metabolism; altered proteins involved in lipid transport (e.g., apolipoprotein B100) and metabolism pathways; and increased lipoprotein lipase activity.

The relative risk of developing T2D was 247% higher among individuals with autism than controls, and was higher among the children with autism than the children without autism. The reasons for higher T2DM and dyslipidemia risks could be multifactorial. Firstly, it could be secondary to the increased prevalence of obesity in autism. Secondly, it could be due to ASD-associated genetic variants (e.g., 16p11.2 deletion, microdeletion 11p14.1). Finally, it could be the result of medications, toxins, prematurity or intrauterine growth retardation. The risk of T1D was 64% higher among individuals with autism than among controls, possibly attributed to an increased risk of autoimmune disease in autism.

An accompanying editorial[1] states that overall, people with autism die much younger than expected, on average by 12 to 30 years earlier. Clinicians should be aware of the specific risk factors among persons with autism, and should implement appropriate preventive measures. To provide appropriate standard of care, clinicians should consider the challenges that these vulnerabilities pose, as well as the structural barriers that people with autism face in accessing high-quality health care.

Reference

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10.14. Quantifying the benefits of inefficient walking: Monty Python inspired laboratory based experimental study

Gaesser GA, Poole DC, Angadi SS

BMJ. 2022 Dec 21;379:e072833.
doi: 10.1136/bmj-2022-072833

Brief summary: It turns out that the common denominator for the treatment of T2D, the metabolic syndrome, and dyslipidemia is physical activity. This physiological study compared the energy expenditure of different walking styles, famously depicted by the Monty Python Ministry of Silly Walks (which was very popular in the UK in the 1970s). If you want to improve your energy expenditure, you should not miss this article from the 2022 Christmas edition of the *BMJ*.

Comment: The authors reviewed the barriers to physical activity, and proposed a new term: PEMPA – practice of effort maximization in physical activity. They assessed the rate of energy expenditure of low efficiency walking compared to high efficiency walking, using Monty Python's Ministry of Silly Walks. It is a must-read article – and then you can learn how to practice these walks here: https://www.youtube.com/watch?v=TNeeovY4qNU.

11. Global Health for the Paediatric Endocrinologist

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In this 8th edition of the Yearbook of Pediatric Endocrinology and diabetes Global Health chapter, we are happy to see increased continuity across all aspects of care especially in pediatric diabetes: from improved access to medicines to e-learning, digital health, epidemiological data specific to low- and middle-income countries and treatment protocols that meet the needs of underserved populations. From a half-full/half-empty glass perspective, we see reasons for hope.

Improving Access to Healthcare in Pediatric Endocrinology and Diabetes

11.1. Reforming the World Health Organization's Essential Medicines List: essential but unaffordable

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JAMA 2022; 328(18):1807–1808.
DOI: 10.1001/jama.2022.19459

Brief summary: The WHO List of Essential Medicines now includes more than 400 medicines. The addition to the list of medicines considered effective, safe, and important for priority public health is made increasingly complicated by the high cost of new drugs. This commentary recommends a new approach whereby consideration would be given separately to economic aspects and to clinical benefits.

Every two years, the WHO publishes a revised List of Essential Medicines for adults (EML, since 1977) and children (EMLc, since 2007). This list is a voluntary guide that helps member states to design their own national EML with the (sadly often unfulfilled) goal of making these medicines available and affordable in the country. Major changes in the diabetes section of the 2021 EML have taken place with the inclusion of long-acting analogues of insulin and of sodium-glucose cotransporter 2 (SGLT-2) inhibitors. While these new drugs are becoming mainstream for the management of diabetes in high-income countries, their high cost keeps them out of reach for patients in many low- and middle-income countries (LMICs) that are still struggling to access human insulin.

The authors suggest that the WHO should separate their assessment of the quality of the medicine by the EML expert committee from the evaluation of the price of the medicines and their cost-effectiveness. This alone is however unlikely to solve the issue of equitable access and new approaches are needed. The WHO has recently implemented a prequalification process for human insulins and for insulin analogues with the aim of increasing the number of available products (there are more than 20 manufacturers of insulin in the world although $>$ 95% of the market is held by Novo Nordisk, Eli Lilly and Sanofi) and, as a consequence, decreasing medicine cost. As of May 2023, only Novo Nordisk's human insulin and Sanofi's glargine have been prequalified. Pooled procurement is another option that is already offered for several medicines (including human insulin) by the Pan American Health Organisation (PAHO, https://www.paho.org/en/paho-strategic-fund) to make drugs more affordable. Finally, as proposed by the authors, the WHO could work with the Medicines Patent Pool (*https://medicinespatentpool.org*) to develop licensing agreements with the patent holders to allow generic manufacturing and supply in LMICs.

11.2. Determining the efficiency path to universal health coverage: cost-effectiveness thresholds for 174 countries based on growth in life expectancy and health expenditures

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Lancet Global Health, 2023;11(6):e833–e842.
DOI: 10.1016/S2214-109X(23)00162-6

Brief summary: This article develops a framework to assess the effect of adoption of new health interventions on rates of healthcare expenditure using a cost-effectiveness threshold. This detailed economic analysis is applied to 174 countries and would allow decision-makers to evaluate the effects of increased expenditure on overall population health.

Health systems face strong pressure to meet the needs of populations, but to do so with scarce resources. With a goal of providing universal health coverage, consideration must be paid to three aspects – who is covered, which services and provided and at what financial cost to the population. As a means of providing further information about the cost-effectiveness of healthcare provision, the authors have developed a framework to assess how adoption and coverage of new interventions will affect the rate of increase in healthcare expenditure and life expectancy at a population level. The model uses the metric of incremental cost-effectiveness ration (ICER) which expressed the efficiency of an intervention in terms of costs per unit of benefit or quality-adjusted life years (QALY). The relationship between the ICER of new interventions and the rate of increase in healthcare expenditure *per capita* and life expectancy at the country level is the basis for their estimation of cost-effectiveness thresholds for these interventions. Based on this 'supply-side' model, the cost-effectiveness thresholds for 174 countries per QALY were calculated and were less than 0.5 gross domestic product (GDP) *per capita* in 96% of low-income countries and in 76% of lower-middle-income countries, and less than 1 GDP in 168 (97%) of countries, which is the current WHO-derived threshold. The authors hope that utilization of a supply-side approach to evaluate cost-effectiveness in healthcare will allow for appropriate prioritization of expenditures and result in decreased health inequities.

11.3. Ten-year experience of a global and freely accessible e-Learning website for pediatric endocrinology and diabetes

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Horm Res Paediatr 2022; 1–10.
DOI: 10.1159/000527984

Brief summary: The authors describe the various applications of the content of ESPE's e-learning website that has been expanding greatly over the last 10 years. The program includes a Limited Resource Setting module that was developed in 2017 and has been translated into French, Spanish, Swahili, and Chinese.

This interesting article retraces the successful development of ESPE's e-learning program since 2012. Thanks to the tenacity and involvement of the program leads and contributors, there has been an increasing use of this freely available tool (*www.espe-elearning.org*). The authors summarize the various ways healthcare trainees

and professionals can benefit from this program including self-directed study, e-learning of pediatric diabetes, healthcare in resource-limited settings, master classes in normal growth and puberty, classroom teaching and continued medical accreditation. It also describes the sustained increase in the number of sessions attended per year (more than 9500 in 2021). Importantly the e-learning program can be accessed on a cell phone, making it particularly appropriate for colleagues practicing in resource-limited settings. Indeed, mobile use has increased more than 6-fold over the last 5 years.

One aspect is particularly interesting. Thanks to Collegium Telemedicus, an online consultation program for health professionals (www.collegiumtelemedicus.org), the option of combining e-learning and e-consultation in resource-limited settings has now been available for several years. Despite the unique opportunity this combination offers, it remains underused: difficult cases can be submitted online, confidentially, in several languages (French, Arabic, English and Spanish) to a group of international colleagues for fast interaction. The simplicity of the website and the opportunity to upload pictures, X-rays and laboratory results make it particularly attractive. It is directly linked to the e-learning program, meaning that in parallel to discussing challenging clinical cases, it is possible to benefit from continuous medical education through the e-learning program.

11.4. Implementing digital systems in diabetes care in low-income and middle-income countries: successes and challenges

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Lancet Diabetes Endocrinol 2023;11(6):387–88.

DOI: 10.1016/S2213-8587(23)00117-1

Brief summary: This commentary focuses on the barriers and opportunities of digital systems with regards to diabetes care in resource-limited settings. It highlights the importance of developing context-specific and cost-effective models of efficient, integrated, and organised diabetes care delivery.

This commentary builds on a more general article on the potential of digital health interventions (1). It focuses on the opportunities for better diabetes care at the patient level. Indeed, with a recent increase in capacity in paediatric diabetes in low- and middle-income countries, the number of patients diagnosed with diabetes has increased exponentially. Challenges in patient management that can be potentially addressed by digital health include (but are not limited to) travel distances from rural areas to the medical center, review of blood glucoses, and patient-specialist interaction for emergencies. Indeed, during the COVID-19 epidemic, pediatric endocrinologists have learned to offer both virtual and in-person visits to maintain patient care while reducing physical contact.

The potential of digital health is not limited to direct patient care. Digital health also plays a major role in the training of health professionals through programs that include a e-learning component (see 11.3) or live, interactive presentations with experts from around the world such as the PEDAF program (https://e-pedaf.org/site/). Virtual registries such as the SWEET project are increasingly integrating countries from low-, middle- and high-income countries to successfully reach commonly agreed goals and bridge 'social and cultural differences among countries, differences in clinical governance, and lack of structured networks of interested parties' (www.sweet-project.org). However, in many instances, work remains to be done to ensure proper access to internet as well as to offer computer education to families, with an emphasis on gender equity, as women are much less likely than men to use mobile internet or to own a smartphone.

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11.5. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study

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Lancet Diabetes Endocrinol 2022; 10 (10): 741–760.
DOI: 10.1016/S2213-8587(22)00218-2

Brief summary: The authors developed a model to estimate the worldwide prevalence of Type 1 diabetes (T1D) in 2040. Compared to 2021, when an estimated 8.4 million individuals had T1D (including 18% < 20 years), the prevalent cases are expected to increase to 13.5–17.4 million in 2040, with the largest relative increase taking place in low- and middle-income countries.

This model, using published data (available from only 97 countries worldwide) provides a global projection of T1D prevalence, incidence and mortality that will serve as a bedrock for later updates as more information becomes available. Besides the projection that the number of patients with T1D will increase by 60–107% in 2040 compared to 2021, the data highlight several important points that all call for better education, better epidemiological data, better understanding of the differences in pathophysiology of T1D between ethnic groups and, potentially, different management approaches in particular in low- and middle-income countries. First, the contrast in remaining life expectancy for 10-year-old children diagnosed with T1D in 2021 between different socio-economic contexts is shocking. In low-income countries, the remaining life expectancy is estimated to be only 13 years, contrasting with 61 years in high-income countries. Second, 47% of the estimated 175 000 deaths due to T1D worldwide in 2021 took place in Sub Saharan Africa and in South Asia. Non diagnosis (estimated in people aged <25 years only) was also the cause of death in a majority (66%) of patients in Sub Saharan Africa and in South Asia.

Finally, separate assumptions were made for Africa, reflecting the fact that the peak age of T1D occurs later in this region, for reasons that remain poorly understood. Specifically, the genetic susceptibility of patients in Sub Saharan Africa and the relative contribution of HLA genes and of other genetic and non-genetic factors are unclear. Indeed, the lower rate of positivity for the islet antibodies traditionally used to confirm an autoimmunity origin of T1D suggest that autoimmunity may not explain all cases of T1D in Sub Saharan Africa and raises the possibility that different therapeutic approaches need to be developed (1).

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11.6. Treatment of diabetic ketoacidosis with subcutaneous regular insulin in a non-ICU setting is effective and economical: A single-center experience

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DOI: 10.25259/JPED_19_2022

Brief summary: Since 2014, children with DKA have been managed on the general ward (instead of the intensive care unit [ICU]) and with SC insulin (instead of IV insulin) at G Kuppuswamy Naidu Memorial Hospital in Coimbatore, India. This retrospective study compares the cost and outcomes of IV (2013–2014) and SC insulin treatment (2017).

In high-income countries (HICs), DKA management commonly includes IV insulin. The 2022 ISPAD Clinical Practice Consensus Guidelines primarily recommend IV insulin infusion, although SC insulin can be considered in children with minimal dehydration who are tolerating oral fluids (1). Admission to ICU should be considered for severe DKA (pH $<$ 7.1). In the Chapter for low- and middle-income countries (LMICs), SC insulin is suggested if IV insulin cannot be safely administered (2).

In the present study, 50 episodes of DKA were treated with either IV ($n=21$) or SC insulin ($n=29$). The time to recovery was shorter (17 hours) in the SC insulin group compared to the IV insulin group (35 hours). There were no cases of cerebral edema or hypoglycemia and no fatal outcomes during the study period. The overall cost of the SC insulin approach was also more than 3 times less than the IV insulin approach. One limitation is that the group of children receiving IV insulin had a slightly more severe metabolic status compared to the group of children receiving SC insulin. Nevertheless, these results have important practical implications. First, in resource-limited settings, best use of the available resources is a priority, in particular when 2 different approaches lead to similar outcomes. Second, the majority of the DKA guidelines are developed based on data published in HICs.

It is suggested that ongoing and future training programs in pediatric diabetology offered in LMICs promote approaches that are appropriate for the level of care of a given region. At the end of the article, the authors remind the reader of a very important point: independently from the protocol itself, the best results are achieved if the medical and nursing staff have developed expertise in the management of DKA in children and if the patients are closely monitored while insulin and rehydration are provided. Indeed, dedication of the health professionals likely remains the most important determinant of treatment success.

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11.7. Survival of children and youth with type 1 diabetes mellitus in Tanzania

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Pediatr Diabetes 2022;1–7.
DOI: 10.1111/pedi.13425

Brief summary: This retrospective study describes a marked increase in the survival of children and youth living with Type 1 diabetes mellitus (T1D) in Tanzania, before (1991–2004), during (2005–2010) and after (2011–2019) implementation of the Life For A Child (LFAC) and Changing Diabetes in Children (CDiC) programs.

This article focuses specifically on diabetes-related mortality. It offers both a message of hope and a candid examination of the issues that youth with diabetes keep facing in Tanzania, similar to other low-resource countries. On the one hand, the authors should be congratulated for their difficult, successful and inspiring work. Over the last 30 years, the number of patients diagnosed with T1D increased more than 14-fold, from 163 before 2005, to 2353 between 2011 and 2019. Many pediatric clinics were developed; mortality, although still significant, decreased from 6 to 2%; the percent of patients lost to follow up also decreased significantly (although it is still 29%, suggesting that mortality, expected to be high in T1D patients without follow up, is likely underestimated at all times).

On the other hand, the authors highlighted a series of issues that keep slowing down further progress. First, although the incidence of T1D was grossly similar in girls and boys, the only factor significantly associated with death in a multivariate analysis was gender, likely reflecting preferential diversion of resources to boys and greater

diabetes-associated stigma in girls. Second, the lack of awareness of diabetes in general negatively affects perception of T1D in the population, slowing advocacy for better management and likely contributing to poor follow up. Finally, the authors highlight the importance of the support received from CDiC (until age 18 years) and LFAC (until 25 years). Both provide not only insulin, but also education for the families and the healthcare workers, HbA1c measurement and glucose strips. Improvement of the situation over the last 12 years is attributed to the implementation of CDiC and LFAC programs although a greater commitment of the healthcare authorities in the funding of diabetes care in general was also observed. Ultimately, it is hoped that support from CDiC and LFAC leads to a full sustainable diabetes care in Tanzania as a part of universal healthcare (see 11.2 in this chapter).

11.8. Insulin thermostability in a real-world setting

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DOI: 10.1016/S2213-8587(23)00028-1

Brief summary: The potency of insulin decreases with increasing temperatures. This pilot study compared the concentration and structure of commonly used types of insulin when exposed to high temperatures for prolonged periods of time.

Manufacturers are required to provide information on how insulin vials and cartridges should be kept by the patient. These recommendations assume that a working fridge is available and that the medicine can be protected from elevated ambient temperatures. For example, according to the manufacturer, a 3 mL glargine cartridge (Eli Lilly, Sanofi) can be used: a) until expiration date if kept unopened at 2–8 °C, b) for 31 days if kept unopened below 30 °C, or c) for 31 days if in-use and kept refrigerated or at room temperature below 30 °C.

The authors tested the potency of various types of insulin for up to 4 months in a real-world setting during the summer in India, where ambient temperature was up to 38.6 °C. Insulin was kept either in a clay pot or on the shelf in a shady part of the home. This pilot study provides reassuring data. The concentration and structure of the various types of insulin tested were found to be mostly within 5% of those found in unopened vials/cartridges kept in the fridge under optimal conditions. As acknowledged by the authors, additional data is needed to confirm that the actual glucose-lowering effect of insulin is also conserved for prolonged periods of time at higher temperatures and that it remains unaffected by ongoing use (drawing insulin once or more every day from the same vial/cartridge).

Interestingly, in September 2022, Novo Nordisk participated in the insulin prequalification program of the WHO and took this opportunity to update the recommendations for the storage of its human insulins (100 IU/mL). They can now be stored at temperatures up to 30°C for four weeks before opening (previously it was 25 °C) (https://extranet.who.int/pqweb/news/first-human-insulins-prequalified). This will prove important for children and families with diabetes living in parts of the world where the climate is hot and humid, where the power grid may not be reliable or where conflicts/war make access to insulin unpredictable. After all, the majority of children with diabetes live in low- and middle-income countries where these conditions are unfortunately too common.

11.9. Global burden of type 2 diabetes in adolescents and young adults, 1990–2019: systematic analysis of the Global Burden of Disease Study 2019

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BMJ 2022; 379:e072385.
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Brief summary: The authors used data from the Global Burden of Disease 2019 to examine the incidence, disability adjusted life years and mortality of early onset type 2 diabetes T2D) in those 15–39 years of age. This showed that early onset T2D is a growing global health problem in adolescents and young adults, particularly in low-middle- and middle-income countries.

Early onset of type 2 diabetes (T2D) is associated with longer lifetime exposure hyperglycemia and earlier onset of complications. The aim of this study was to provide more information about attributable risk factors for early onset T2D in different countries, analyzing secular trends and variations based on sociodemographic index. Significant estimation and modeling were needed due to limitations of the Global Burden of Disease 2019 survey. This study, however, found the highest age standardized incidence rate and age standardized disability adjusted life years (DALY) in countries with low-middle and middle sociodemographic index. This may be attributable to the rapid social and economic changes in these countries. Countries with low sociodemographic index had the lowest age standardized incidence rate, but the highest age standardized mortality rate, possibly due to poorer quality of diabetes care. At a regional level, the greatest burden of early onset T2D was seen in parts of Oceania and South Asia. Western Europe and southern Latin American had the fastest increase in age standardized incidence rate with the UK and Canada having the fastest increase on a country level. The age-standardized mortality rate in high, high-middle and low sociodemographic index countries showed a declining trend while this was unchanged in countries with a middle sociodemographic or increased in those with low-middle sociodemographic index. This may be due to slower changes in health systems compared to epidemiologic changes. High body mass index (BMI) was the main attributable risk factor for all regions by sociodemographic index. From 1990 to 2019, the proportion of T2D cases attributable to high BMI increased from 45% to 69% in women, and from 40% to 66% in men globally.

11.10. Type 1 diabetes in diverse ancestries and the use of genetic risk scores

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Brief summary: This review article discusses the influence of genetics on type 1 diabetes (T1D), particularly with regard to differences across diverse genetic ancestries, and the development of validated genetic risk scores (GRS) for use various populations. These may contribute to disease prevention and treatment.

The autoimmune destruction of pancreatic beta cells is triggered by the interaction of genetic predisposition and environmental factors. T1D has a major genetic risk factor, the human MHC on chromosome 6p21.3 with HLA class I and class II genes contributing 33–50% of genetic risk. As a result, there is an increased interest in developing genetic risk scores (GRS) for non-European individuals by improving methodology and mapping for global populations. By review of published literature, including the Type 1 Diabetes Genetics Consortium (T1DGC), a study examining the genetics of T1D includes African ancestry and multi-ethnic samples, shows that the primary risk allele in Asian individuals is *HLA-DR3* with *HLA-DR4* conferring lower risk, the opposite of that seen in Europeans. The findings are similar in India. A protective African-specific *HLA-DR3* haplotype has been identified in non-Hispanic Black individuals and despite conferring protection in European populations, *HLA-DR7* confers risk in this population. Hispanic individuals in the US have a stronger association with *HLA-DR3* than non-Hispanic individuals, but the complexity of this demographic makes it difficult to describe as a single population.

The goal of GRS is to predict future disease and is dependent on the amount of heritable risk captured by association with genetic variants. GRS-1 and GRS-2 perform well in European populations. In a US sample, GRS-1 performed well in Hispanic and non-Hispanic While individuals, but not in non-Hispanic Black individuals. Other GRS have been developed in Japan and included non-HLA variants to improve differentiation. A GRS

developing using information from African ancestry risk alleles show strong performance in individuals of African ancestry and outperformed a larger GRS of European ancestry. Overall, the development of a cross-population GRS for T1D remains an ongoing challenge with an ideal solution being a unified model that performs well regardless of ancestry. Improved knowledge of genetic factors in T1D will enable more detailed evaluation of currently unknown factors that initiate T1D and allow for improved and equitable care.

11.11. The phenotype of type 1 diabetes in sub-Saharan Africa

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Front Public Health 2023 Jan 27;11:1014626.
DOI: 10.3389/fpubh.2023.1014626

Brief summary: This review article draws on existing studies of type 1 diabetes (T1D) in sub-Saharan Africa, examining differences in phenotype, genetic susceptibility and rates of autoimmunity within this population. Lack of large prospective studies with well-standardized methodologies are noted in this population, making more definitive studies necessary.

This article reviews studies reporting the clinical features of patients with T1D in sub-Saharan Africa (SSA), examining both those at or close to diagnosis and those with longer duration diabetes. Understanding the phenotype and etiology of T1D in SSA in challenging due to lack of robust epidemiological and clinical research, difficulties making an appropriate diagnosis and mortality attributable to poor access and management. Case definition is complicated by atypical presentations and poor differentiation between T1D and T2D, including a high rate of lean T2D, high prevalence of ketosis-prone diabetes and diabetes associated with chronic malnutrition. Limited access to classification tests adds to the difficulty. As a result, phenotypic studies may be examining populations with mixed etiologies. With these caveats, a later peak age of onset of T1D is disproportionately reported in SSA, with a peak about a decade later than that observed in Caucasian populations. This may be due to heterogeneity in phenotype or high and early mortality among younger patients with T1D resulting in lack of diagnosis in this age group. Reported incidence of diabetic ketoacidosis ranges from 20 to 80% depending on the population studied. Immunological studies have consistently reported lower rates of islet autoantibodies in SSA that in developed countries which suggests a possible difference in etiological underpinnings. Based on current evidence, GAD antibodies appear to be the single best islet autoantibody to test for in African populations. Differences in genetic susceptibility are also noted with HLA DR3 conferring the highest susceptibility for T1D in SSA.

Endocrinology

11.12. Ethnic and national differences in congenital adrenal hyperplasia incidence: a systematic review and meta-analysis

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Horm Res Paediatr 2022 Aug 16; 1–10.
DOI: 10.1159/000526401

Brief summary: This paper reviews the incidence of congenital adrenal hyperplasia (CAH) across 58 studies (31 countries). The overall CAH incidence was 1:9498. Geographic variation was observed with a higher incidence in Eastern Mediterranean and South-East Asia and a lower incidence in countries of the Western Pacific region.

Untreated CAH is a recessive condition associated with high mortality and morbidity and early diagnosis and management are key. Unfortunately, neonatal CAH screening often remains limited to high income countries

and access to affordable, lifesaving medicines (hydrocortisone and fludrocortisone) is poor in many low and middle income countries (LMICs) (www.clanchildhealth.org/mates4kids.html). One of the basic areas of research is simply to map the incidence of this condition.

These authors identified 58 published studies on CAH incidence. Several interesting aspects are emerging. First, CAH incidence tends to be higher in the Middle East, a region associated with a high degree of consanguinity, and in Southeast Asia, making these countries prime targets for resource allocation. Second, the authors were unable to find CAH data from large population studies for sub-Saharan Africa where neonatal screening is not routinely performed. Based on North American data, the incidence is assumed to be lower in Sub Saharan Africans compared to Whites, Hispanics and Asians, but this extrapolation is clearly unsatisfactory. Third, they found a high incidence of 1:6084 in China, based on a respectable number of tests ($n = 2\,834\,792$). However, a subsequent even larger Chinese study reported a much lower incidence (1:23 024), based on 7.85 million tests in 41 different regions (1).

Overall, these data raise important points. First, neonatal screening should use identical diagnostic criteria across counties. This lack of standardization makes it very difficult to identify priority regions. Second, besides the lack of resources, other reasons why CAH screening is difficult to establish in LMICs are the lack of education of families on the importance of participating in screening, and lack of infrastructure making collection, transportation and sample analysis within an appropriate timeframe difficult. Finally, the present algorithm for CAH screening, based on 17OHP determination followed by liquid chromatography tandem mass spectrometry (LC–MS/MS) is cumbersome and costly. New approaches, such as genetic analysis (2) or point of care testing are needed to spearhead the development of reliable screening.

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11.13. Measuring contraceptive method mix, prevalence, and demand satisfied by age and marital status in 204 countries and territories, 1970–2019: a systematic analysis for the Global Burden of Disease Study 2019

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Lancet 2022; 400: 295–327.
DOI: 10.1016/S0140-6736(22)00936-9.

Brief summary: An analysis of contraception data in 204 countries and territories between 1970 and 2019 shows that 162.9 million women had unmet needs for contraception (65.5% in sub-Saharan Africa and in south Asia). Demand was not satisfied for 64.8% of women aged 15–19 years, the highest proportion of any age group.

This study included women aged 15–49 years and analysed contraceptive prevalence rate (CPR) and 'demand satisfied'. We highlight this article because of the importance of the results for the youngest age group (15–19 years), commonly cared for by pediatric endocrinologists or by adult endocrinologists with a focus on adolescence. As a reminder, the WHO list of essential medicines for children (EMLc) includes ages 0–12 years, and oral contraceptives are therefore included in the adult list.

The encouraging finding of this study is that CPR and 'demand satisfied' have increased steadily between 1979 and 2019 in all countries and for all age groups. However, the 15–19 years age group still ranks lowest across all sociodemographic markers (married or unmarried, geographical location) for both CPR and 'demand satisfied', in particular in Africa, Middle East and South Asia. This article, in a non-judgmental way, highlights the

negative consequences of the lack of access to contraception on gender equity (delaying childbearing to later in life allows women to pursue education, to gain work experience and to have better access to paid employment) while acknowledging that being married and having children can lead to financial and social security. They also highlight some barriers such as culture (i.e. traditions, religion) and cost (birth control pill in particular, preferred by younger women who want reversible methods) that negatively affect access to contraception.

This article should help pediatric endocrinologists reflect on the role of contraception while balancing culture, gender equity and successful future for their adolescent female (and male) patients. We all have a role to play in supporting United Nations Sustainable Development Goal (SDG) 3.7 which aims to ensure by 2030 '*universal access to sexual and reproductive health-care services, including for family planning, information and education, and integration of reproductive health into national strategies*' (www.un.org/development/desa/pd/data/sdg-indicator-371-contraceptive-use).

11.14. The association between dietary intake and cardiometabolic risk factors among obese adolescents in Indonesia

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BMC Pediatr. 2022 May 12;22(1):273.
DOI: 10.1186/s12887-022-03341-y

Brief summary: This cross-sectional study found high prevalences of high dietary intakes of fat and sugar and inadequate intakes of fiber among obese Indonesian adolescents, and a statistically significant correlation between fiber intake and HDL cholesterol levels.

Low and middle-income countries are experiencing nutritional transitions with increasing prevalence of obesity and cardiovascular disease risks. This cross-sectional study performed in Indonesia aimed to explore the relationship between excess intake of nutrients and cardiovascular disease risk. The study assessed dietary intake in 179 adolescents using a semi-quantitative food frequency recall questionnaire. Participants were between 15 and 18 years of age and classified as obese based on WHO, IOTF and CDC criteria. Laboratory evaluation for LDL, HDL, triglycerides, fasting blood glucose, insulin and HbA1c were performed. A multivariable linear regression model was used to evaluate the relationship.

Compared to dietary recommendations from WHO guidelines, 98% of participants had inadequate intake of fiber, 65% had excess intake of fat and 36% had excess intake of total sugar. There was a statistically significant correlation between fiber intake and level of HDL cholesterol with a non-statistically significant correlation between sugar intake and HbA1c concentrations. Unhealthy dietary intake among obese adolescents in Indonesia is highly prevalent and may lead to obesity and other obesity related cardiovascular diseases.

11.15. Genotype, mortality, morbidity, and outcomes of 3β-hydroxysteroid dehydrogenase deficiency in Algeria

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DOI: 10.3389/fendo.2022.867073

Brief summary: This study describes the genetic and clinical characteristics of 3βHSD2 deficiency in children seen at a single center in Algeria. It describes clinical outcomes, including the frequency of adrenal rest tumors in this population.

3β-hydroxysteroid dehydrogenase 2 deficiency (3βHSD2) is a rare form of congenital adrenal hyperplasia. This mixed longitudinal and cross-sectional study was performed in a single Algerian center between 2007 and 2021, and characterized 14 cases (6 males, 8 females) from 10 families. Consanguinity was present in eight families. All patients presented with severe salt-wasting during infancy, except for two who were diagnosed by family screening. While associated with a disorder of sex development (DSD) in all male patients, this was not the principal reason for referral. Females were often misdiagnosed as having 21-hydroxylase deficiency and therefore were managed inappropriately based on 17OHP levels. Genetic analysis of this cohort identified the p.Pro222Gln null mutation in 12 of the 14 patients. The remaining two patients (sisters) were homozygous for a novel 12bp deletion (c453-464del). All patients were treated with hydrocortisone (mean dose 15.2 mg/m^2/day), but not all patients regularly received fludrocortisone as it was not reliably available. Premature pubarche was noted in the majority of patients, both male and female. Four girls reached menarche during the study period, but three had oligomenorrhea and met criteria for PCOS. Two males were diagnosed with testicular adrenal rest tumor by ultrasound at 5 and 10 years of age, presumably due to inadequate treatment. Adrenal tumors were found in two female patients, both initially thought to have 21-hydroxylase deficiency and therefore likely inadequately treated.

Based on patients seen for CAH at this site, 3bHSD2 deficiency appears more prevalent in Algeria than elsewhere and with high mortality and significant morbidity. Lack of appropriate diagnosis and resulting inappropriate monitoring, as well as poor access to treatment with fludrocortisone result in significant issues in disease management.

11.16. Syrian females with congenital adrenal hyperplasia: a case series

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J Med Case Rep 2022 Oct 15;16(1):371.
DOI: 10.1186/s13256-022-03609-y

Brief summary: This case series of five patients with 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) treated in Damascus, Syria is presented to highlight the current management, including the difficulties of sex assignment, in this cultural context.

In Syria, female infants with 21-hydroxylase deficiency CAH and resulting virilization are generally considered to be male until proven otherwise. This case series reports on the care of five children born with CAH treated at three hospitals in Damascus, Syria between 2017 and 2020. Consanguinity was present in all five families. All five children were treated with hydrocortisone and those with salt-wasting were also treated with fludrocortisone. Two genetically female children were raised as male. One had hysterectomy and oophorectomy and her masculine appearance was preserved in the absence of gonads. The other only had one reconstructive surgery at the age of two years and was later raised as a boy. The authors note that this was the result of parental decision-making despite the counseling of both geneticist and endocrinologist, presumably due to regional and cultural traditions to 'have a male that can be relied upon'. Interestingly, both had younger female siblings with CAH who were raised as female. Two other genetic female children had reconstructive surgery at less than 2 years of age and were raised as female. One reached normal menarche and carried a healthy pregnancy. The fifth patient was diagnosed at 4 years of age due to clitoromegaly and premature pubarche and was raised as female. Despite some menstrual irregularities in her teen years, she had two healthy pregnancies.

The authors advocate for improved multi-disciplinary support for genetic females diagnosed with CAH to provide psychological support, appropriate surgical intervention and reproductive assistance to support them in their natal sex.

11.17. Clinical profile and aetiologies of delayed puberty: a 15 years' experience from a tertiary centre in Sudan

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DOI: 10.1515/jpem-2022-0243

Brief summary: This retrospective study describes patients diagnosed with delayed puberty in a single tertiary care center in Sudan. The frequencies of various etiologies of delayed puberty are described.

Delayed puberty is a common cause for referral to endocrinology clinics, usually due to concerns about height or future fertility. This retrospective study summarises the characteristics of patients with delayed puberty seen in the endocrinology unit at Gaffar Ibn Auf Children's Hospital, Sudan. 136 patients met the criteria for delayed or arrested puberty using standard definitions. The etiology was classified based on gonadotropin and sex steroid measurements, and GnRH stimulation testing, as: constitutional, functional, or permanent hypogonadotropic, permanent hypergonadotropic or unclassified.

The series comprised 83 males (61.1%) and 53 females (38.9%) who presented with short stature in the majority ($n=71$, 52.2%), followed by both short stature and delayed puberty ($n=37$, 27.2%), and delayed puberty alone ($n=28$, 20.6%). The median age of presentation for males was 16 years and for females was 14 years. The most common etiologies were permanent hypogonadotropic hypogonadism (37.5%) and functional hypogonadotropic hypogonadism (36%) with constitutional delay of growth and puberty (CGDP) found in only 14.7%. These data are in contrast with other studies where CGDP is the most common cause of delay. Hypergonadotropic hypogonadism was diagnosed in 11.7%, the majority of whom were female.

This pattern of delayed puberty etiology dominated by permanent hypogonadotropic hypogonadism and functional hypogonadotropic hypogonadism is different from that seen in developed countries, but similar to patterns seen in other underdeveloped countries. Functional hypogonadotropic hypogonadism was associated with underlying chronic disease in 65% of patients. These results may reflect some referral bias with a low rate of CDGP, but also the importance of appropriate chronic disease treatment, general health and nutrition reflected in the high rate of functional hypogonadism.

12. The Year in Science and Medicine

Christa Flück, Ken Ong

Genetics

12.1. A draft human pangenome reference

Wen-Wei Liao, Mobin Asri, Jana Ebler, Daniel Doerr, Marina Haukness, Glenn Hickey, Shuangjia Lu, Julian K Lucas, Jean Monlong, Haley J Abel…., Erik Garrison, Tobias Marschall, Ira M Hall, Heng Li, Benedict Paten
Nature 617, 312–324 (2023).
https://doi.org/10.1038/s41586-023-05896-x

Brief summary: The Human Pangenome Reference Consortium reports a first draft of the human pangenome reference due to replace the existing reference GRCh38 (1, 2). It is an updated, high-quality, graph-based, telomere-to-telomere representation of global genomic diversity including common variants (single-nucleotide variants, structural variants and functional elements).

The human reference genome is the fundamental, open-access resource of modern human genetics and genomics. It was released in its first version more than 20 years ago (1, 2). This reference sequence is needed to provide a system for coordinated reporting and comparing results across genetic and genomic studies. In the updated version, the researchers integrated a large set of reference human genome sequences (known as the Pangenome) that represent the extent of human genetic diversity much better than the GRCh38 genome reference which was composed of a single mosaic sequence representing up to 70% of a single person. In addition, using long-read sequencing technologies (more than 10ãkb long), gaps and errors have been largely removed.

Overall, the new Pangenome ensures that the results of genetic research apply to a wider range of people, and it may help in the identification of so far unsolved, pathogenic genetic variations.

References

1. Initial sequencing and analysis of the human genome. Lander, E. S. *et al*. *Nature* **409**, 860–921 (2001). doi.org/10.1038/35057062.
2. The sequence of the human genome. Venter, J. C. *et al*. *Science* **291**, 1304–1351 (2001). DOI: 10.1126/science.1058040.

12.2. Genomic diagnosis of rare pediatric disease in the United Kingdom and Ireland

Wright CF, Campbell P, Eberhardt RY, Aitken S, Perrett D, Brent S, Danecek P, Gardner EJ, Chundru VK, Lindsay SJ, Andrews K, Hampstead J, Kaplanis J, Samocha KE, Middleton A, Foreman J, Hobson RJ, Parker MJ, Martin HC, Fitz Patrick DR, Hurles ME, Firth HV for the DDD Study
N Engl J Med 2023 Vol. 388 Issue 17 Pages 1559–1571.
DOI: 10.1056/NEJMoa2209046

Brief summary: In this large-scale, multicenter DNA sequencing study, probands (and families; $n = 13\,449$) with previously undiagnosed, severe, likely monogenic, complex developmental disorders from 24 centers in the UK and Irland were studied by whole exome sequencing and microarray analysis. Multimodal data analysis yielded a diagnosis in 41%, more likely with TRIO family analysis. Probands with a history of extreme prematurity, in utero exposure to antiepileptics and maternal diabetes as well as of African descent were less likely to have a genetic diagnosis identified.

In the field of rare diseases, genome sequencing is instrumental to identify a large number of underlying monogenic molecular causes. High throughput methods, decreasing prices and thus larger availability has given

an additional spark to the field. This study shows that a genome approach combined with detailed phenotyping and sophisticated data analysis, integrating both genetic and clinical data and using an iterative analysis approach, can improve the diagnostic yield remarkably.

The study also points to well-known environmental factors that were identified in probands who remained without a genetic diagnosis. In the Discussion the authors suggest correctly that in such patients, gene variants of less severe effect size, but nevertheless contributing to the phenotype, might have been missed as this all depends on the data analysis plan.

Overall, not only developmental disorders but also other disorders, such as differences of sex development, have a heterogenous genetic architecture. In these groups of disorders, the discovery of underlying large burden, highly penetrant *de novo* variants is easier, while in the remaining individuals rare and common incompletely penetrant variants and nongenetic causes may contribute to the disease and are much more difficult to identify.

Note: This paper was published with an Editorial (1).

Reference

1. Genomics in clinical practice. Jennifer E. Posey and James R. Lupski. *N Engl J Med* 388;17 April 27, 2023. DOI: 10.1056/NEJMe2302643.

12.3. Genetic effects on the timing of parturition and links to fetal birth weight

Pol Solé-Navais, Christopher Flatley, Valgerdur Steinthorsdottir, Marc Vaudel, Julius Juodakis, Jing Chen, Triin Laisk, Abigail L LaBella, David Westergaard, Jonas Bacelis …., Ge Zhang, Bo Jacobsson

Nat Genet **55**, 559–567 (2023).
https://doi.org/10.1038/s41588-023-01343-9

Brief summary: This maternal genome-wide meta-analysis of gestational duration ($n = 195\,555$) found 22 associated loci and an enrichment in genes expressed during labour. The related meta-analysis of preterm delivery (18 797 cases, 260 246 controls) revealed seven associated loci and large genetic similarities with gestational duration. Maternal alleles that increase gestational duration had a negative effect on birth weight.

Preterm delivery is one of the biggest risk factors for early neonatal and childhood morality. Both maternal and fetal factors seem to influence timing of parturition, however, these factors are incompletely understood. Preterm delivery has been recognized as a syndrome with various mechanisms involved, among them genetic factors of both the mother and the fetus. The presented study enhances our knowledge of the genetic effects on the timing of parturition and the inter-relationship between mother and child regarding gestational duration and birth weight. They identify an unexpected joint effect of some maternal alleles that increase gestational duration are also associated with lower birth weight (i.e., opposite to the expected effect of longer gestation on higher birth weight). They term this phenomenon 'antagonistic pleiotropy'. We suggest that a longer duration of pregnancy might be an appropriate compensation for maternal factors that restrict fetal growth.

These findings may help in better understanding the molecular signaling of parturition in humans and could eventually lead to the discovery of novel drug targets for tocolysis or labor induction.

12.4. Genetic insights into the social organization of Neanderthals

Skov L, Peyregne S, Popli D, Iasi LNM, Deviese T, Slon V, Zavala EI, Hajdinjak M, Sumer AP, Grote S, Bossoms Mesa A, Lopez Herraez D, Nickel B, Nagel S, Richter J, Essel E, Gansauge M, Schmidt A, Korlevic P, Comeskey D, Derevianko AP, Kharevich A, Markin SV, Talamo S, Douka K, Krajcarz MT, Roberts RG, Higham T, Viola B, Krivoshapkin AI, Kolobova KA, Kelso J, Meyer M, Paabo S, Peter BM

Nature 2022 Vol. 610 Issue 7932 Pages 519–525.
https://doi.org/10.1038/s41586-022-05283-y

Brief summary: Genomic information (nuclear, Y-chromosome and mitochondrial DNA data) of 13 Neanderthals from two neighboring caves in Siberia have been analysed to infer their social community

organization. The data show greater diversity of maternal lineages, which is best explained by female-biased migration between communities.

This study is fascinating as it illustrates what information can be gained with genetic data and analysis beyond the medical field. Based on the shorter average coalescent time for the Y chromosomes (which are paternally inherited) than for the mitochondrial DNA (mtDNA, which is maternally inherited) and shared mtDNA variants between the individuals of the two caves, it can be inferred that these small Neanderthal communities were predominantly linked by female migration.

Neanderthals lived between 400 000 to 40 000 years ago and were a species of early humans. From the study, it can be assumed that they lived in small communities of about 20 individuals together. Their social system was likely patrilocal, which means that wives typically moved to live with their husbands' family after marriage.

12.5. Perspectives of rare disease experts on newborn genome sequencing

Gold NB, Adelson SM, Shah N, Williams S, Bick SL, Zoltick ES, Gold JI, Strong A, Ganetzky R, Roberts AE, Walker M, Holtz AM, Sankaran VG, Delmonte O, Tan W, Holm IA, Thiagarajah JR, Kamihara J, Comander J, Place E, Wiggs J, Green RC
JAMA Network Open. 2023;6(5):e2312231.
DOI: 10.1001/jamanetworkopen.2023.12231

Brief summary: This survey study addressed the question whether rare disease experts ($n = 238$) would advise genetic neonatal screening for treatable genetic disorders. Most experts (87.9%) agreed that genetic analysis for a limited number of monogenic treatable conditions should be available to all newborns.

Newborn screening has been introduced for diagnosing a few treatable congenital disorders at birth in apparently healthy newborns, in order to identify and treat them before they become severely sick. Originally newborn screening was based on biochemical markers, such as TSH to detect congenital hypothyroidism and 17-hydroxyprogesterone for congenital adrenal hyperplasia. Lately, genetic screening for some disorders without biochemical markers has been introduced in several countries, e.g. for adrenoleukodystrophy and cystic fibrosis.

In theory, genetic screening can be rapidly expanded without limitations by use of efficient techniques, such as whole exome sequencing, but the question remains whether we should really do it?

Newborn genome sequencing (NGseq) is able to screen for thousands of genes associated with disorders that cannot be assessed by laboratory assays. But it should be discussed critically as we do not have data for its possible effects on our medical, psychosocial and economic systems. So far, surveys suggested that individuals and especially parents have a positive opinion regarding NGseq, while pediatricians are more critical although still rather positive. The opinion of medical geneticists and other rare disease specialists, who actually would be responsible for implementing NGseq, was to date missing and is now available through this study. More than 200 rare disease specialists would advise with a concordance of 85% for a limited NGseq screening for 25 gene-disease pairs; these relate mostly to metabolic disorders, but include also genes that cause endocrine diseases (*OTC*, *G6PC*, *SLC37A4*, *CYP11B1*, *ARSB*, *F8*, *F9*, *SLC2A1*, *CYP17A1*, *RB1*, *IDS*, *GUSB*, *DMD*, *GLUD1*, *CYP11A1*, *GALNS*, *CPS1*, *PLPBP*, *ALDH7A1*, *SLC26A3*, *SLC25A15*, *SMPD1*, *GATM*, *SLC7A7*, and *NAGS)*.

Thus it remains to be seen how the original newborn screening will be expanded by NGseq in the near future.

Steroids

12.6. Classic and 11-oxygenated androgens in serum and saliva across adulthood: a cross-sectional study analyzing the impact of age, body mass index, and diurnal and menstrual cycle variation

Schiffer L, Kempegowda P, Sitch AJ, Adaway JE, Shaheen F, Ebbehoj A, Singh S, McTaggart MP, O'Reilly MW, Prete A, Hawley JM, Keevil BG, Bancos I, Taylor AE, Arlt W

European Journal of Endocrinology, 2023, 188, 1–15.
https://doi.org/10.1093/ejendo/lvac017

Brief summary: In this cross-sectional study, 11-oxygenated androgens were measured in morning serum samples from 290 healthy adults (125 men, age 22–95 years; 165 women, age 21–91 years) by LC–MSMS (liquid chromatography, tandem mass spectrometry) to generate normative values across the lifespan. In a subset of volunteers ($n = 83$), additional measurements were performed in saliva to assess diurnal and menstrual cycle-dependent variation. In general, it was observed that classic but not 11-oxygenated androgens decline with age, and that with increasing BMI classic androgens decrease while active 11-oxygenated androgens increase.

In the last two decades, several studies showed that the circulating androgen pool results not only from classic androgen biosynthesis in the gonads and adrenals, but also from synthesis through alternative pathways and peripheral metabolism. Thereby 11-oxygenated androgens were found to play a key role, especially in androgen excess disorders such as PCOS (polycystic ovary syndrome) and congenital adrenal hyperplasia (e.g. due to 21-hydroxylase deficiency and P450 oxidoreductase deficiency), where they might be used as disease markers for diagnosis and monitoring. But so far, comprehensive data were missing to show the physiological levels and variability of these androgens in a larger cohort of healthy persons of both sexes with variable BMI, throughout life, menstrual cycles and diurnal rhythm. Therefore, this study provides much more than just normative values. It informs us about the physiology of these 'new steroids' which will likely soon also be used for routine clinical diagnostic and therapeutic control. In the study some of the androgen metabolites were also measured in saliva, which is an easy to obtain, alternative biomaterial to serum that might be difficult to obtain repetitively for questions regarding diurnal and cycle variabilities.

Lastly, in addition to the description of age, sex and cycle specific characteristics of circulating 11-oxygenated androgens in humans, the study provides important insight into the effect of body weight (BMI) on these androgens. Overall, an increase in BMI went parallel with an increase in 11- oxygenated androgens, with some sex specific differences. Furthermore, the observation that adrenal derived precursor 11- oxygenated androgens (11OHA4 and 11KA4) do not change with increasing BMI in females, while the activated products do, informs an important peripheral conversion in the adipose tissue.

12.7. Preoperative circulating 11-oxygenated androgens are associated with metastasis-free survival in localized prostate cancer

Dahmani C, Caron P, Simonyan D, Lacombe L, Aprikian A, Saad F, Carmel M, Chevalier S, Levesque E, Guillemette C
J Urol 2023 Feb;209(2):337–346.
https://doi.org/10.1097/JU.0000000000003049

Brief summary: In the prospective PROCURE study cohort ($n = 1783$), 11-oxygenated androgens were studied in all men with newly diagnosed localized prostate cancer before undergoing radical prostatectomy. Data were related to clinical outcomes (e.g. metastatic disease). Levels of the adrenal androgen precursor 11b-OH-androstenedione were associated with progressive disease, while levels of the predominant bioactive 11-ketotestosterone and its metabolite 11-ketoandrosterone were associated with better, metastasis-free survival.

This study solidifies the role of adrenal 11-oxygenated androgens in prostate cancer, with the largest cohort to date, 1793 men, in which these androgens were profiled. Prior to this study, predominant *in vitro* studies, using prostate cancer cell lines, drove the 11-oxygenated androgen research, while few clinical investigations focused either on smaller cohort sizes or castration-resistant prostate cancer or only profiled 11-oxygenated adrenal androgen precursors. This current paper does a deep-dive into circulatory 11-oxygenated androgen precursors, androgens and their downstream metabolites and associates these androgens with metastasis-free survival. The findings support the future use of these androgens in the clinical work-up of prostate cancer patients to personalize hormonal strategies.

12.8. Interpretation of steroid biomarkers in 21-hydroxylase deficiency and their use in disease management

Sarafoglou K, Merke DP, Reisch N, Claahsen-van der Grinten H, Falhammar H, Auchus RJ

J Clin Endocrinol Metab, 2023, Mar 23;dgad134. Online ahead of print.

https://doi.org/10.1210/clinem/dgad134

Brief summary: In this Minireview, one pediatric and one adult case with 21-hydroxylase deficiency (21OHD) are discussed with respect to different clinical questions and steroid biomarkers reflecting their diagnosis, treatment and disease control. Basics of the disease mechanisms with different aspects throughout life (childhood, adulthood, sex, fertility and pregnancy) are discussed to lay grounds for the interpretation and use of laboratory data, including the newer 11-oxygenated androgens, for clinical decision making of optimal treatment.

Congenital adrenal hyperplasia due to 21OHD caused by *CYP21A2* variants is a fairly common genetic disorder that requires personalized care throughout life. The diagnosis is made ideally by genetic analysis, or by biochemical investigations of a panel of steroids (mostly immunoassays) or a steroid profile (LC–MSMS) under basal and/or ACTH stimulated conditions. By contrast, optimal treatment of CAH by hormonal replacement of glucocorticoids (GC) and mineralocorticoids remains a major challenge but is crucial to avoid severe short- and long-term adverse effects of undertreatment and overtreatment. The many challenges faced in clinical care for CAH patients are comprehensively explained in this Minireview that provides the underlying evidence and highlights our current gaps in knowledge and needs for improvement.

However, the Minireview also highlights the recent advances in steroid profiling that have and will bring newer disease biomarkers into play, the adrenal derived 11-oxy androgens. Although promising, much remains to be evaluated on their performance in different clinical scenario of the disease. In addition, current monitoring of GC treatment is mostly done by a single measurement of adrenal biomarkers reflecting disease control at one specific time point and does not reflect complete information of disease control and androgen exposure overall. But large diurnal variability exists in both the underlying biological system as well as the GC therapy currently provided in most cases by hydrocortisone which has a short half-life, and requires individualized decisions on dose, timing and interval.

There is hope that newer monitoring options (e.g. daily profiling of a biomarker as in diabetes) or novel drugs might give us better tools at hand to optimize treatment for our patients with CAH and improve their long-term outcome in the near future.

A similar review on the same topic, including steroid disorders beyond 21-hydroxalyse deficiency, was published in April 2023 (1).

Reference

1. The clinical and biochemical significance of 11-oxygenated androgens in human health and disease. Karl-Heinz Storbeck and Michael W. O'Reilly. *Eur J Endocr.* 2023 Apr 5;188(4):R98–R109.DOI: 10.1093/ejendo/lvad047.

Basic Research

12.9. Formin-mediated nuclear actin at androgen receptors promotes transcription

Knerr J, Werner R, Schwan C, Wang H, Gebhardt P, Grotsch H, Caliebe A, Spielmann M, Holterhus PM, Grosse R, Hornig NC

Nature 2023 Vol. 617 Issue 7961 Pages 616–622.

https://doi.org/10.1038/s41586-023-05981-1

Brief summary: Two unrelated patients with a disorder of sex development (DSD) phenotype of partial androgen insensitivity (PAIS) showed heterozygous variants in the *DAAM2* gene. Their genital skin fibroblasts showed reduced dihydrotestosterone-stimulated androgen receptor (AR) activity. Extensive basic studies

revealed the underlying mechanism of the DSD in which DAAM2-regulated actin polymerization at the ligand-inducible androgen receptor is required for androgen-stimulated AR activation.

DSD due to androgen insensitivity/resistance syndrome (AIS) is one of the most common causes of 46,XY DSD. *AR* gene variants with complete loss of function lead to complete AIS and sex reversal, while partial activity (PAIS) can manifest with a broad range of DSD phenotypes. However, *AR* gene variants are found in less than half of individuals with a PAIS phenotype. Previously, the same group of researchers reported that aberrant CpG methylation within the proximal *AR* promoter plays an important role in the control of *AR* gene expression (1). In this current report, they show that even factors that indirectly regulate the androgen-stimulated AR included in the DNA polymerization machinery for gene transcription can have a crucial effect on its gene expression and function.

To date, half of investigated patients with a 46,XY DSD remain without molecular diagnosis when using whole exome sequencing and/or array CGH and an 'opinionated' analytical pipeline. This work illustrates very nicely where we can find additional regulatory factors of known DSD genes playing important roles in sex development.

Reference

1. Epigenetic repression of androgen receptor transcription in mutation-negative androgen insensitivity syndrome (AIS Type II). Nadine C Hornig *et al. J Clin Endocrinol Metab*. 2018 Dec 1;103(12):4617–4627. doi: 10.1210/jc.2018-00052.

12.10. Delivery of low-density lipoprotein from endocytic carriers to mitochondria supports steroidogenesis

Zhou YX, Wei J, Deng G, Hu A, Sun PY, Zhao X, Song BL, Luo J
Nat Cell Biol. 2023 Jul;25(7):937–949.
https://doi.org/10.1038/s41556-023-01160-6

Brief summary: Genome-wide small hairpin RNA screening revealed a specialized role for the protein phospholipase D6 (PLD6) located at and highly expressed in the outer mitochondrial membrane of cells in steroidogenic organs. Here PLD6 promotes the entrance of LDL/LDLR complex into the mitochondria where LDL-carried cholesterol is released for steroid hormone biosynthesis.Thereby the mitochondrial redox-sensitive CISD2 protein was found to support the trafficking of the LDL/LDLR complex from the cell membrane to the mitochondria.

Cholesterol is the essential and only substrate for initiating steroidogenesis in steroid-producing organs, including the adrenals, gonads and placenta. The first and rate-limiting step for all steroid hormone production takes place in the mitochondrium where the side-chain cleavage enzyme machinery turns cholesterol over to pregnenolone. Thus cholesterol supply plays a pivotal role. In endocrinology, disorders that affect cholesterol supply to this first step of steroidogenesis are well-known and include steroidogenic acute regulatory protein (STAR) deficiency and Smith Lemli Opitz syndrome (DHCR7), which are both causes of adrenal insufficiency and/or differences of sex development. While STAR deficiency has been shown to affect cholesterol transport to the inner mitochondrial membrane, DHCR7 mutations affect enzyme activity of 3β-hydroxysterol-Δ7 reductase which catalyzes the last reaction in cholesterol biosynthesis.

It is thought that about 20% of cholesterol import is STAR independent in the adrenals and gonads. Thus it is conceivable that variants in genes involved in the newly described CISD2 and PLD6 dependent LDL/LDLR transport pathway could also lead to adrenal and gonadal steroid disorders. Additional hints supporting this hypothesis this are: a) mutations in *LDLR* in humans and mice have been reported to decrease steroid hormone production, b) patients with abetalipoproteinemia who lack circulating LDL have deficient acute cortisol production, c) *Pld6*-deficient mice are infertile, and d) *Cisd2*-deficient mice have low testosterone levels and testicular atrophy.

12.11. Estradiol regulates leptin sensitivity to control feeding via hypothalamic Cited1

Gonzalez-Garcia I, Garcia-Clave E, Cebrian-Serrano A, Le Thuc O, Contreras RE, Xu Y, Gruber T, Schriever SC, Legutko B, Lintelmann J, Adamski J, Wurst W, Muller TD, Woods SC, Pfluger PT, Tschop MH, Fisette A, Garcia-Caceres C
Cell Metabolism 35, 438–455, 2023.
https://doi.org/10.1016/j.cmet.2023.02.004

Brief summary: This basic science study in mice shows how melanocortin neurons integrate reproductive signaling with energy homeostasis. In melanocortin neurons, estradiol (E2) enhances the anorectic action of leptin. Cited1 is enriched in these neurons and its loss exacerbates diet-induced obesity in female mice. Using several specific mouse models it is demonstrated how hypothalamic Cited1, via ERα and Stat3 interactions, link the effects of E2 and leptin on food intake.

It is well known that leptin deficiency leads to obesity and hypogonadotropic hypogonadism in both sexes. In addition, existence of a sex-specific crosstalk between the reproductive system and energy homeostasis is also established. Female mammals, including humans, seem quite protected against metabolic diseases by E2 during their reproductive years, but are at higher risk of diet-induced obesity after menopause when E2 production is lost.

However, the exact interplay between the two endocrine regulatory systems was not known. More so perhaps because of the difference in the nature of the signaling pathways by which they act. Leptin, a peptide hormone, acts on membrane receptors at the cell surface signaling through JAK/STAT. By contrast E2, a steroid hormone, binds mostly to nuclear receptors (e.g. ERα) which then regulate gene transcription. But the group of Gonzalez-Garcia et al based their study on the finding that both hormones are specifically expressed in the same melanocortin neurons in the hypothalamus. They found that Cited1 is the mediator between leptin-JAK/STAT and E2/ERα to enhance POMC transcription and inhibit food intake.

Understanding of the mechanism how E2 regulates leptin sensitivity in melanocortin neurons is essential for the development of novel, sex-specific treatment options against diet-induced obesity. This knowledge might even lead to an explanation for diet-induced obesity in some women in whom variants in the involved genes are found.

Results of this study and its significance were summarized in a Spotlight article (1).

Reference

1. Estradiol and leptin: no engagement without CITED1. Olga Barca-Mayo and Miguel Lo´pez. *Trends in Endocrinology & Metabolism*, July 2023, Vol. 34, No. 7. *https://doi.org/10.1016/j.tem.2023.04.002*.

Food for Thought

12.12. Associations between infant screen use, electroencephalography markers, and cognitive outcomes

Law EC, Han MX, Lai Z, Lim S, Ong ZY, Ng V, Gabard-Durnam LJ, Wilkinson CL, Levin AR, Rifkin-Graboi A, Daniel LM, Gluckman PD, Chong YS, Meaney MJ, Nelson CA
JAMA Pediatrics 2023 Mar 1;177(3):311–318.
DOI: 10.1001/jamapediatrics.2022.5674

Brief summary: In the prospective population-based Growing Up in Singapore Toward Healthy Outcomes (GUSTO) study ($n = 437$ children, 51.9% boys), the effect of parent-reported screen time in infancy was tested on EEG characteristics and correlated to cognitive outcome. Mean daily screen time at 12 months of age was 2 hours. Infant screen time was associated with altered cortical EEG activity at 18 months and was suggested to explain observed alterations in attention and executive functions at 9 years, including impaired higher order cognitive skills essential for self-regulation, learning, and academic achievement, as well as mental health.

How much screen time is healthy for our kids? The American Academy of Child and Adolescent Psychiatry recommends limiting screen time for infants aged 18–24 months to watching only educational programs occasionally (1). For children aged 2–5 years, non-educational screen time should be limited to ∼1 hour per weekday and 3 hours on weekend days (1). Infants exposed to screens are regarded particularly vulnerable to executive function deficits as these functions develop in the first years of life and are highly susceptible to environmental influences. This study is interesting as it shows a dose-dependent association between infant screen time, cortical activity, and cognitive function.

It is well known that excessive screen time is also associated with other adverse health-related outcomes, e.g. a more sedentary lifestyle and thus increased risk of obesity, cardiovascular, metabolic and mental health disorders, especially also in adolescents and adults.

Reference

1. https://www.aacap.org/AACAP/Families_and_Youth/Facts_for_Families/FFF-Guide/Children-And-Watching-TV-054.aspx#:∼:text=Between%2018%20and%2024%20months,limit%20activities%20that%20include%20screens.

12.13. The artificial sweetener erythritol and cardiovascular event risk

Witkowski M, Nemet I, Alamri H, Wilcox J, Gupta N, Nimer N, Haghikia A, Li XS, Wu Y, Saha PP, Demuth I, Konig M, Steinhagen-Thiessen E, Cajka T, Fiehn O, Landmesser U, Tang WHW, Hazen SL
Nat Med. 2023 Mar;29(3):710–718.
https://doi.org/10.1038/s41591-023-02223-9

Brief summary: This observational study in three different cohorts found an increased risk for atherothrombotic disease associated with the commonly used sugar substitute erythritol. Untargeted metabolomics (in cohort 1, $n=1157$) and targeted metabolomics (in cohorts 2 and 3, $n=2149$ USA and $n=833$ Europe, respectively) investigations revealed an increased risk for major adverse cardiovascular events (MACE) including death, myocardial infarction and stroke in persons with circulating levels of multiple polyol sweeteners such as erythritol. At physiological levels, erythritol enhanced human platelet reactivity *in vitro*in a dose-dependent manner; and increased thrombosis formation in a mouse model.

Erythritol is a 4-carbon sugar alcohol which is present in fruits and vegetables in very low amounts. Its sweetness intensity is low and therefore it is often used in high doses as a 'natural sweetener'. Erythritol is commonly used as an artificial sugar either alone or in combination with other sugar substitutes in many food items. People in USA consume on average about 30 g per day erythritol, which corresponds to the amount of one can of an artificially sweetened drink. Erythritol is not metabolized and is excreted in the urine. The current study shows that after consuming a 'usual' amount of erythritol, plasma levels remain elevated for up to 2 days, and these levels were associated with significantly increased risk of thrombosis.

First sugar and now sugar alternatives seem to be a threat to our health. Sugar alternatives have long been recommended to reduce sugar and calorie intake to oppose the obesity epidemic. Especially people at risk for obesity-related metabolic or cardiovascular disorders (e.g. people with diabetes) are still advised to switch to such sugar substitutes. So far, these compounds are generally considered safe by regulatory agencies, but their widespread use shows already traces in our environment (in soil and tap water). Long-term adverse consequences are largely unknown and have not been expected. Only recently studies revealed adverse associations of erythritol (and other sugar replacement compounds) with longer term health related outcomes, such as obesity and diabetes. Studies such as this one by Witkowski et al. are therefore extremely important to inform us on the (new) artificial compounds that we introduce into our food chain with the best intentions, but then turn out quite the contrary.

12.14. Social jet lag and (changes in) glycemic and metabolic control in people with type 2 diabetes

Bouman EJ, Beulens JWJ, den Braver NR, Blom MT, Remmelzwaal S, Elders PJM, Rutters F
Obesity 2023 Apr;31(4):945–954.
DOI: 10.1002/oby.23730

Brief summary: This cross-sectional and longitudinal study, nested in the Diabetes Care System cohort study from the West Friesland region of the Netherlands ($n = 990$), assessed 'social jet lag' as the difference in midpoint of sleep in hours between weekdays and weekend days. Among working individuals with type 2 diabetes (T2D), this discordance between between social and biological rhythms (social jet lag) was associated with poor metabolic control. By contrast, among retired individuals, greater social jet lag was associated with better metabolic control.

Our body clocks integrate internal physiological and behavioral data (e.g. sleep time and wake-up schedules) and constantly try to align them with environmental factors in the best interest of overall health. While several studies showed a negative effect of disturbed synchronization of circadian rhythms on psychological and metabolic outcomes, less is known of this effect in T2D, especially also when considering possible confounders such as sex, age, education, work status, and diabetes complications. The presented study closes this gap in knowledge.

In this study, participants were categorized in three groups: low (≤ 1 hour; 35%), intermediate (44%) and high social jet lag (≥ 2 hours; 21%). Interesting findings that might concern not only T2D individuals, include: a) daily sleeping time was similar in all three groups, b) people with high social jet lag were younger and working, c) working people with higher social jet lag had worse metabolic disease markers (HbA1c, blood pressure, cholesterol ratio), while d) a mixed picture was observed in retired people with rather better disease markers in low and high social jet lag groups compared to intermediate group, and e) BMI was not associated with social jet lag in working people, but was negatively associated with social jet lag in retired persons.

Social jet lag is also a big subject for debate in adolescent medicine when it comes to topics such as sleep and fatigue, obesity, and school performance. The authors emphasise that age, lifestyle and working status matter when looking at the influence of social jet lag, and that the underlying mechanisms are complex and largely unknown. In a related commentary, Till Roenneberg writes (1): '*While enforced social jetlag disrupts health, voluntary sleep extension on weekends might protect it.*' And: '*A reduction of enforced social jetlag should therefore be central to strategies to prevent disease*'; e.g. through more flexible work schedules, later school start times for adolescents or eliminating daylight saving time.

Reference

1. How can social jetlag affect health? Till Roenneberg. *Nature Reviews Endocrinology*, Volume 19, July 2023: 383–384, 384. https://doi.org/10.1038/s41574-023-00851-2.

12.15. Adult height and health-related quality of life in patients born small for gestational age treated with recombinant growth hormone

Rodrigez JMR, Toda LI, Lopez ID, Munoz JB, Fresno LS, Hernandez EF, de Arriba Munoz A
Sci Rep. 2023 Feb 23;13(1):3135.
https://doi.org/10.1038/s41598-023-30281-z

Brief summary: In this cross-sectional study, Health related quality of life (HRQoL) was assessed in 50 adults of 4 Spanish hospitals with a previous diagnosis of small for gestational age (SGA) who had received growth hormone treatment (rhGH) during childhood. Tests revealed lower scores on mental health domains than on domains related to physical health. Remarkably, no correlation was found between HRQoL in adulthood and final height, rhGH treatment and timing of puberty.

How important are physical characteristics, such as height, for wellbeing in children, adolescents and adults? This seems an ever-returning question that can be examined from numerous perspectives and it is likely to be

changing over time, according to societal changes, and of course differ between cultures. Therefore, similar study designs can provide mixed results on this topic.

However, the important finding of this study is not that final adult height, achieved by growth hormone treatment in short SGA children, had no influence on HRQoL in adulthood. Instead, it should be noted that ex-SGA individuals (especially with lower catch-up growth) had lower mental health parameters and therefore may have unmet needs for support. The authors therefore advise to include assessments for HRQoL, neuro-cognitive and psychiatric functioning in their transition to adult age, and to address this issue by future prospective studies.

12.16. The landscape of retesting in childhood-onset idiopathic growth hormone deficiency and its reversibility: a systematic review and meta-analysis

Laurer E, Sirovina A, Blaschitz A, Tischlinger K, Montero-Lopez R, Hortenhuber T, Wimleitner M, Hogler W
Euro J Endocrinol (2022) 187, 265–277.
https://doi.org/10.1530/EJE-21-1179

Brief summary: In this meta-analysis, data of 2030 patients with idiopathic growth hormone deficiency (IGHD) extracted from 25 studies were reanalyzed for reversal of GHD on GH retesting. The reversibility of IGHD varied depending on GH retest cut-offs and testing time-point/age. Higher GH cut-offs and earlier testing resulted in lower GHD reversal rate, but even with a cut-off of 7.7–10 ng/mL the reversal rate was 55%, and retesting before final height revealed also a reversal rate of 48%.

GHD reversal is an important topic in pediatric endocrine health care to protect children and families from unnecessary long-term rhGH treatment and related clinical visits, and to save unnecessary drug costs. Currently, most pediatric endocrinologists use a threshold peak GH value of ∼7 ng/mL on stimulation tests to diagnosis GHD in children. Guidelines recommend retesting only after full pubertal development and attaining final height (using a threshold peak GH value then of 3–5 ng/mL). The presented systematic review provides an excellent summary of GHD retesting practices over the last decades.

Two hypotheses have been proposed to explain GHD reversal in children with IGHD: first, maturation of the GH axis related to puberty and exposure to sex hormones; second, false-positive initial GH stimulation tests leading to a false diagnosis of IGHD due to diagnostic inaccuracy of our current GH testing.

Given the high GHD reversal rates after retesting, as presented in several studies and confirmed in this meta-analysis, it is time to rethink our practice with respect to both time of retesting as well as initial test methods and cut-offs. Updated guidelines for the management of patients with IGHD are therefore needed.

12.17. Metformin: update on mechanisms of action and repurposing potential

Foretz M, Guigas B, Viollet B
Nat Rev Endo 2023; 19(8):460–476.
https://doi.org/10.1038/s41574-023-00833-4

Brief summary: This review provides an update on the possible actions of metformin beyond glucose regulation in the liver. It summarizes recent findings regarding its action in the gastrointestinal tract, on gut microbiota and tissue-resident immune cells and thereby gives insight into studies addressing its immune-modulating and anti-inflammatory effects for possible use as treatment against inflammation- and age-related diseases, as well as cancer. Current knowledge of metformin's several mechanisms of action at the molecular level is also summarized.

Metformin (1,1-dimethylbiguanide hydrochloride) was synthesized one hundred years ago and was found to lower blood glucose levels leading to its current widespread use as an medication in type 2 diabetes mellitus (T2D) as well as many other metabolic-related conditions. However, it remains a fascinating agent that is still

extensively studied as its actions expand beyond the field of glucose regulation and the liver and reach the level of systems biology through its modulation of the inflammasome and immune system.

Will we all soon take metformin as a preventive treatment against aging and low-grade inflammation-related disorders? There are still many open questions to understand the complex actions and possible benefits of metformin.

It is also important to note that with thousands of people taking metformin, this compound is accumulating in our environment. Metformin is not metabolized in the human body but is excreted in urine and feces to reach waste waters. Out there it is biotransformed by microbial degradation and photolysis to numerous organic products that may endanger our global environmental health. Therefore studies of these aspects are also needed.

Of note: Same authors wrote an excellent review on metformin and glucoregulation in T2D in 2019 (1).

Reference

1. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. Foretz, M., Guigas, B. & Viollet, B. *Nat. Rev. Endocrinol*. 15, 569–589 (2019). *DOI: 10.1038/s41574-019-0242-2.*

13. Editors' Choice

Ken K Ong, Christa E Flück

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13.1. Taurine deficiency as a driver of aging

Singh P, Gollapalli K, Mangiola S, Schranner D, Yusuf MA, Chamoli M, Shi SL, Lopes Bastos B, Nair T, Riermeier A, Vayndorf EM, Wu JZ, Nilakhe A, Nguyen CQ, Muir M, Kiflezghi MG, Foulger A, Junker A, Devine J, Sharan K, Chinta SJ, Rajput S, Rane A, Baumert P, Schonfelder M, Iavarone F, di Lorenzo G, Kumari S, Gupta A, Sarkar R, Khyriem C, Chawla AS, Sharma A, Sarper N, Chattopadhyay N, Biswal BK, Settembre C, Nagarajan P, Targoff KL, Picard M, Gupta S, Velagapudi V, Papenfuss AT, Kaya A, Ferreira MG, Kennedy BK, Andersen JK, Lithgow GJ, Ali AM, Mukhopadhyay A, Palotie A, Kastenmuller G, Kaeberlein M, Wackerhage H, Pal B, Yadav VK

https://www.science.org/doi/10.1126/science.abn9257

Science 2023;380:eabn9257.

In Brief: The authors describe a wide range of studies. Firstly, observational studies showed that circulating taurine concentrations decline with age in mice, monkeys, and humans – and in the latter, low taurine was associated with metabolic disease (abdominal fat, blood pressure, Type 2 diabetes). They then gave oral taurine to mice throughout their lives and showed that this increased lifespan by 10–12% and improved functional outcomes of almost all tissues studied (bone, muscle, pancreas, brain, fat, gut, and immune system). Similarly, they found that taurine supplementation improved almost all known cellular mechanisms of ageing, including: DNA damage repair, telomere protection, epigenetic markers of ageing, mitochondrial function and inflammation.

Comment: These are truly remarkable, almost unbelievable findings. The authors observe consistent findings across a comprehensive range of study designs, from observational and experimental studies across diverse species to human observational phenotypic and genetic associations. Taurine is one of the most abundant amino acids. Notably, oral supplementation (to mice and worms) was sufficient to improve not only lifespan but also 'healthspan', the duration of good health.

The data certainly support the authors' proposal that trials of oral taurine supplementation in humans are now needed. However, they cite a recent meta-analysis of such randomised controlled trials in humans, which found no benefit of taurine supplementation. The authors do not comment on whether the doses used in previous unsuccessful trials are adequate according to the doses used in their successful experiments. However, the lack of any apparent toxic effect of oral taurine in humans means this should not be a restriction.

An interesting and relevant observation is that taurine appears to be more important during early life development. Accordingly, early life taurine deficiency leads to growth retardation and osteoporosis, and maternal supplementation during pregnancy increases offspring postnatal bone mass. So it might be important to start taking taurine supplements as soon as possible!

13.2. Somatic mutation rates scale with lifespan across mammals

Cagan A, Baez-Ortega A, Brzozowska N, Abascal F, Coorens THH, Sanders MA, Lawson ARJ, Harvey LMR, Bhosle S, Jones D, Alcantara RE, Butler TM, Hooks Y, Roberts K, Anderson E, Lunn S, Flach E, Spiro S, Januszczak I, Wrigglesworth E, Jenkins H, Dallas T, Masters N, Perkins MW, Deaville R, Druce M, Bogeska R, Milsom MD, Neumann B, Gorman F, Constantino-Casas F, Peachey L, Bochynska D, Smith ESJ, Gerstung M, Campbell PJ, Murchison EP, Stratton MR, Martincorena I

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Nature 2022;604:517–524.
https://www.nature.com/articles/s41586-022-04618-z

In brief: To document variations in rates of somatic mutations, the authors performed whole-genome sequencing of DNA from cells of intestinal crypts across 16 diverse mammalian species, spanning huge 40 000-fold variations in body mass and 30-fold variations in lifespan. The somatic mutation rates per year varied greatly across species and showed a much stronger inverse relationship with species lifespan than with species body mass.

Comment: Peto's paradox describes the observation that larger species do not have higher rates of cancer than smaller species, despite containing several thousand-fold more cells, each of which is prone to developing an oncogenic somatic mutation. The prevailing hypothesised explanation was that larger organisms have developed efficient cancer suppression mechanisms. This landmark paper turns that hypothesis on its head by performing detailed genomic analyses across 16 diverse species, including colobus monkey, cat, cow, dog, ferret, giraffe, harbour porpoise, horse, human, lion, mouse, naked mole-rat, rabbit, rat, ring-tailed lemur and tiger. They found wide differences in somatic mutation rates per genome, highest in mice (796 substitutions per year) and fortunately it was lowest in humans (47 substitutions per year). Notably, there was an almost perfect correlation between somatic mutation rate and species lifespan, which attenuated the weaker relationship between somatic mutation rate and body mass.

Somatic mutations may occur in cells due to a number of reasons, including endogenous DNA damage, errors during cell division, or damage by environmental mutagens. The authors studied intestinal crypt cells as these are less affected by environmental factors and so indicate endogenous processes. The findings imply that somatic mutations are evolutionarily constrained according to lifespan and this involves a number of mechanisms such as DNA damage repair, and avoidance of DNA polymerase slippage and DNA assembly errors. Remarkably, by the end of their lifespan, species differ relatively little in their estimated mutation load per cell. Together with the tight correlation with lifespan, these findings further imply that somatic mutation rate is the major determinant of differences in lifespan across species.

From a developmental perspective, as well as the obvious insights into the mechanisms of ageing, it is recently recognised that DNA damage repair and efficiency and accuracy of DNA replication may also influence early life cell and tissue growth rate. For example, the same mechanisms that protect against DNA damage to delay menopause also contribute to the establishment of a larger oocyte pool at birth (1).

Reference

1. Ruth KS, Day FR, Hussain J, *et al*. Genetic insights into biological mechanisms governing human ovarian ageing. *Nature*. 2021 Aug;596(7872):393–397.

13.3. Evolutionary constraint and innovation across hundreds of placental mammals

Christmas MJ, Kaplow IM, Genereux DP, Dong MX, Hughes GM, Li X, *et al.*
https://www.science.org/doi/10.1126/science.abn3943
Science 2023;380:eabn3943.

In brief: The authors of this large international project describe the generation of 'Zoonomia', which is a large comparative genomics resource covering 240 different mammalian species. Among their many findings, the authors use this resource to: i) document the vast range of biodiversity on earth, ii) identify 3.6 million genomic sites that are perfectly conserved across species, indicating genes and regulatory regions that are likely essential for mammalian survival, iii) identify genes and regulatory elements that likely underlie species-distinct mammalian traits, such as olfaction, temperature responses, hibernation, immunity and other adaptations to the environment.

Comment: This work documents and celebrates the huge diversity among mammalian species, with important insights into the genes and regulatory regions that are essential across all species, and those that have allowed different species to evolve distinct functions to survival and adapt to different circumstances.

91% of the human genome aligns to at least five other species, and 11% aligns to ≥95% (≥228) of all studied species. These 11% most 'constrained' genes are enriched for core molecular processes, such as post-transcriptional regulation (e.g. mRNA processing) and embryonic development (e.g. cell–cell signaling by wnt). By contrast, genes that are most diverse across species are those that regulate interactions with the environment, including innate and adaptive immune responses, skin development, smell, and taste – this finding illustrates the powerful evolutionary processes that enable species to optimally adapt to their environments.

Sometimes, there are important trade-offs. A notable example is absence of the gene *CMAH* (as occurs in humans and 40 other species, including bats) confers resistance to infection by pathogens that are dependent on the sialic acid metabolite Neu5Gc (e.g. malaria) but increases susceptibility to viruses that bind Neu5Ac, notably severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

A particularly interesting section among the many findings is the mapping of likely genes to distinct mammalian traits. Olfactory abilities vary hugely between species. Rodents have the most olfactory receptor genes. Cetaceans (whales and dolphins) have the fewest. 22 species are deep hibernators – they are able to lower their core body temperature <18 °C for prolonged periods. Efficient degradation of damaged mitochondria appears to allow metabolic depression during hibernation. The authors explain that understanding the processes of hibernation, as well as cellular recovery from cooling and rewarming, could inform healthcare in critical settings, as well as a possible future 'Sci-fi' mechanism to enable humans to hibernate during long-distance spaceflight!

Biodiversity is in peril, largely due to human expansion and 'civilisation'. This paper highlights the importance of studying and protecting biodiversity, which likely has countless lessons to enable new discoveries in human healthcare and survival.

13.4. Evolution of the germline mutation rate across vertebrates

Bergeron LA, Besenbacher S, Zheng J, Li P, Bertelsen MF, Quintard B, Hoffman JI, Li Z, St Leger J, Shao C, Stiller J, Gilbert MTP, Schierup MH, Zhang G
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Nature 2023;615:285–291.
https://www.nature.com/articles/s41586-023-05752-y

In Brief: The authors conducted genome sequencing on 151 mother–father–offspring trios from 68 vertebrate animal species in order to estimate and compare germline mutation rates (GMRs). They found a 40-fold variation in GMR per generation between the species. Higher GMRs were observed in species that have a longer generation time, older age at puberty and fewer offspring per generation.

Comment: Mutations in germline DNA during gametogenesis are the cause of a multitude of rare *de novo* genetic disorders in humans. However, the rate of DNA mutation is also an important determination of the pace of evolution, the process through which species adapt to achieve optimal survival and reproductive capacity for their surrounding environments. It has long been suggested that rates of DNA mutation vary between species, but until now it was too challenging to demonstrate this due to the need for large-scale sequence data in pedigrees across many different species.

The authors find there is indeed substantial variation in DNA mutation rates. GMR per generation varied 40-fold between species – and it was even larger when they considered differences in inter-generation time, up to 120-fold variation in GMR *per year*. Other notable findings were a large parental sex-difference in mammals and birds, with higher mutation rates in the parental germline compared to the maternal germline, which could be explained by the larger number of cell divisions needed to generate paternal compared to maternal germ cells. Domesticated animals had much higher yearly mutation rates than non-domesticated animals, likely due to their strong artificial selection for short generation times.

Finally various life-history traits (generation time, age at puberty and offspring per generation) explained 18% of the variation in GMR. These findings suggest that GMR itself may be under evolutionary selection. While age at

menopause was not on their list of examined traits (only humans and a very few other species undergo this phenomenon), recent human evidence shows that genetic susceptibility to earlier age at menopause also confers higher maternal GMR (1). This may be logical as, although they reflect contrasting periods of reproduction, both traits rely on optimal generation and maintenance of germline DNA.

Reference

1. Stasa Stankovic, Saleh Shekari, Qin Qin Huang, Eugene J. Gardner, Nick D. L. Owens, Ajuna Azad, *et al*. Genetic susceptibility to earlier ovarian ageing increases de novo mutation rate in offspring. *medRxiv* June 2022. https://doi.org/10.1101/2022.06.23.22276698.

13.5. Intermittently scanned continuous glucose monitoring for type 1 diabetes

Leelarathna L, Evans ML, Neupane S, Rayman G, Lumley S, Cranston I, Narendran P, Barnard-Kelly K, Sutton CJ, Elliott RA, Taxiarchi VP, Gkountouras G, Burns M, Mubita W, Kanumilli N, Camm M, Thabit H, Wilmot EG, FLASH-UK Trial Study Group Diabetes, Endocrinology, and Metabolism Centre, Manchester Royal Infirmary, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, UK.,

N Engl J Med 2022;387:1477–1487.

https://www.nejm.org/doi/full/10.1056/NEJMoa2205650

In brief: This multicenter, randomised controlled trial in 156 adults (mean age 44 years) with type 1 diabetes (T1D) showed benefits of intermittently scanned continuous glucose monitoring (CGM) (intervention) compared to usual monitoring of blood glucose levels by fingerprick testing on: lower HbA1c (−0.5%; $P<0.001$), 130 minutes/day longer duration of 'in target' glucose levels, and 43 minutes/day shorter time spent with hypoglycaemic blood glucose levels.

Comment: I admit that it has been a very long time since your Yearbook editor (KO) last managed patients with T1D. However, I still follow this literature and highlight from time-to-time papers of notable significance in my opinion. A major longstanding challenge is to persuade patients with T1D to regularly monitor their capillary blood glucose levels with sufficient frequency to inform their self-management. By automating the process, this FLASH-UK trial showed that users of their mobile phone-linked CGM system very frequently monitored their glucose levels during the 24-week trial period: mean(S.D.) 11.0(6.2) number of sensor scans/day. By contrast, usual fingerprick monitoring users started quite well, 4.2(2.1) tests/day at baseline, but this rate declined substantially to 0.6(1.1) tests/day at 24 weeks. This marked increase in frequency of monitoring led to very significant improvements in blood glucose control.

These findings have important translational lessons for the management (and in particular self-management) of other health conditions. The first lesson is that reducing the individual burden of lifestyle behavioural interventions may allow substantially larger changes. It is so much easier to scan a subcutaneous sensor with your phone than to obtain and measure a fingerprick blood sample. Similarly, it would be much easier to follow a healthy diet if the majority of manufactured foods are formulated according to healthy nutritional profiles, and if unhealthy profile foods are not placed at supermarket checkouts or advertised intensely by your traditional or social media channels. Secondly is the concept of 'agency'. Individuals are more motivated to make changes if they are empowered to make choices and take actions. Rather than following a fixed protocol of diet and insulin dosing, the intervention group received information and instruction on how to use scanned glucose level data to make changes to their treatment and lifestyles.

Of course we do not (yet!) have such sensors to monitor daily changes in thyroid function, 17 hydroxy progesterone, or biomarkers of sufficiency of glucocorticoid or mineralocorticoid replacement. But the more we educate and help our patients to understand and contribute to chronic disease management, the more we will see patient engagement and concordance with their treatments.

13.6. Multi-ancestry genome-wide study in >2.5 million individuals reveals heterogeneity in mechanistic pathways of type 2 diabetes and complications

Ken Suzuki, Konstantinos Hatzikotoulas, Lorraine Southam, Henry J Taylor, Xianyong Yin, Kim M Lorenz, *et al*.
medRxiv March 2023.
https://doi.org/10.1101/2023.03.31.23287839
https://www.medrxiv.org/content/10.1101/2023.03.31.23287839v1

In brief: The authors report the largest genetic study to date of Type 2 diabetes (T2D), pooling genome-wide association study (GWAS) data from 2 535 601 individuals (39.7% non-European ancestry), including 428 452 T2D cases. They identified 1289 independent association signals at genome-wide significance ($P < 5 \times 10^{-8}$). These T2D GWAS signals could be separated into eight nonoverlapping clusters, characterised by distinct cardiometabolic disease associations.

Comment: The literature on genetics of T2D requires some effort to follow due to relatively frequent updates in differing populations. By contrast this paper is a major landmark, increasing three-fold the effective sample size compared to the previous largest studies. Notably as well as sample size, they highlight that nearly half of the T2D signals would not have been identified without the inclusion of under-represented ancestry groups, i.e. non-Europeans, who bring a richer, more diverse, extent of genetic variation.

The large number of independent T2D signals allowed a well-powered approach to identify distinct genetic clusters that contribute to this heterogeneous disease. These clusters are 1) beta-cell dysfunction with positive or 2) negative association with proinsulin, 3) insulin resistance mediated via obesity, 4) lipodystrophy, 5) liver/lipid metabolism, 6) residual glycaemic effects, 7) accumulations of body fat, and 8) the metabolic syndrome. These clusters showed differing strength of associations with other insulin resistance related disorders. For example, gestational diabetes was most strongly associated with the beta-cell dysfunction/positive proinsulin association cluster. Polycystic ovary syndrome was most strongly associated with the insulin resistance/obesity cluster. Coronary artery disease risk was positively associated with the lipodystrophy cluster and the insulin resistance/obesity cluster.

The authors conclude that one day we may be able to offer genetically-informed diabetes care and prevention. If this does indeed become a reality, it is important to ensure global access to such advances.

13.7. Bridging the gaps: recent advances in diagnosis, care, and outcomes in congenital hyperinsulinism

Rosenfeld E, De Leon DD
Division of Endocrinology and Diabetes, Children's Hospital of Philadelphia. Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.,
Curr Opin Pediatr 2023;35:486–493.
https://journals.lww.com/co-pediatrics/abstract/2023/08000/bridging_the_gaps__recent_advances_in_diagnosis,.15.aspx

In brief: The authors review advances in our understanding of congenital hyperinsulinism (CHI), including newly described molecular mechanisms, new treatments, and improved understanding of long-term outcomes.

Comment: We apologise for the absence of the chapter on neonatal endocrinology in this year's edition of the yearbook. It will be back next year! In the meantime, this review provides an excellent update on new advances in CHI, covering new molecular discoveries through to new treatments.

The authors reflect that while there are >20 genes known to cause CHI, its aetiology remains unknown in > 20% of all cases, and in ~50% of diazoxide-responsive CHI cases. This year mutations in the gene *HK1* encoding hexokinase were discovered by whole genome sequencing (see paper 13.8 in this chapter). Other recent studies reported mutations in *SLC25A36* as a cause of the CHI-hyperammonemia phenotype, characterized by both fasting and protein-induced hypoglycemia and persistent hyperammonemia (1). *SLC25A36* encodes a carrier that transports pyrimidine and guanine nucleotides across the inner mitochondrial membrane. Uridine supplementation was given to one child, resulting in improved glycemic control and also normalized thyroid function and growth.

There have been advances in CHI imaging. Current best practice in multidisciplinary hyperinsulinism centres is ^{18}F-DOPA PET/CT, which has >90% sensitivity to detect focal pancreatic lesions. However, more accurate imaging would potentially allow even more cases to be treated by surgery. There is promising data that 68Ga-NODAGA-exendin-4 PET/CT imaging may have even greater sensitivity and at lower radiation doses.

Continuous glucose monitoring (CGM) is a promising management tool. However despite its large evidence base in patients with type 1 (see paper 13.5 in this chapter) and type 2 diabetes, its use in CHI is still limited to small case series, and reliable detection of hypoglycemia appears more challenging than detection of hyperglycemia (2).

Diazoxide remains the only FDA approved medication for the treatment of CHI, 40 years after its first reported use. A recent paper describes that lanreotide, a synthetic version of somatostatin, significantly improved fasting tolerance and 42% of this large series of 58 CHI patients were able to discontinue other CHI treatments (3). New potential treatments in the testing pipeline include a soluble Glucagon analogue, a GLP-1 receptor antagonist, and an allosteric inhibitor of the insulin receptor.

Reference

1. Shahroor MA, Lasorsa FM, Porcelli V, *et al*. PNC2 (SLC25A36) deficiency associated with the hyperinsulinism/hyperammonemia syndrome. *J Clin Endocrinol Metab* 2022; 107:1346–1356.
2. Worth C, Dunne MJ, Salomon-Estebanez M, *et al*. The hypoglycaemia error grid: a UK-wide consensus on CGM accuracy assessment in hyperinsulinism. *Front Endocrinol (Lausanne)* 2022; 13:1016072.
3. Cuff H, Lord K, Ballester L, *et al*. The use of lanreotide in the treatment of congenital hyperinsulinism. *J Clin Endocrinol Metab* 2022; 107:e3115–e3120.

13.8. Non-coding variants disrupting a tissue-specific regulatory element in HK1 cause congenital hyperinsulinism

Wakeling MN, Owens NDL, Hopkinson JR, Johnson MB, Houghton JAL, Dastamani A, Flaxman CS, Wyatt RC, Hewat TI, Hopkins JJ, Laver TW, van Heugten R, Weedon MN, De Franco E, Patel KA, Ellard S, Morgan NG, Cheesman E, Banerjee I, Hattersley AT, Dunne MJ, International Congenital Hyperinsulinism Consortium, Richardson SJ, Flanagan SE
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Nat Genet 2022;54:1615–1620.
https://www.nature.com/articles/s41588-022-01204-x

In brief: The authors performed whole genome sequencing on 135 patients with congenital hyperinsulinaemia (CHI) who had negative genetic testing for previously known CHI genes. They identified nine different non-coding de novo variants (carried by 14 probands) located in a regulatory region of *HK1* intron 2 that co-segregated with disease in families.

Comment: *HK1* is a 'disallowed gene' in the liver and pancreatic beta cells. This means it is expressed in all other tissues, but it is actively silenced in these two specific tissues. Here, through epigenetic and functional studies, the authors show that the CHI-associated variants cause *HK1* to be inappropriately expressed in pancreatic beta cells. This leads to inappropriate insulin secretion during hypoglycemia and the cases presented with severe CHI from birth and were unresponsive to medical treatments.

Hexokinase has very high affinity for glucose, even higher than the glucose affinity of glucokinase, which is the canonical glucose sensor in pancreatic beta cells. The identified pathogenic mutations were located in a non-coding part of *HK1* which appears to bind to transcription factors (including FOXA2 and NKX2) needed for the silencing of *HK1*. Notably, inactivating mutations in *FOXA2* are a previously recognised cause of CHI with hypopituitarism. These findings indicate that disallowed genes require ongoing active silencing throughout life.

Finally, the authors note that there are a further 60 genes that are selectively silenced in beta cells. These genes, and their epigenetic regulators, should also be candidates for novel CHI genes.

13.9. Obesity in pregnancy

Creanga AA, Catalano PM, Bateman BT
Johns Hopkins Bloomberg School of Public Health, and the Department of Johns Hopkins School of Medicine, Baltimore. The Mother Infant Research Institute, Tufts Medical Center, and the Department of Obstetrics and Gynecology, Tufts University School of Medicine, Boston, USA.,
N Engl J Med 2022;387:248–259.
https://www.nejm.org/doi/full/10.1056/NEJMra1801040

In brief: The authors provide a comprehensive review of the health challenges of obesity in pregnancy, covering its epidemiology, associated risks for pregnancy, childbirth, fetus and offspring. They also discuss the evidence for lifestyle interventions during pregnancy and also the benefits and risks of bariatric surgery prior to conception.

Comment: Remarkably in the USA in 2020, only two out of every five pregnant women had a normal weight status – of the others just under half had overweight and just over half had obesity. Obesity has adverse effects on all maternal outcomes throughout the course of reproduction, from subfertility and miscarriage through to pregnancy, in particular gestational diabetes and hypertensive disorders of pregnancy but also depression and anxiety, on labour and childbirth, and even after birth on risks of postpartum haemorrhage, infection and likelihood of successful lactation. Risks for the fetus are also substantially raised – much of this can be explained by those maternal disorders of hyperglycemia, hypertension, preterm birth, and instrumental delivery. Risks for neural tube defects may reflect higher folic acid requirements by women with obesity. However, the full extent of the significantly elevated risks of stillbirth and congenital malformations associated with maternal obesity remains unexplained.

Of particular interest is the section of this review covering the benefits and risks of bariatric surgery in the mother before conception. Notably, data are lacking on the effectiveness and safety of new GLP1 and GIPR receptor agonists during pregnancy as this group is deliberately excluded from such trials due to lack of knowledge about potential teratogenicity. Therefore bariatric surgery will likely continue to be increasingly used. It has been shown to restore ovulation and improve fertility, and reduce risks of gestational diabetes, hypertensive disorders and the associated newborn complications. However, there is a risk of surgical complications and also micronutrient deficiencies in the mother, which impact adversely on fetal development. Therefore the American College of Obstetrics and Gynaecology recommends that women delay pregnancy for at least 12 to 18 months after surgery or until they achieve a stable post-surgery weight.

13.10. Race-dependent association of high-density lipoprotein cholesterol levels with incident coronary artery disease

Zakai NA, Minnier J, Safford MM, Koh I, Irvin MR, Fazio S, Cushman M, Howard VJ, Pamir N
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J Am Coll Cardiol 2022;80:2104–2115.
https://www.jacc.org/doi/10.1016/j.jacc.2022.09.027

In Brief: In this US cohort study of 30,239 Black and White individuals aged $>=45$ years followed up for a median of 10 years, low baseline levels of HDL-Cholesterol (HDL-C) were associated with increased risk of coronary heart disease (CHD) in White (HR: 1.22; 95% CI: 1.05–1.43) but not in Black (HR: 0.94; 95% CI: 0.78–1.14) adults (*P*-interaction by race $=0.08$).

Comment: The established clinical risk factors for coronary heart disease (CHD) were originally based on data from the famous Framingham Heart Study in the 1970s. While weightings of the contributing factors have been updated with extended data, the original Framingham Heart Score has stood the test of time, encompassing age, sex, LDL cholesterol, HDL cholesterol, blood pressure (and hypertension treatment), diabetes, and smoking. However the original Framingham Heart Study was universally of white European origin. Although the town of

Framingham and surrounding community has since increased in racial diversity, large sample sizes are needed to robustly detect population group differences.

These notable findings highlight the need to check the validity of so-called 'established' risk markers for disease in different populations. In contrast to the new findings with HDL-C, the authors observed the expected associations between LDL-cholesterol and triglycerides with increased risk of CHD in both races, which supports the validity of the study design. The authors comment that the findings are consistent with those in other studies of racially diverse cohorts, which have reported weaker or null heart disease associations with HDL-C. Black Americans have a lower overall risk of CHD compared with White Americans, but higher risk of fatal CHD events. Further work is needed to calibrate new heart disease prediction scores based on robust data in non-white populations.

Of note, *high* HDL-C was not associated with CHD risk in either White or Black Americans, suggesting that in White Americans the relationship between HDL-C and CHD is non-linear (low levels are harmful but high levels are not protective). Indeed, beyond its role as a predictor, the causal effect of HDL-C on CHD remains unestablished.

13.11. Etiology of the broad avoidant restrictive food intake disorder phenotype in Swedish twins aged 6 to 12 years

Dinkler L, Wronski ML, Lichtenstein P, Lundstrom S, Larsson H, Micali N, Taylor MJ, Bulik CM
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JAMA Psychiatry 2023;80:260–269.
https://jamanetwork.com/journals/jamapsychiatry/fullarticle/2801119

In brief: The authors analysed data from the nationwide Child and Adolescent Twin Study in Sweden (CATSS) to estimate the heritability of avoidant restrictive food intake disorder (ARFID) as defined by *DSM-5* criteria. Of the 16 951 twin pairs born between 1992 and 2010, 682 (2.0%) children were identified to have ARFID. By modelling shared risk in monozygous compared to dizygous twin pairs, they estimated the heritability of ARFID as 79% (95%CI, 70–85).

Comment: While ARFID is not known to have an endocrine aetiology, we may see these patients in our clinics due to its impact on poor growth and delayed pubertal maturation. It is a cause of significant individual, family, and social impairment and can be life threatening.

ARFID is distinguishable from anorexia nervosa by its earlier age at onset, even from infancy, and by the lack of motivation by body image concerns or drive for thinness. Instead, it may be characterised by a lack of interest in food, fear of choking, vomiting or allergic reaction, or linked to autistic traits such as sensory sensitivity to food texture, smell, or taste. It is even thought to be on the rise in adults, linked to the popularity of severe restriction or food avoidance diets.

The headline finding of this paper is striking. A condition that was thought to be triggered by a wide range of stochastic factors, from acute allergies to distressing early experiences of eating, is now shown to be highly heritable – indeed, extremely heritable. There are few complex diseases or traits that have such a high heritability estimate, 79% is around the same heritability as adult height. The authors comment that this estimate is similar or higher than for other mental health traits: autism 64–91%; schizophrenia 79%; ADHD: 77–88%; bipolar disorder: 50–71%. By contrast, heritability estimates for other eating disorders are lower: anorexia nervosa 48–74%; bulimia nervosa 55–61%; binge-eating disorder 39–45%.

Now that ARFID can be considered a highly genetic condition, the next step is to explore the underlying genetic factors, molecular mechanisms and biological pathways that contribute to its pathogenesis. Hopefully this will reveal effective preventive and therapeutic interventions.

13.12. Embryo model completes gastrulation to neurulation and organogenesis

Amadei G, Handford CE, Qiu C, De Jonghe J, Greenfeld H, Tran M, Martin BK, Chen DY, Aguilera-Castrejon A, Hanna JH, Elowitz MB, Hollfelder F, Shendure J, Glover DM, Zernicka-Goetz M
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Nature 2022;610:143–153.
https://www.nature.com/articles/s41586-022-05246-3

In brief: The authors created mouse embryos in the laboratory from a combination of multiple stem cell lines. These embryos were developed *ex vivo* up to the equivalent of day 8.5 post-fertilization. Embryos developed within an extraembryonic yolk sac and were similar to whole natural embryos, with defined forebrain and midbrain regions, a beating heart-like structure, a neural tube and somites, a tail bud containing neuromesodermal progenitors, a gut tube, and also primordial germ cells. Using this model, they showed that ES-C knockout of *Pax6* causes ventral domain expansion of the neural tube.

In the same month, another paper by Tarazi *et al*. (1) from the Weizmann Institute of Science, Israel, reported a similar 'synthetic' mouse embryo model containing all embryonic and extraembryonic compartments, and organ progenitors within complex extraembryonic compartments similar to day 8.5 mice embryos.

Comment: These two papers describe major advances in producing embryos 'in a dish', past gastrulation (the milestone when the three primary germ layers ectoderm, endoderm and mesoderm are formed), up to roughly mid-gestation (in the mouse). To do this, they generated and cultured together stem cells from all three blastocyst cell types: embryonic stem cells (ES-C), trophoblast stem cells (TS-C), as well as extraembryonic endoderm stem cells (XEN-C). These cells have remarkable innate properties of self-organization, and after 8.5 days they had self-formed 'embryoids' that resembled whole natural embryos.

Both groups then showed how these embryoid models can be used as powerful models to understand mechanisms of disease. Amadei *et al*. studied embryoids formed from *Pax6*-knockout ES-Cs, to study its impact on early neuronal development. *Pax6* is involved in eye and brain development and disruptive mutations cause optic nerve hypoplasia and have been implicated in congenital hypopituitarism. Tarazi *et al*. modified their embryoids by making their primordial germ cells (PGCs) fluoresce. Remarkably, they could detect PGCs at day 5, specifically at the site of the putative primitive streak. These PGCs then showed 'normal' migration to the posterior ventral location by day 8.

More work is needed, in particular to improve the efficiency in generating such embryoids (currently is only around 0.1 to 0.5%). However, these model systems have high potential as disease models to study the impacts of genetic factors on early development as well as to screen chemicals for teratogenic properties.

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14. Selected Papers by Ze'ev Hochberg

The Yearbook 2023 Team

Preface

This year we have included a Chapter in memoriam to Ze'ev Hochberg who sadly passed away at the beginning of 2023. He was the Director of Pediatric Endocrinology at the Rambam Medical Center in Haifa, Professor of Pediatric Endocrinology in the Technion, and a Fellow of the Rappaport Research Institute in Haifa. He also was a very active ESPE member, past president of ESPE, founder of the Global Pediatric Endocrinology and Diabetes (GPED) as well as the ESPE Winter School, leader of several ESPE summer schools and schools in Africa. His research focus was on basic principles of child growth and development, evolutionary perspectives and mechanisms. For his significant contributions, Ze'ev received several international awards and prizes, among them ESPE's prestigious Andrea Prader Prize.

Lastly, Ze'ev also established the ESPE Yearbook (with Jean-Claude Carel) and was its Editor-in-Chief for many years. Therefore, to reflect and honor his contributions to science and medicine, we have put together a potpourri of articles that he published over the years, showing the broad spectrum of his research interests that connected very many of us.

14.1. Energy trade-off and 4 extreme human body types

Ze'ev Hochberg, Kerstin Albertsson-Wikland, Florian Privé, Alina German, Anton Holmgren, Lisa Rubin, Michael Shmoish
J Clin Endocrinol Metab 2023 Apr 13;108(5):e89–e97.
doi: 10.1210/clinem/dgac665

Brief summary: In this paper introduced a new energy trade-off score (ETOS) and index to characterize four extreme human body types regarding height and weight in young adulthood (e.g. *tall-slender, short-stout, short-slender, tall-stout*) for growth patterns and underlying genetic background. Growth data of 1889 subjects (996 girls) of the GrowUp 1974 Gothenburg study were investigated for the four body types showing that the two trade-off body types *tall-slender* and *short-stout* differed in height, weight, BMI and ETOS and mirrored each other. While BMI trajectories for the trade-off groups increased or decreased constantly over time, ETOS trajectories showed phasic changes suggesting a specific role of energy trade-off during infancy and puberty. GWAS were run for ETOS, height and BMI using data extracted from the UK Biobank and yielded several genes that have been previously been associated with changes in weight and/or height. Several loci were found strongly associated with ETOS, and comprised genes were enriched in pathways of metabolism, chromatin organization, regulation of PTEN and WNT signaling.

The trade-off theory states that, in human evolution, changes in two energy consuming traits are competing, meaning that they come at a cost to each other. Examples of this include early reproduction, which comes at the cost of shorter height and increased BMI, or the inverse trade-off between weight and height with undernutrition and overnutrition. The introduction of a new ETOS and distinct body types allows now to interrogate existing data for this theory not only to find growth patterns and time windows where ETO is most effective, but also to identify its underlying genetic basis and (disease) mechanisms. In the future, this might help to identify people at risk earlier and provide them with more specific treatments.

This paper, published in April 2023, is the most recent of the 280 PubMed cited papers of Ze'ev Hochberg. It shows that he was involved in research projects addressing the most important questions of today's human health and medicine using the newest approaches and providing excellent analytical expertise.

14.2. Prediction of adult height by machine learning technique

Michael Shmoish, Alina German, Nurit Devir, Anna Hecht, Gary Butler, Aimon Niklasson, Kerstin Albertsson-Wikland, Ze'ev Hochberg

J Clin Endo Metab 2021 Jun 16;106(7):e2700–e2710.
doi: 10.1210/clinem/dgab093

Brief summary: Growth data from three independent longitudinal cohort studies (Gothenburg GrowUp 1974 (n 1596); Gothenburg GrowUp 1990 (n 1890); Edinburgh Growth Study (n 145)) were used to train machine learning (ML) to predict adult height (AH) based on growth measurements until the age of 6 years. Five ML algorithms were tested. A random forest model predicted best, with sex and height at age 3.4–6.0 years being the most influencing factors. The model was cross-validated using unrelated data and revealed a prediction accuracy of $r = 0.88$, with an overprediction of AH in short children and an underprediction in tall children. The average prediction error for AH was -0.4 ± 4.0 cm.

Assessing growth in children and predicting adult height is the daily business of a pediatric endocrinologist. Tools used are longitudinal height measurements that are recorded on growth charts to visualise an individual's growth trajectory against a selected reference population and/or radiologic evaluation of skeletal maturation in comparison to a healthy reference population. In the latter approach, AH is then most often predicted according to Tanner-Whitehouse and/or Bayley-Pinneau methods, or the newer online version BoneXpert. Accuracy of these methods is generally quite disappointing with a standard error of about 5–6 cm.

Electronic health records are in standard use in most health care centers and accumulate huge amounts of patients' data. The use of such data for personalized, real-time decision-making is currently implemented in many aspects of medicine. So it is nice to see that this is also the case for growth assessments and AH prediction. Remarkably, the ML algorithm developed by the investigators performed better for boys and girls at age 6 years than all other older classic prediction methods. It is able to explain 75–77% of the variability in AH, while the remaining variability is due to pubertal growth and remains unexplained and unpredictable by the algorithm.

This study published in 2021 shows that Ze'ev Hochberg never got tired to learn and apply novel methods to his research which he intended to translate into clinical practice in order to improve children's healthcare. Moreover, the collaborative network between Haifa, London and Goteborg supporting this study shows that he was an internationally acknowledged team player and leader.

14.3. People are taller in countries with better environmental conditions

German A, Mesch G, Hochberg Z
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Front. Endocrinol 2020; 11:106.
doi: 10.3389/fendo.2020.00106

Brief summary: 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 too 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.

14.4. Steroid metabolomic signature of insulin resistance in childhood obesity

Gawlik AM, Shmoish M, Hartmann MF, Wudy SA, Hochberg Z
Diabetes Care. 2020 Feb;43(2):405–410.
doi: 10.2337/dc19-1189

Prof Hochberg was an exceptional paediatrician, endocrinologist and paediatric endocrinologist. He had an inquisitive mind, interest in various and wide fields, love for music, operas, books and people. Even in the field of endocrinology, he always researched beyond the visible. His groundbreaking contributions to the field of paediatric endocrinology have left an indelible mark on the medical community.

Comment: To distinguish among children with obesity, between those with and without the metabolic syndrome, Professor Hochberg and his colleagues investigated the steroid metabolomic profile. They focused specifically on 31 steroid metabolites found in urine samples, using gas chromatography–mass spectrometry. By collecting urine samples over a 24-hour period, they observed a notable distinction between the two groups. The metabolomic profile of children with compared to without insulin resistance exhibited significantly higher levels of steroids from all three adrenal pathways (adrenal androgens, glucocorticoids, and mineralocorticoids). Moreover, children with insulin resistance had heightened activities of 5α-reductase and 21-hydroxylase, but reduced activity of 11βHSD1. These findings effectively establish a strong correlation between insulin resistance, hyperinsulinemia and increased production of adrenal steroids, thus suggesting that the adrenal glands are a target of insulin resistance or hyperinsulinemia.

This work reflects the scientific path of Professor Hochberg and his international collaboration. His research encompassed a wide range of projects, and excelled in providing comprehensive descriptions of diverse phenomena, while delving deeply into the fundamental processes underlying them.

Note, this paper was included in the 2020 Yearbook, highlighting its importance.

14.5. Emerging adulthood, a pre-adult life-history stage

Hochberg Z, Konner M
Front Endocrinol (Lausanne) 2020; 10:918.
https://pubmed.ncbi.nlm.nih.gov/31993019/

Brief summary: This review uses an evolutionary approach to provide an interesting discussion on a proposed period of development called 'emerging adulthood'. The authors explain that it can be seen not only as a sociological transition period but also as a biological life-history phase.

The central theme of this review is 'emerging adulthood', which is the concept that an additional 4-6-year pre-adult period should be included in models of human development. The concept of emerging adulthood was initially introduced by Arnett [1] as '*a phase of life between adolescence and full-fledged adulthood, with distinctive demographic, social, and subjective psychological features*'. This emerging adulthood phase exists also in other mammals and it is likely the result of genetic and cultural evolution.

Importantly, the authors highlight that this phase is characterized by further brain maturation, involving in particular neocortical association areas in the frontal lobes. Consequently, emerging adulthood is characterized by progressive increases in individuals' cognitive abilities, which reach their peak around the 4th decade. Emerging adulthood is also a social stage, being an important period of learning about intimacy and mutual support, intensification of pre-existing friendships, family-oriented socialization, and the attainment of those social skills needed for mating and reproduction.

This life-history stage applies to individuals aged between 18–25 years, a critical period when people become progressively economically independent by training and/or education. Emerging adults are still learning and maturing and thus they have a level of 'vulnerability' and require protection.

Although not discussed in the review, emerging adulthood is also an important concept in medicine when managing patients with chronic conditions. In this context, emerging adulthood indicates a period of gradual transition from paediatric to adult care. Awareness of the dynamic aspects of this phase should be considered to support emerging adults and make the transition process as smooth as possible [2,3]. A key goal of the transition process should be to optimize health outcomes and increase opportunities for young people with chronic conditions to achieve their maximum potential.

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14.6. Outcomes of pubertal development in girls as a function of pubertal onset age

German A, Shmoish M, Belsky J, Hochberg Z

Eur J Endocrinol. 2018; 179(5):279–285.

PMID: 30087116.

https://academic.oup.com/ejendo/article-abstract/179/5/279/6655429?redirectedFrom=fulltext&login=false

Brief summary: This prospective study, including 380 American girls followed from birth to age 15.5 years between 1991 and 2006, showed the predictive nature of age at onset of puberty for the subsequent progression and duration of pubertal maturation.

Ze'ev Hochberg's scientific and clinical curiosity is an inspiration for us all. Ze'ev approached the topic of puberty using evolutionary and developmental biology principles, according to which maturational traits respond to environmental factors in order to assure survival and fecundity. This paper illustrates his constant search for clinical insights with a focus on puberty.

Age at onset of puberty is characterized by a wide physiological variability, which is specific to human and non-human primates (1). The authors hypothesized that age at onset of puberty determines pubertal and growth tempo. The study included 380 girls between 1991 and 2006, followed longitudinally through thelarche, pubarche and menarche.

Girls with early pubertal onset (EO; thelarche at mean 9.0 ± 0.1 years) also showed earlier pubarche and menarche than girls with later onset (LO; thelarche at mean 11.0 ± 0.5 years). However, the tempo (duration) of pubertal progression was longer in EOs than in LOs.

This study helps us to understand pubertal tempo and progression, which today represent an extremely frequent cause for referral to our clinics.

Reference

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14.7. Early adiposity rebound and premature adrenarche

Chrisanthi Marakaki, Olga Karapanou, Alexandros Gryparis, Ze'ev Hochberg, George Chrousos, Anastasios Papadimitriou

J Pediatr 2017 Jul;186:72–77.

doi: 10.1016/j.jpeds.2017.03.058

Brief summary: In this cross-sectional study, the growth pattern from birth until diagnosis of premature adrenarche (PA) was assessed in 82 children (16 boys) compared to 63 controls (15 boys). PA children were taller, heavier and had an accelerated linear growth. PA children also showed earlier adiposity rebound (AR) irrespective of whether they had obesity or not. A sex dimorphism was observed. The authors hypothesized that both earlier AR and accelerated linear growth may trigger adrenal androgen production and PA.

Adrenarche remains a poorly understood developmental event. Neither its normal regulation, nor its disease-related relevance when occurring too early, are understood. Several studies (including this one) describe significant differences in clinical and biochemical characteristics of PA compared to normal-timed adrenarche. As several studies also find a correlation of PA with later adverse outcome concerning metabolic and reproductive disorders, it is important to put all possible effort in research to understand the regulation of normal adrenarche and the pathogenesis underlying PA. We need to understand in order to be able to treat and prevent as necessary.

This descriptive study clearly shows that PA is not (only) the result of early childhood obesity. It also shows that the growth pattern of PA is different long before the diagnosis is made; it is in fact already visible by an earlier AR. Whether this is an early sign of the underlying "disease" or in fact the trigger of PA remains an open question.

Ze'ev Hochberg was very much interested in the infancy-childhood-adolescence transition events and underlying mechanisms (1-3). He suggested that the AR may be the first sign of juvenility or the signal that turns on the transition from childhood to juvenility.

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14.8. Child health, developmental plasticity, and epigenetic programming

Hochberg Z, Feil R, Constancia M, Fraga M, Junien C, Carel JC, Boileau P, Le Bouc Y, Deal CL, Lillycrop K, Scharfmann R, Sheppard A, Skinner M, Szyf M, Waterland RA, Waxman DJ, Whitelaw E, Ong K, Albertsson-Wikland K

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doi: https://academic.oup.com/edrv/article/32/2/159/2354728

Endocr Rev 2011;32:159–224.

In Brief: This manuscript was prepared from presentations given at the ESPE New Inroads for Child Health (NICHe) conference held in May 2009 in Marstrand, Sweden. It reviewed the concept of plasticity in developmental programming and evidence for the role of epigenetic mechanisms. It became widely accepted as a leading reference on this topic with currently >750 citations in Google Scholar.

Comment: Ze'ev Hochberg had a brilliant, creative mind. He was also enormously ambitious – not for himself, but for the wide dissemination and successful implementation of his projects that aimed to bring about improvements in education and specialist training, and to foster deeper interactions between leading researchers. As well as the Yearbook (and PETCA and many other highly valued ESPE activities), this review paper represents another of Ze'ev's creations.

New Inroads for Child Health (NICHe) was a series of ESPE mini-conferences, which brought together researchers at the forefront of their fields (many of whom came from fields beyond paediatric endocrinology or even clinical science) in order to generate new research directions to address key issues in child health and development. The first NICHe conference had been held in Stockholm and Ze'ev's arrangement to include the involvement of the Nobel Committee for Physiology or Medicine no doubt helped in persuading many leading international researchers to attend! Another major 'draw' for attendees was the idyllic setting of this conference on developmental plasticity and epigenetic programming on Marstrand, a historical fortress island on the West coast of Sweden, which was facilitated by Kirsten Albertsson-Wikland and generously supported by the Vaxthuset Foundation for Children.

The sections in this review bring together key observations in paediatric endocrinology (plasticity in human growth, postnatal development, intrauterine programming, impact of assisted reproduction) with a deep understanding of epigenetics and molecular regulation – it remains essential reading for those embarking on research in this field.

14.9. Juvenility in the context of life history theory

Hochberg Z
Arch Dis Child. 2008 Jun;93(6):534–9.
doi: 10.1136/adc.2008.137570.
Epub 2008 Mar 12.
https://pubmed.ncbi.nlm.nih.gov/18337281/

In this review, Prof Zeev Hochberg presents the characteristics and the function of the juvenile stage of life of human beings from a broad evolutionary perspective.

Homo sapiens is unique in having four postnatal pre-adult life stages: infancy, childhood, juvenility and adolescence. Unlike humans, all other mammals (including the great apes) transit directly from infancy to juvenility and then to adulthood, without the childhood and adolescence stages. This review highlights the importance of these intermediary growth stages in human evolution with a specific emphasis on juvenility.

The onset of juvenility coincides with the eruption of permanent molars around 6 years, at the end of brain growth, and is highly associated with brain weight. Unlike the secular trend, the age of transition to juvenility has not changed throughout the 200 000 years of modern humans.

The juvenile stage of life is presented by three distinct endocrine and body composition changes: adrenarche (the onset of adrenal androgen generation), growth pattern (decelerating from a linear childhood growth velocity) and adiposity rebound (acceleration of body mass index).

Serum DHEA and DHEAS rise progressively throughout juvenility, is required to prepare the central nervous system for adolescence, both in its psychosocial sense and in setting the scene for pubertal maturation of the hypothalamic–pituitary–gonadal axis. Increased adrenal androgens are also related with enhanced GH-IGF-1 axis activity, increased muscle mass and bone mineral; and increased leptin and body fat.

Adiposity rebound may be the first clinical sign of juvenility, which corresponds with the second rise in the age-related BMI curve that occurs between ages 4 and 6. Increases in DHEAS levels correlate positively and coincide with increases in BMI. The aim of adiposity rebound in the transition to juvenility is linked to energy supply. The energy that was allocated to brain growth in childhood is stored as abdominal fat in juvenility, in order to support the energetically costly accelerated growth during the subsequent adolescence.

The growth declines in juvenility to the slowest rate after a period of constant growth rate in childhood. This accompanies a clear acceleration in lower limb growth. This pattern of growth is required for living within the social hierarchy as longer lower limbs relative to body mass reduce the energetic cost of human walking as the juvenile joined the hunter-gatherer adult society without posing the physical threat of a large body.

Finally, the timing of transition from childhood to juvenility has significant impact on body composition and metabolic adaptation. Given as an example is that an early adiposity rebound is observed in overweight children and is associated with an increased risk of being overweight. Another example is early juvenility and premature adrenarche seen in babies born small for gestational age. This juvenile adaptive response generates a thrifty phenotype characterized by ovarian hyperandrogenism, hyperinsulinemia, and dyslipidemia in the female later in life to enhance self-sufficient energy supplies, survival schedules and behavioral strategies that yield the highest fitness against environmental constraints.

Ze'ev introduced this new concept of juvenility to try to explain and understand the enigma of adrenarche. He described juvenility as part of a strategy in the transition from a period of total dependence on the family for provision and security to self-supply; it is assigned with a predictive adaptive response of body composition and energy metabolism. The juvenile stage offers opportunities to prepare for the physical and social complexity of adolescence as well as adulthood.

14.10. The importance of adrenocortical glucocorticoids for adrenomedullary and physiological response to stress: A study in isolated glucocorticoid deficiency

Nehama Zuckerman-Levin, Dov Tiosano, Graeme Eisenhofer, Stefan Bornstein, Ze'ev Hochberg
J Clin Endocrinol Metab. 2001; 86(12): 5920–4.
PMID: 11739465
https://pubmed.ncbi.nlm.nih.gov/11739465/

The adrenal cortex and medulla are intimately linked both anatomically and functionally in the adrenal gland (**1, 2**). Glucocorticoids are essential for the survival and maintenance of chromaffin cells, as well as the production of epinephrine (**3, 4**). At the transcriptional level, the expression of phenylethanolamine N-methyltransferase (PNMT), the enzyme that converts norepinephrine to epinephrine, depends on glucocorticoids (**5**). The effect of impaired adrenal function on sympatho-adrenomedullary function has been studied previously in ACTH deficiency (**6**) and in mice (**7**) or humans (**8**) with 21-hydroxylase deficiency. Severe impairment of adrenomedullary function was characterized by alterations in chromaffin cell migration, development, structure, and catecholamine synthesis.

The aim of the present study was to examine the hypothesis that in patients with isolated glucocorticoid deficiency, epinephrine would be suppressed despite replacement therapy, that norepinephrine might be compensatory, and that the physiological response would reflect these changes.

To this end, patients with isolated glucocorticoid deficiency were subjected to three levels of acute stress: assumption of upright posture, cold pressor and exercise. Their catecholamine and physiological response were monitored. Patients with isolated glucocorticoid deficiency had severe adrenomedullary dysfunction, characterized by a minimal resting production of epinephrine (6 ± 2 pg/mL compared with 64 ± 22 pg/mL of the controls) and a minimal response to stress. A slight compensatory increase of norepinephrine was noted in response to cold pressor test (754 ± 200 pg/mL compared with 431 ± 73 pg/mL of the control). The physiological response is characterized by low systolic blood pressure and high pulse rate in rest and mild stress and in a pressor response to exercise (diastolic 87 ± 5 mmHg, compared with 73 ± 2 mmHg of the control).

These findings suggest that intra-adrenal glucocorticoids are essential for epinephrine secretion, that norepinephrine may be compensatory, and that these result in a distinct physiological response. The implications of the pressor response to exercise, the declining pulse pressure, and the increased pulse response indicate a lower physical fitness in patients with adrenal insufficiency.

This group of patients with ACTH unresponsiveness has been reported previously to have early and extreme postnatal clinical hypoglycemia. Although isolated glucocorticoid deficiency might contribute to neonatal hypoglycemia (**9**), the medullary underdevelopment and epinephrine deficiency might also contribute to its severity. Furthermore, adrenocortical insufficiency is often characterized by changes in cardiac function and blood pressure, which can be partly reestablished by glucocorticoid replacement therapy (**10**). This study showed for the first time that the adrenal medulla is an essential player in the fine-tuning of the cardiovascular response to mild stress.

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14.11. Effect of thyroid hormone and growth hormone on recovery from hypothyroidism of epiphyseal growth plate cartilage and its adjacent bone

Lewinson D, Harel Z, Shenzer P, Silbermann M, Hochberg Z

Endocrinology. 1989 Feb;124(2):937–45.

doi: 10.1210/endo-124-2-937.

PMID: 2912707

Ze'ev Hochberg was fascinated by growth, a dominant topic during his long-standing career. He published 153 articles on growth according to PubMed, the first 1980 [1] and the last in April this year [2]. Synergy between thyroid hormones and growth hormone was recognized decades ago [3] but Ze'ev Hochberg added an important piece of knowledge on the impact of hypothyroidism on the growth plate as well as the effects of thyroid hormones, growth hormone and the combination of both on recovery of the growth plate chondrocytes. He has shown that thyroid hormones were central for the recovery of the epiphyseal growth plate cartilage of hypothyroid rats, while growth hormone alone had no effect. Growth hormone together with thyroid hormones were synergistic. The main effects of thyroid hormones were 1) increased proliferation in the growth plate cartilage, and 2) hypertrophy of mature chondrocytes, both crucial for normalization of growth plate width, morphology, and function.

With Ze'ev Hochberg we have not only lost a clinician with enormous experience and expertise, but a highly innovative teacher and researcher in pediatric endocrinology and dedicated member of our Society, the Yearbook being the ultimate proof of all his dedication for our field.

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14.12. The effect of single-dose radiation on cell survival and growth hormone secretion by rat anterior pituitary cells

Hochberg Z, Kuten A, Hertz P, Tatcher M, Kedar A, Benderly A
Radiat Res. 1983 Jun;94(3):508–12.

Brief summary: this early experimental study analyzed the effects of radiation on growth hormone secretion and short-term cell survival in a cell culture model of dispersed rat anterior pituitary cells.

At the time of this study, growth retardation had already been recognized as a significant adverse effect of brain irradiation in humans and experimental animals. Such growth retardation had been associated with impaired secretion of growth hormone in children, but a comparable correlation had not been confirmed in rats.

After a single dose of radiation, survival curves of different rat anterior pituitary cells (classified according to hematoxylin-eosin immunostaining into acidophil, basophil, and chromophobe pituitary cells) were analyzed and compared with control cell cultures unexposed to radiation.

Acidophilic pituicytes were the cells most damaged by irradiation and were sensitive to a single radiation dose higher than 300 rad. On the contrary, basophilic pituicytes showed remarkable resistance to doses as high as 1000 rad. Acidophil cells are characterized by the biosynthesis and secretion of growth hormone and prolactin. Basophil cells synthetize and secrete TSH, ACTH, FSH and LH. Chromophobe cells are resting cells without any hormonal immunoreactivity.

This seminal study showed for the first time that irradiation can selectively damage different pituitary cells depending on the dose used, and that growth hormone-secreting cells show the greatest sensitivity to irradiation-induced damage. The results are consistent with current clinical evidence that lower doses of radiation therapy (18–50 Gy, mainly used in cranial and total body irradiation for leukaemias) produce selective hypothalamic–pituitary dysfunction, which usually manifests as isolated growth hormone deficiency. In contrast, higher doses, such as those used in the treatment of brain tumors, can produce direct damage to the anterior pituitary gland leading to multiple pituitary hormone deficiencies. Growth hormone deficiency represents the earliest and most common endocrine defect induced by brain irradiation, even after relatively low doses. In contrast, central adrenal insufficiency (ACTH deficiency), central hypothyroidism (TSH deficiency), and hypogonadotropic hypogonadism (LH/FSH deficiency) are less common, appear later, and are usually observed only after high doses of cranial irradiation.

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