Editor's Choice

Jean-Claude Carel and Ze'ev Hochberg

New mechanism: understanding declining juveniles

DHEA suppresses longitudinal bone growth by acting directly at the growth plate through estrogen receptors

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Background: Dehydroepiandrosterone (DHEA) is produced by the adrenal cortex and is the most abundant steroid in humans. Although in some physiological and pathological conditions the increased secretion of DHEA and its sulfated form is associated with accelerated growth rate and skeletal maturation, it is unclear whether DHEA can affect longitudinal bone growth and skeletal maturation by acting directly at the growth plate.

Results: In this study, DHEA suppressed metatarsal growth, growth plate chondrocyte proliferation, and hypertrophy/differentiation. In addition, DHEA increased the number of apoptotic chondrocytes in the growth plate. In cultured chondrocytes, DHEA reduced chondrocyte proliferation and induced apoptosis. The DHEA-induced inhibition of metatarsal growth and growth plate chondrocyte proliferation and hypertrophy/differentiation was nullified by culturing metatarsals with DHEA in the presence of ICI 182,780, an inhibitor of estrogen receptor, but not in the presence of Casodex, an inhibitor of androgen receptor. Lastly, nuclear factor- κ B DNA-binding activity was inhibited by the addition of DHEA in the medium of cultured chondrocyte.

Conclusions: DHEA suppressed bone growth by acting directly at the growth plate through estrogen receptor. Such growth inhibition is mediated by decreased chondrocyte proliferation and hypertrophy/differentiation and by increased chondrocyte apoptosis.

Androgens in general enhance growth, as is well known from the adolescent growth spurt. Many of us were puzzled for years why is it that following the transition from childhood to juvenility through adrenarche, children decelerate their growth. This study provides evidence for a direct inhibitory effect of adrenal DHEA on the growth plate enchondral ossification process. To do so, DHEA is aromatized to estrone, which utilizes estrogen receptors and nuclear factor- κ B DNA-binding activity to slow growth.

The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know

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This article summarizes the new 2011 report on dietary requirements for calcium and vitamin D from the Institute of Medicine (IOM). An IOM Committee charged with determining the population needs for these nutrients in North America conducted a comprehensive review of the evidence for both skeletal and extraskeletal outcomes. The Committee concluded that available scientific evidence supports a key role of calcium and vitamin D in skeletal health, consistent with a cause-and-effect relationship and providing a sound basis for determination of intake requirements. For extraskeletal outcomes, including cancer, cardiovascular disease, diabetes, and autoimmune disorders, the evidence was inconsistent, inconclusive as to causality, and insufficient to inform nutritional requirements. Randomized clinical trial evidence for extraskeletal outcomes was limited and generally uninformative. Based on bone health, recommended dietary allowances (RDAs; covering requirements of $\geq 97.5\%$ of the population) for calcium range from 700 to 1,300 mg/day for life-stage groups at least 1 year of age. For vitamin D, RDAs of 600 IU/day for ages 1–70 years and 800 IU/day for ages 71 years and older, corresponding to a serum 25-hydroxyvitamin D level of at least 20 ng/ml (50 nmol/l), meet the requirements of at least 97.5% of the population. RDAs for vitamin D were derived based on conditions of minimal sun exposure due to wide variability in vitamin D synthesis from ultraviolet light and the risks of skin cancer. Higher values were not consistently associated with greater benefit, and for some outcomes U-shaped associations were observed, with risks at both low and high levels. The Committee concluded that the prevalence of vitamin D inadequacy in North America has been overestimated. Urgent research and clinical priorities were identified, including reassessment of laboratory ranges for 25-hydroxyvitamin D, to avoid problems of both under- and overtreatment.

This report by the by the US-based Institute of Medicine (IOM; has anyone else missed the existence of this institute?) drew much interest and fire. It comes on the background of the past 2 years, when vitamin D supplement sales to consumers have increased markedly. The IOM, who reported to the media before they did so to an audience of scientists, recommends that vitamin D decisions are based on serum 25(OH)D levels needed to ensure skeletal health only (20 ng/ml), as in their judgment there was insufficient evidence to make any recommendations with respect to nonskeletal benefits. In a comment (that we second) that they called 'Why the IOM recommendations for vitamin D are deficient' [*J Bone Miner Res* 2011;26:455–457], Heaney and Holick draw attention to a problem in logics: 'Since the panel did not know whether there might be nonskeletal benefits and at what blood levels, it is patently incorrect to say that they know that people are getting enough.' To contest that vitamin D requirements are those they claim goes well beyond what the panel admits it knows. Current studies suggest quite convincingly that instead of 20 ng/ml, a serum level of 30 ng/ml is closer to the bottom end of the acceptable range for skeletal health. In a large randomized study, raising serum 25(OH)D level from 21 to 29 ng/ml produced a 33% reduction in all major osteoporotic fractures [*BMJ* 2003;326:469–474]. A meta-analysis showed that reduction with vitamin D does not occur reproducibly below serum 25(OH)D levels of 30 ng/ml and for some fractures even 40 ng/ml. Finally, they argue with the IOM recommended intake (600 IU/day for all individuals up through to age 70): 600 IU/day, if the body's sole input of vitamin D, would not be enough to produce a value of even 10 ng/ml, let alone 20 ng/ml or above; each additional 100 IU of vitamin D per day raises the serum 25(OH) D concentration by ~ 1 ng/ml.

Neural sirtuin 6 (Sirt6) ablation attenuates somatic growth and causes obesity

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Proc Natl Acad Sci U S A 2010;107:21790.

Background: In yeast, Sir2 family proteins (sirtuins) regulate gene silencing, recombination, DNA repair, and aging via histone deacetylation. Most of the seven mammalian sirtuins (Sirt1–Sirt7) have been implicated as NAD⁺-dependent protein deacetylases with targets ranging from transcriptional regulators to metabolic enzymes.

Results: This paper reports that neural-specific deletion of sirtuin 6 (Sirt6) in mice leads to postnatal growth retardation due to somatotrophic attenuation through low growth hormone (GH) and insulin-like growth factor 1 (IGF1) levels. However, unlike Sirt6 null mice, neural Sirt6-deleted mice do not die from hypoglycemia. Instead, over time, neural Sirt6-deleted mice reach normal size and ultimately become obese. Molecularly, Sirt6 deletion results in striking hyperacetylation of histone H3 lysine 9 (H3K9) and lysine 56 (H3K56), two chromatin marks implicated in the regulation of gene activity and chromatin structure, in various brain regions including those involved in neuroendocrine regulation.

Conclusions: On the basis of these findings, the authors propose that Sirt6 functions as a central regulator of somatic growth and plays an important role in preventing obesity by modulating neural chromatin structure and gene activity.

In yeast, SIR2 is the mechanism for lifespan extension caused by caloric restriction. Longevity has been previously discussed as a feature of GH deficiency in experimental animals and humans. We are now told that mice that specifically lack Sirt6 in the brain (BS6ko) display postnatal growth retardation catch-up growth and obesity, which may be related to the latter. Pituitaries from BS6ko mice showed GH deficiency despite normal levels of GHRH and somatostatin. Sirt6 is known to deacetylate the two chromatin marks H3K9 and H3K56. Human SIRT6 associates specifically with telomeres, and SIRT6 depletion led to telomere dysfunction with end-to-end chromosomal fusions and premature cellular senescence. Moreover, SIRT6-depleted cells exhibited abnormal telomere structures that resemble defects observed in Werner syndrome, a premature aging disorder. This study suggests a role for Sirt6 in somatic growth and adult-onset obesity in mouse.

Food for thought: growth in age

Effects of a growth hormone-releasing hormone antagonist on telomerase activity, oxidative stress, longevity, and aging in mice

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Proc Natl Acad Sci U S A 2010;107:22272–7.

Background: Both deficiency and excess of growth hormone (GH) are associated with increased mortality and morbidity. GH replacement in otherwise healthy subjects leads to complications, whereas individuals with isolated GH deficiency such as Laron dwarfs show increased lifespan.

Methods: The study determined the effects of treatment with the GH-releasing hormone (GHRH) receptor antagonist MZ-5-156 on aging in SAMP8 mice, a strain that develops with aging-cognitive deficits

and has a shortened life expectancy. Starting at age 10 months, mice received daily s.c. injections of 10 µg/mouse of MZ-5-156.

Results: Mice treated for 4 months with MZ-5-156 showed increased telomerase activity, improvement in some measures of oxidative stress in brain, and improved pole balance, but no change in muscle strength. MZ-5-156 improved cognition after 2 and 4 months, but not after 7 months of treatment (ages 12, 14, and 17 months, respectively). Mean life expectancy increased by 8 weeks with no increase in maximal lifespan, and tumor incidence decreased from 10 to 1.7%.

Conclusions: These results show that treatment with a GHRH antagonist has positive effects on some aspects of aging, including an increase in telomerase activity.

Among hormones that are implicated in aging is GH, whose levels decrease dramatically with aging, and some of the findings in aging are the opposite of those found in acromegaly. This has led to the proposal that replacement of GH will reverse or slow the aging process. However, several clinical trials showed that replacement of GH is associated with increased mortality, as in acromegaly. Transgenic mice overexpressing GH have a decreased lifespan and evidence of accelerated aging in several organs and tissues, including the brain. This study tested an antagonist of GHRH for effects on lifespan, telomerase activity, tumor incidence, muscle strength, oxidative stress in brain, body weights, serum glucose, serum triglycerides, and cognition in aged SAMP8 mice. The SAMP8 mouse strain has a natural mutation that results in increases in amyloid precursor protein (APP) and amyloid beta peptide (AβP) with aging, and it develops age-related cognitive impairments, which are severe by age 12 months, increased oxidative stress levels in brain, and decreased brain acetylcholine levels. Most indices were affected, including some measures of oxidative stress, telomerase activity, median life expectancy, tumor incidence, and learning and memory. These results not only indicate that GHRH antagonists can have positive effects on the aging process, but also reveal some of the mechanisms involved through antioxidant effects, decreasing tumor incidence, and increasing telomerase activity.

New hormones: same same but different

Tissue distribution and cardiac metabolism of 3-iodothyronamine

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Endocrinology 2010;151:5063–73.

Background: 3-Iodothyronamine (T1AM) is a novel relative of thyroid hormone, able to interact with specific G-protein-coupled receptors, known as trace amine-associated receptors. Significant functional effects are produced by exogenous T1AM, including a negative inotropic and chronotropic effect in cardiac preparations.

Methods: This work was aimed at estimating endogenous T1AM concentration in different tissues and determining its cardiac metabolism. A novel HPLC tandem mass spectrometry assay was developed, allowing detection of T1AM, thyronamine, 3-iodothyroacetic acid, and thyroacetic acid.

Results: T1AM was detected in rat serum, at the concentration of 0.3 ± 0.03 pmol/ml, and in all tested organs (heart, liver, kidney, skeletal muscle, stomach, lung, and brain), at concentrations significantly higher than the serum concentration, ranging from 5.6 ± 1.5 pmol/g in lung to 92.9 ± 28.5 pmol/g in liver. T1AM was also identified for the first time in human blood. In H9c2 cardiomyocytes and isolated perfused rat hearts, significant Na⁺-dependent uptake of exogenous T1AM was observed, and at the steady-state total cellular or tissue T1AM concentration exceeded extracellular concentration by more than 20-fold. In both preparations, T1AM underwent oxidative deamination to 3-iodothyroacetic acid. T1AM deamination was inhibited by iproniazid but not pargyline or semicarbazide, suggesting the involvement of both monoamine oxidase and semicarbazide-sensitive amine oxidase. Thyronamine and thyroacetic acid were not detected in heart. Finally, evidence of T1AM production was observed in cardiomyocytes exposed to exogenous thyroid hormone, although the activity of this pathway was very low.

It isn't new – thyronamines (TAM), a class of endogenous signaling compounds exhibiting structural similarity to the thyroid hormone L-thyroxine, were recognized in the 1950s, and have now made a comeback. Interacting with specific G-protein-coupled receptors, they show a variety of pharmacological effects including reduction of fat mass, hyperglycemia, and reduction of the respiratory quotient. For those of us who have not followed the field, this review article summarizes the current knowledge on TAM.

New paradigms: fathers matter

Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals

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Cell 2010;143:1084–96.

Background: Epigenetic information can be inherited through the mammalian germline and represents a plausible transgenerational carrier of environmental information.

Methods: To test whether transgenerational inheritance of environmental information occurs in mammals, this study carried out an expression-profiling screen for genes in mice that responded to paternal diet.

Results: Offspring of males fed a low-protein diet exhibited elevated hepatic expression of many genes involved in lipid and cholesterol biosynthesis and decreased levels of cholesterol esters, relative to the offspring of males fed a control diet. Epigenomic profiling of offspring livers revealed numerous modest (!20%) changes in cytosine methylation depending on paternal diet, including reproducible changes in methylation over a likely enhancer for the key lipid regulator Ppara.

Conclusions: These results, in conjunction with recent human epidemiological data, indicate that parental diet can affect cholesterol and lipid metabolism in offspring and define a model system to study environmental reprogramming of the heritable epigenome.

Chronic high-fat diet in fathers programs β -cell dysfunction in female rat offspring

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Nature 2010;467:963–6.

Background: The global prevalence of obesity is increasing across most ages in both sexes. This is contributing to the early emergence of type 2 diabetes and its related epidemic. Having either parent obese is an independent risk factor for childhood obesity. Although the detrimental impacts of diet-induced maternal obesity on adiposity and metabolism in offspring are well established, the extent of any contribution of obese fathers is unclear, particularly the role of non-genetic factors in the causal pathway.

Results: Here they show that paternal high-fat-diet (HFD) exposure programs β -cell 'dysfunction' in rat F1 female offspring. Chronic HFD consumption in Sprague-Dawley fathers induced increased body weight, adiposity, impaired glucose tolerance and insulin sensitivity. Relative to controls, their female offspring had an early onset of impaired insulin secretion and glucose tolerance that worsened with time, and normal adiposity. Paternal HFD altered the expression of 642 pancreatic islet genes in adult female offspring ($p < 0.01$); genes belonged to 13 functional clusters, including cation and ATP binding, cytoskeleton and intracellular transport. Broader pathway analysis of 2,492 genes differentially expressed ($p < 0.05$) demonstrated involvement of calcium-, MAPK- and Wnt-signaling pathways, apoptosis and the cell cycle. Hypomethylation of the IL-13ra2 (IL-13R) gene, which showed the highest fold difference in expression (1.76-fold increase), was demonstrated.

Conclusions: This is the first report in mammals of non-genetic, intergenerational transmission of metabolic sequelae of a HFD from father to offspring.

Quite surprisingly, a father's diet can affect his daughters' health. When male rats were fed a high-fat diet, their body weight and body fat expectedly increased, and they exhibited glucose intolerance and insulin resistance to the hormone insulin. Unexpectedly, their daughters developed in adulthood but not earlier impaired glucose tolerance and insulin secretion. Moreover, the gene-expression profile of the daughters' pancreatic islet cells was abnormal. Thus, the fathers' high-fat diets must have transmitted this (probably epigenetic) pattern through their sperm, which then promoted an adult-onset disease in the daughters. While GWAS have explained only a small fraction of the weight variation, trans-generational epigenetic effects of diets may play a role in the tendency of obesity to cluster in families.

A smile lights on first-borns

First-borns carry a higher metabolic risk in early adulthood: evidence from a prospective cohort study

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Background: Birth order has been associated with early growth variability and subsequent increased adiposity, but the consequent effects of increased fat mass on metabolic risk during adulthood have not been assessed. We aimed to quantify the metabolic risk in young adulthood of being first-born relative to those born second or subsequently.

Method and Results: Body composition and metabolic risk were assessed in 2,249 men, aged 17–19 years, from a birth cohort in southern Brazil. Metabolic risk was assessed using a composite Z score integrating standardized measurements of blood pressure, total cholesterol, high-density lipoprotein, triglycerides and fat mass. First-borns had lower birth weight Z score ($\Delta = -0.25$, 95% CI $-0.35, -0.15$, $p < 0.001$) but showed greater weight gain during infancy (change in weight Z score from birth to 20 months: $\Delta = 0.39$, 95% CI $0.28-0.50$, $p < 0.0001$) and had greater mean height ($\Delta = 1.2$ cm, 95% CI $0.7-1.6$, $p < 0.0001$) and weight ($\Delta = 0.34$ kg, 95% CI $0.13-0.55$, $p < 0.002$) at 43 months. This greater weight and height tracked into early adulthood, with first-borns being significantly taller, heavier and with significantly higher fat mass than later-borns. The metabolic risk Z score was significantly higher in first-borns.

Conclusions: First-born status is associated with significantly elevated adiposity and metabolic risk in young adult men in Brazil. The results, linking cardiovascular risk with life history variables, suggest that metabolic risk may be associated with the worldwide trend to smaller family size and it may interact with changes in behavioral or environmental risk factors.

First-borns are exciting, but seem to need special attention – they tend to be heavier and develop the metabolic syndrome. In the context of childhood obesity, it is valuable to investigate life history traits that have been known to be associated with the metabolic syndrome. These include age at maturation and adult size. Studies have focused on maternal and infant pregnancy weight gain, intrauterine growth retardation, maternal smoking or preterm. Work by Ong and Dunger suggested that first-born infants grow faster than later-born infants and that the in-utero growth of first-born babies may be restrained as they have lower birth weight and accelerated post-natal catch-up growth [Pediatr Res 2002;52:863–867], both of which are risk factors for obesity, cardiovascular and metabolic diseases in adult life. The new study shows that birth order is associated with increased body mass, adiposity and metabolic risk, according to conventional physiological and biochemical markers and after adjustment for multiple confounding variables associated with maternal and offspring socio-demographic status and health. The first-born effect was however tested in a cohort of young

men and therefore further studies are required to establish with greater confidence the magnitude of the effect in other populations, and the potential variability in populations living in industrialized and non-industrialized settings which may be exposed to different dietary and lifestyle factors. Globally, there is a trend towards lower fertility rate, such that an increasing proportion of individuals will be first-borns, which influence the obesity statistics.

Concepts revised: conflicting interests

High-resolution analysis of parent-of-origin allelic expression in the mouse brain

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Science 2010;329:643–8.

Background: Genomic imprinting results in preferential expression of the paternal or maternal allele of certain genes.

Methods: This study performed a genome-wide characterization of imprinting in the mouse embryonic and adult brain.

Results: This approach uncovered parent-of-origin allelic effects of more than 1,300 loci. They identified parental bias in the expression of individual genes and of specific transcript isoforms, with differences between brain regions. Many imprinted genes are expressed in neural systems associated with feeding and motivated behaviors, and parental biases preferentially target genetic pathways governing metabolism and cell adhesion. They observed a preferential maternal contribution to gene expression in the developing brain and a major paternal contribution in the adult brain.

Conclusion: Parental expression bias emerges as a major mode of epigenetic regulation in the brain.

Genomic imprinting appears to occur only in mammals among vertebrates. Several research groups identified about 100 imprinted genetic loci in mammals. This paper now expanded this list to over 1,300 loci. As shown in the case of pseudohypoparathyroidism and PWS, imprinted gene renders the organism functionally haploid at that locus, and permits the expression of phenotypes from mutations that would normally be recessive. Such imprinting precludes the protection of a back-up copy afforded by a diploid genome. It has been postulated that imprinting is a consequence of parental conflict over resource allocation to the: it is in the father's 'interest' to maximize maternal resources devoted to his progeny, whereas the mother's 'interest' would be to allocate resources more fairly to current and future offspring, who might conceivably result from mating with other males. For interesting reading on this concept, the reader is referred to article by David Haig [*Proc Natl Acad Sci USA* 2010;107(suppl 1):1731–1735]. The newly described imprinted loci are scattered across all autosomes, and two-thirds of these exist as clusters containing two or more loci, a feature also observed with previously described imprinted genes. Most imprinted genes in the brain (60%) are maternally expressed, whereas there is a distinct paternal expression bias (70% of genes) in the preoptic area and medial prefrontal cortex. X-linked imprinting was much sought in the case of Turner syndrome, where some lose their maternal and other their paternal X chromosome. Analysis of all SNP reads from the two X chromosomes demonstrates a bias for preferential transcription from the maternal chromosome. Normalizing the SNP reads for this maternal bias still yields 11 new candidate imprinted loci (with either a maternal or a paternal bias in allele expression). It seems reasonable to assume that similar analysis in other brain regions and perhaps elsewhere in the body will reveal many additional genes that are also imprinted. In other words, the selective pressure to imprint genes appears to be operating on a scale not previously appreciated.

Psychosocial risk and correlates of early menarche in Mexican-American girls

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Am J Epidemiol 2011;173:1203–10.

Background: Mexican-American girls have one of the fastest rates of decline in age at menarche. To date, no study has addressed the role of psychosocial factors on age at menarche in this population.

Method: The authors used data from a longitudinal cohort of Mexican-American girls from the Houston, Texas, metropolitan area recruited in 2005 and investigated associations between family life and socio-economic environment and age at menarche in 523 girls.

Results: After adjusting for maternal age at menarche, daughter's age, and body mass index at baseline, perception of family life environment as conflict-prone was significantly associated with an earlier age at menarche (<11 years). Additionally, there was a 2-fold higher risk (odds ratio = 2.22, 95% CI 1.12, 4.40) of early menarche among daughters of mothers who were single parents compared with those who were not. Girls who matured early had a 2.5-fold increased risk (odds ratio = 2.69, 95% CI 1.04, 6.96) of experimenting with cigarettes compared with those who had an average-to-late age at menarche ≥ 11 years).

Conclusion: Important information is provided regarding the role of family life environment and single parenting on age at menarche in Mexican-Americans. Awareness of the impact of the family life environment and fathers' absence during the early years should be emphasized when addressing early age at menarche across cultures.

The earlier occurrence of puberty in girls is a hot topic nowadays and several studies in all continents have documented this phenomenon. However, several key aspects of this phenomenon are still mysterious, including the mechanism underlying this earlier activation of the gonadotropic axis and the long-term developmental consequences of this earlier start of adolescence. This epidemiological paper provides clues for both aspects of the conundrum. In this cohort study of Mexican-American girls, it was found, as shown before, that earlier age at menarche was associated with an almost 3-fold increase of cigarette smoking, although the cognitive susceptibility was not dependent on age at menarche, suggesting more vulnerability to peer pressure in girls with early menarche. In addition, those educated by a single mother or in an environment perceived as conflicting had an increased risk of early menarche. Altogether, these epidemiological data support the notion that the psychosocial environment, either perinatal or later in life, has a strong impact on the biology of puberty. They converge with observations made in pygmies, suggesting life history trade-off mechanisms triggering early puberty in situations of threatening environment and 'danger signals' [1]. In addition, they confirm earlier findings indicating that household composition alters the age at menarche and that absence of the father in the household is associated with earlier menarche [2]. One further important point illustrated by this study is the need to adjust for socio-demographic factors if one wants to evaluate the long-term psychosocial consequences of early puberty.

Evidence that nasal insulin induces immune tolerance to insulin in adults with autoimmune diabetes

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Diabetes 2011;60:1237–45.

Background: Insulin in pancreatic β cells is a target of autoimmunity in type 1 diabetes. In the NOD mouse model of type 1 diabetes, oral or nasal administration of insulin induces immune tolerance to insulin and protects against autoimmune diabetes. Evidence for tolerance to mucosally administered insulin or other autoantigens is poorly documented in humans. Adults with recent-onset type 1 diabetes in whom the disease process is subacute afford an opportunity to determine whether mucosal insulin induces tolerance to insulin subsequently injected for treatment.

Methods: 52 adults with recent-onset, noninsulin-requiring type 1 diabetes were randomized to nasal insulin or placebo for 12 months. Fasting blood glucose and serum C-peptide, glucagon-stimulated serum C-peptide, and serum antibodies to islet antigens were monitored three times monthly for 24 months. An enhanced ELISpot assay was used to measure the T-cell response to human proinsulin.

Results: β -Cell function declined by 35% overall, and 23 of 52 participants (44%) progressed to insulin treatment. Metabolic parameters remained similar between nasal insulin and placebo groups, but the insulin antibody response to injected insulin was significantly blunted in a sustained manner in those who had received nasal insulin. In a small cohort, the interferon- γ response of blood T cells to proinsulin was suppressed after nasal insulin.

Conclusion: Although nasal insulin did not retard loss of residual β -cell function in adults with established type 1 diabetes, evidence that it induced immune tolerance to insulin provides a rationale for its application to prevent diabetes in at-risk individuals.

This paper could appear as one in a long series of failures or half successes that have paved the way of immune intervention at the early phases of diabetes and the three papers that appeared in July of 2011 illustrate this chronic disappointment, including the failure of GAD vaccination to alter the course of diabetes [3–5]. So what is special with this paper where nasal insulin in adults with recent onset ‘slow type 1 diabetes’ failed (once more) to alter the course of β -cell destruction? Indeed this paper is the first where T-cell response was adequately monitored during the trial and where the authors were able to demonstrate suppression of T-cell response to insulin after nasal administration. Demonstrating an immune effect is essential if we want to progress on the path towards finding an efficient immune intervention in type 1 diabetes. This new paradigm contrasts with previous studies where the only readout of the intervention was β -cell function and its correlates such as HbA1c and insulin dose. It teaches us that future trials should first focus on immune effects before even considering clinically relevant metabolic effects.

Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans

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Sci Transl Med 2011;3:70ra13.

Background: Mutations in growth signaling pathways extend lifespan, as well as protect against age-dependent DNA damage in yeast and decrease insulin resistance and cancer in mice.

Methods: Ecuadorian individuals who carry mutations in the growth hormone receptor (GHR) gene that lead to severe GHR and IGF-1 (insulin-like growth factor-1) deficiencies were monitored for 22 years. The information was combined with surveys to identify the cause and age of death for individuals in this community who died before this period.

Results: The individuals with GHR deficiency exhibited only one nonlethal malignancy and no cases of diabetes, in contrast to a prevalence of 17% for cancer and 5% for diabetes in control subjects. A possible explanation for the very low incidence of cancer was suggested by in vitro studies: serum from subjects with GHR deficiency reduced DNA breaks but increased apoptosis in human mammary epithelial cells treated with hydrogen peroxide. Serum from GHR-deficient subjects also caused reduced expression of RAS, PKA (protein kinase A), and TOR (target of rapamycin) and upregulation of SOD2 (superoxide dismutase 2) in treated cells, changes that promote cellular protection and lifespan extension in model organisms. Reduced insulin concentrations were observed (1.4 vs. 4.4 μ U/ml in unaffected relatives) as well as a very low HOMA-IR (homeostatic model assessment-insulin resistance) index (0.34 vs. 0.96 in unaffected relatives) in individuals with GHR deficiency, indicating higher insulin sensitivity, which could explain the absence of diabetes in these subjects.

Conclusion: These results provide evidence for a role of evolutionarily conserved pathways in the control of aging and disease burden in humans.

Data has accumulated in the animal field, both in rodents and in worms, linking the growth hormone-IGF-I axis to aging and cancer. Data in humans is less clear and associations have been found between IGF-I levels and risks of certain cancers at the population level. There are very few 'clean' models of growth hormone-IGF-I axis defects in humans and most patients with growth hormone deficiency have complex diseases and combined pituitary deficiencies that hamper a clear interpretation of the data. Laron syndrome, due to molecular defects in the growth hormone receptor genes, constitutes an isolated defect of growth hormone signal transduction but is so rare in most parts of the world that it is merely impossible to perform epidemiological studies. This year, two studies from isolates of patients with growth hormone receptor defects have been presented. The study cited here comes from Ecuador where an isolate of individuals arising from a Jewish population that fled Spain in the 15th century has a high incidence of such mutations and allowed the follow-up of 99 mutated individuals. The striking effect was a decreased cancer and diabetes risk, contrasting with increased mortality in childhood. The causes of death contrasted between patients and controls, with an increase in cardiac disease, alcohol-related and convulsive disorders in affected patients, contrasting with an increase in cancer, stroke and diabetes in controls. In vitro studies were consistent with anti-aging and anti-DNA damage mechanisms promoted by reduced growth signaling, as seen in model organisms. Similar data were obtained from an observational multinational study collecting oncologic events from Laron syndrome patients from throughout the world [6]. Altogether, these human observations give credit to those performed in the laboratory, in a much more controlled environment. They do not inform us on the overall health of these individuals who have a decreased quality of life associated with their extreme short stature. The significance of these results should be carefully weighed and not extrapolated to more common variations of growth hormone signaling such as acromegaly, growth hormone deficiency or growth hormone treatment which are transient in the majority of cases.

Fertility and ovarian function in high-dose estrogen-treated tall women

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J Clin Endocrinol Metab 2011;96:1098–105.

Background: High-dose estrogen treatment to reduce final height of tall girls has been shown to interfere with fertility. Ovarian function has not been studied. Fertility and ovarian function were evaluated in tall women who did or did not receive such treatment in adolescence.

Methods: Retrospective cohort study of 413 tall women aged 23–48 years, of whom 239 women had been treated. A separate group of 126 fertile, normoovulatory volunteers aged 22–47 years served as controls.

Results: Fertility was assessed in 285 tall women (157 treated, 128 untreated) who had attempted to conceive. After adjustment for age, treated women were at increased risk of experiencing subfertility (odds ratio (OR) 2.29, 95% CI 1.38–3.81) and receiving infertility treatments (OR 3.44, 95% CI 1.76–6.73). Moreover, fecundity was notably affected because treated women had significantly reduced odds of achieving at least one live birth (OR 0.26, 95% CI 0.13–0.52). Remarkably, duration of treatment was correlated with time to pregnancy ($r = 0.23$, $p = 0.008$). Ovarian function was assessed in 174 tall women (119 treated, 55 untreated). 39 women (23%) exhibited a hypergonadotropic profile. After adjusting for age category, treated women had significantly higher odds of being diagnosed with imminent ovarian failure (OR 2.83, 95% CI 1.04–7.68). Serum FSH levels in these women were significantly increased, whereas antral follicle counts and serum anti-müllerian hormone levels were decreased.

Conclusion: High-dose estrogen-treated tall women are at risk of subfertility in later life. Their fecundity is significantly reduced. Treated women exhibit signs of accelerated ovarian aging with concomitant follicle pool depletion, which may be the basis of the observed subfertility.

High-dose estrogens for tall stature is certainly among the most controversial treatments in pediatric endocrinology. The controversy even increased when an Australian case-control study identified a decrease in fertility in tall women who had received such treatments in childhood [7]. Estrogen treatments for tall stature have been particularly used in the Netherlands, the country where the average height of the population is highest. This case-control study confirmed the previous findings and found a decrease in fecundity which had not been identified before. In addition, ovarian function seemed to be altered in a proportion of women. Similar studies were performed by the same group on a smaller population of tall men treated with high-dose testosterone in childhood and found no effect on fertility and semen quality, although there were some minor variation of testosterone levels [8]. Altogether, there are now two papers consistently indicating an effect of high-dose estrogen on reproductive capacities later in life, with no obvious confounder. In addition to the reluctance of most pediatric endocrinologists to use these treatments, the demand has declined, making life easier for us.

Important clinical observation: cyclophosphamide for acquired hypothalamic dysfunction

Cyclophosphamide for rapid-onset obesity, hypothalamic dysfunction, hypoventilation, and autonomic dysregulation syndrome

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Background: Patients with rapid-onset obesity, hypothalamic dysfunction, hypoventilation, autonomic dysregulation, and neural crest tumor syndrome have poor long-term outcomes.

Results: A patient who was treated successfully with high-dose cyclophosphamide immunoablation is reported.

Conclusion: This experience offers a novel therapeutic approach and an indirect insight into the underlying pathogenesis of this syndrome.

Acquired hypothalamic dysfunction, associated with obesity, hypoventilation and ganglioneuromas, is a severe pediatric endocrine disorder that has been described in the literature under various names and acronyms [9–11]. Although a genetic basis has been sought and not found so far, there are several features that are consistent with an acquired and autoimmune origin. Several features lead the authors to evaluate the effect of immunosuppression in one such patient with severe disease. These features included the lymphocytic infiltrate in the brain of deceased patients, and the analogy with opsoclonus myoclonus ataxia syndrome, an autoimmune paraneoplastic syndrome associated with neuroblastoma. The authors used cyclophosphamide, rituximab, prednisone, cyclosporin A and intravenous immunoglobulins and obtained an effect, in particular with high-dose cyclophosphamide. Although this is a single case report and the effect should be confirmed in prospective studies, this is an important paradigm shift for one of the severe, potentially lethal diseases seen by pediatric endocrinologists.

Nature or nurture: all in the genes

Common genetic determinants of vitamin D insufficiency: a genome-wide association study

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Background: Vitamin D is crucial for maintenance of musculoskeletal health, and might also have a role in extraskeletal tissues. Determinants of circulating 25-hydroxyvitamin D concentrations include sun exposure and diet, but high heritability suggests that genetic factors could also play a part. The authors aimed to identify common genetic variants affecting vitamin D concentrations and risk of insufficiency.

Methods: A genome-wide association study of 25-hydroxyvitamin D concentrations in 33,996 individuals of European descent from 15 cohorts. Five epidemiological cohorts were designated as discovery cohorts ($n = 16,125$), five as in-silico replication cohorts ($n = 9,367$), and five as de-novo replication cohorts ($n = 8,504$). 25-Hydroxyvitamin D concentrations were measured by radioimmunoassay, chemiluminescent assay, ELISA, or mass spectrometry. Vitamin D insufficiency was defined as concentrations <75 or 50 nmol/l. We combined results of genome-wide analyses across cohorts using Z score-weighted meta-analysis. Genotype scores were constructed for confirmed variants.

Results: Variants at three loci reached genome-wide significance in discovery cohorts for association with 25-hydroxyvitamin D concentrations, and were confirmed in replication cohorts: 4p12 (overall $p = 1.9 \times 10^{-109}$; 11q12 ($p = 2.1 \times 10^{-27}$) near DHCR7), and 11p15 ($p = 3.3 \times 10^{-20}$) near CYP2R1). Variants at an additional locus (20q13, CYP24A1) were genome-wide significant in the pooled sample ($p = 6.0 \times 10^{-10}$). Participants with a genotype score (combining the three confirmed variants) in the highest quartile were at increased risk of having 25-hydroxyvitamin D concentrations <75 nmol/l (OR 2.47, 95% CI 2.20–2.78, $p = 2.3 \times 10^{-48}$) or lower than 50 nmol/l (1.92, 1.70–2.16, $p = 1.0 \times 10^{-26}$) compared with those in the lowest quartile.

Conclusion: Variants near genes involved in cholesterol synthesis, hydroxylation, and vitamin D transport affect vitamin D status. Genetic variation at these loci identifies individuals who have a substantially raised risk of vitamin D insufficiency.

Rickets has been considered as an environmental condition since its early description in the 17th century and it has been known that children exposed to fewer sunny days had a higher risk of the disease. However, as for most diseases, all those exposed to the environmental exposure do not get ill, suggesting a genetic basis. To further understand the mechanisms underlying the variation of 25-hydroxyvitamin D, a GWAS approach was undertaken in more than 30,000 individuals and allowed the identification of 3 loci affecting vitamin D levels. It is of interest that a significant proportion of the variance of vitamin D levels was explained by the 3 loci, unlike for some of the traits such as height or age at menarche and there was a 2- to 2.5-fold increase in the risk of vitamin D deficiency between the lower and upper quartile score, based on a combination of alleles. It is also of interest that the loci identified harbor genes of biological plausibility: DHCR7 is the gene involved in Smith-Lemli-Opitz syndrome encodes the enzyme 7-dehydrocholesterol (7-DHC) reductase, which converts 7-DHC to cholesterol and removes the substrate from the biosynthesis pathway of vitamin D₃. CYP2R1 encodes a hepatic microsomal enzyme that underlies the 25-hydroxylation of vitamin D. Last, GC encodes vitamin D binding protein synthesized in the liver that binds and transports vitamin D and its metabolites. Therefore, this GWAS is also quite unique for identifying genes involved in the vitamin D synthesis or transport pathway. From a practical point of view, these findings raise several questions: First, should we start measuring free vitamin D rather than total vitamin D if a fraction of the hormone is bound to the binding protein? Second, should we modulate the dose of vitamin D we give children to their genotype? Giving cod liver oil will never be simple any more...

New concepts: deeper in the metabolome and prediction of metabolic disease

Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans

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Background: Dyslipidemia is an independent risk factor for type 2 diabetes, although exactly which of the many plasma lipids contribute to this remains unclear.

Methods: Liquid chromatography/mass spectrometry-based lipid profiling was performed in 189 individuals who developed type 2 diabetes and 189 matched disease-free individuals, with over 12 years of follow-up in the Framingham Heart Study.

Results: Lipids of lower carbon number and double bond content were associated with an increased risk of diabetes, whereas lipids of higher carbon number and double bond content were associated with decreased risk. This pattern was strongest for triacylglycerols (TAGs) and persisted after multivariable adjustment for age, sex, BMI, fasting glucose, fasting insulin, total triglycerides, and HDL cholesterol. A combination of 2 TAGs further improved diabetes prediction. To explore potential mechanisms that modulate the distribution of plasma lipids, lipid profiling during oral glucose tolerance testing, pharmacologic interventions, and acute exercise testing was performed. Levels of TAGs associated with increased risk for diabetes decreased in response to insulin action and were elevated in the setting of insulin resistance. Conversely, levels of TAGs associated with decreased diabetes risk rose in response to insulin and were poorly correlated with insulin resistance.

Conclusion: A relationship between lipid acyl chain content and diabetes risk is identified, demonstrating how lipid profiling could aid in clinical risk assessment.

Prediction of metabolic syndrome diabetes and cardiovascular diseases is a major challenge with the increased prevalence of obesity and sedentary lifestyles in the developed world and we know that current tools are not operative at the individual level. This paper addressed whether the composition of triglycerides could aid the prediction of diabetes risk in individual who participated in the Framingham Heart Study and had samples from the initiation of the study. They used a case-control approach, selecting individuals who had developed diabetes, and then matching them with controls with a logistic model that included age, BMI, fasting glucose, and hypertension status. Lipid profiling showed strong associations between length and number of double bond content of lipids and future diabetes risk with odd ratios between the lower and upper quartiles of risk of 0.3 and more than 4 for protective and at risk associations. A companion paper, published by the same group in *Nature Medicine*, also reported the association of diabetes risk with amino-acid profiling [12]. Altogether, these will be essential tools in the future to evaluate our patients at metabolic risk to establish risk levels and propose adapted therapies.

Sexism at work

Medically underserved girls receive less evaluation for short stature

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Background: To determine if gender is associated with diagnostic evaluation by primary care pediatricians caring for children with growth-faltering.

Methods: This was a retrospective study of children who were attending four urban pediatric primary care practices affiliated with a tertiary pediatric hospital. Growth-faltering was defined as height at the <5th percentile or a Z score decrease of ≥ 1.5 SDs before 18 months of age or ≥ 1 SD thereafter. For each child, height Z score, age, gender, race, insurance, diagnostic tests, and subspecialist appointments were examined.

Results: Of 33,476 children, 3,007 had growth-faltering (mean height: -1.5 ± 1.0 vs. 0.3 ± 0.9 SDs in those without growth-faltering). Boys comprised 53% of the growth-faltering group (vs. 51% of the non-faltering group; $p < 0.01$). Among children with growth-faltering, 2.8% had endocrinology appointments (vs. 0.8% of others; $p < 0.0001$) and 6% had gastroenterology appointments (vs. 1.5% of others; $p < 0.0001$). Subspecialty care was not associated with gender. Pediatricians ordered diagnostic tests for a significantly greater proportion of children with growth-faltering than others. In a multivariate analysis of height Z score among children with growth-faltering, tests for chromosomes (1.4% of short girls vs. 0.4% of short boys; $p < 0.005$) and growth hormone/insulin-like growth factor axis (0.9% of short girls vs. 1.8% of short boys; $p < 0.05$) were associated with gender. 35% of the girls for whom chromosome testing was performed were 12 years old or older.

Conclusions: Patterns in diagnostic testing of children with growth-faltering by their pediatricians may lead to underdiagnosis of Turner syndrome and growth hormone deficiency among girls.

Referral patterns in pediatric endocrinology are widely variable around the world and very little work has been done to identify the factors involved. Last year we saw in the *Yearbook* that factors associated with the case, but also with the family and the physician, modulated the referral pattern of primary care pediatricians [13]. In this paper, the factors associated with referral and testing of children seen in primary care in Philadelphia County were evaluated. One of the important findings not cited in the abstract of the paper is that of those with growth-faltering, a small proportion (3%) was seen by a pediatric endocrinologist or gastroenterologist (6%). Gender was not associated with referral pattern, whereas those with more severe short stature or older were more prone to be referred. Among those referred, girls had more karyotypes performed but less growth hormone-IGF-I testing done. Altogether, this and other studies show how loosely defined our referral and evaluation patterns are. One of the main challenges in the next decade will be to establish evidence-based criteria for referral and evaluation of possible endocrine conditions in children seen in primary care.

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