A new marker of testicular function

Insulin-like peptide 3 (INSL3) in men with congenital hypogonadotropic hypogonadism/Kallmann syndrome and effects of different modalities of hormonal treatment: a single-center study of 281 patients

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J Clin Endocrinol Metab 2014;99:E268–E275

Context: Insulin-like factor 3 (INSL3) is a testicular hormone secreted during fetal life, the neonatal period, and after puberty.

Objective: To measure INSL3 levels in a large series of men with congenital hypogonadotropic hypogonadism (CHH)/Kallmann syndrome (KS), in order to assess its diagnostic value and to investigate its regulation.

Patients and Methods: The authors studied 281 CHH/KS patients (91 untreated, 96 receiving T, and 94 receiving combined gonadotropin therapy (human chorionic gonadotropin, hCG, and FSH)) and 72 age-matched healthy men. Serum INSL3 was immunoassayed with a validated RIA.

Results: Mean (±SD) INSL3 levels (pg/ml) were 659 ± 279 in controls and lower (60 ± 43; p < 0.001) in untreated CHH/KS patients, with no overlap between the two groups, when the threshold of 250 pg/ml was used. Basal INSL3 levels were lower in both untreated CHH/KS men with cryptorchidism than in those with intra-scrotal testes and in patients with testicular volumes <4 ml. Significant positive correlations between INSL3 and both serum total T and LH levels were observed in untreated CHH/KS. Mean INSL3 levels remained low in T-treated CHH/KS patients and were significantly higher in men receiving combined hCG-FSH therapy (p < 0.001), but the increase was lower cryptorchid patients. FSH-hCG combination therapy or hCG monotherapy, contrary to T and FSH monotherapies, increased INSL3 levels in CHH/KS.

Conclusions: INSL3 is as sensitive a marker as T for the evaluation of altered Leydig cell function in CHH/KS patients. INSL3 levels correlate with LH levels in CHH/KS men showing, together with the rise in INSL3 levels during hCG therapy, that INSL3 secretion seems not constitutively secreted during adulthood but is dependent on pituitary LH.

Produced by Leydig cells constitutively and independently of the gonadotropins, INSL3 is a central player in testicular physiology, and deserves attention by the pediatric endocrinologist. Interacting with the Leydig cell’s specific receptor RXFP2, it controls testicular steroidogenesis, and in male germ cells, it synergizes with Sertoli cell products to support spermatogenesis. This article makes it a new marker for Leydig cell differentiation and functional capacity. Basal INSL3 levels were lower in untreated HH and in men with cryptorchidism than in those with intra-scrotal testes and in patients with testicular volumes <4 ml. It correlated positively with serum testosterone. This marker could be useful in puberty, or in the diagnosis and monitoring of treatment of hypogonadal patients.
**Appetite and growth: a longitudinal sibling analysis**

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*JAMA Pediatr* 2014;168:345–350

**Context:** Identifying early markers of future obesity risk can help target preventive interventions. Several studies have shown that a heartier appetite in infancy is a risk factor for more rapid weight gain, but to date no investigations have been able to rule out familial confounding.

**Objectives:** To use a sibling design (data from same-sex, dizygotic twin pairs) to test the hypothesis that sibling differences in infant appetite predicted differential weight gain during childhood.

**Methods:** Gemini is a population-based twin cohort among the general United Kingdom population born between March 1, 2007, and December 15, 2007. Growth trajectories were analyzed from birth to age 15 months. Appetite-discordant pairs were selected from 800 non-identical, same-sex twin pairs. Exposures appetite during the first 3 months of life was assessed with the food responsiveness (FR) and satiety responsiveness (SR) subscales from the Baby Eating Behavior Questionnaire. Discordance was defined as a within-pair difference of at least 1 SD. A mean of 11.5 weight measurements per child were available between birth and age 15 months. Multilevel models, adjusted for sex and birth weight, compared growth curves for the higher-appetite vs. lower-appetite twins.

**Results:** 172 pairs were discordant for SR, and 121 pairs were discordant for FR. Within-pair analyses showed that those with higher FR and those with lower SR grew faster than their sibling. At age 6 months, those with higher FR were 654 (95% CI 395–913) g heavier and at age 15 months were 991 (95% CI 484–1,498) g heavier. For sibling pairs discordant for SR, the weight differences between siblings were 637 (95% CI 438–836) g at age 6 months and 918 (95% CI 569–1,267) g at age 15 months.

**Conclusions:** A heartier appetite (indexed with higher FR or lower SR) in early infancy is prospectively associated with more rapid growth up to age 15 months in a design controlling for potential familial confounding, supporting a causal role for appetite in childhood weight gain. Appetite could be an early marker for risk of weight gain in the current obesogenic environment and might be a potential target for preventive interventions.

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**Satiety mechanisms in genetic risk of obesity**

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*JAMA Pediatr* 2014;168:338–344

**Context:** A better understanding of the cause of obesity is a clinical priority. Obesity is highly heritable, and specific genes are being identified. Discovering the mechanisms through which obesity-related genes influence weight would help pinpoint novel targets for intervention. One potential mechanism is satiety responsiveness. Lack of satiety characterizes many monogenic obesity disorders, and lower satiety responsiveness is linked with weight gain in population samples.

**Objective:** To test the hypothesis that satiety responsiveness is an intermediate behavioral phenotype associated with genetic predisposition to obesity in children.

**Methods:** Cross-sectional observational study of a population-based cohort of twins born January 1, 1994, to December 31, 1996 (Twins Early Development Study). Participants included 2,258 unrelated children (53.3% female; mean [SD] age, 9.9 [0.8] years), one randomly selected from each twin pair. Exposure genetic predisposition to obesity. They created a polygenic risk score (PRS) comprising 28 common obesity-related single-nucleotide polymorphisms identified in a meta-analysis of obesity-related genome-wide association studies. Satiety responsiveness was indexed with a standard psychometric scale (Child Eating Behavior Questionnaire). Using 1990 United Kingdom reference data, body mass index SD scores and waist SD scores were calculated from parent-reported anthropometric data for each child. Information on satiety responsiveness, anthropometrics, and genotype was available for 2,258 children. They examined associations among the PRS, adiposity, and satiety responsiveness.
Results: The PRS was negatively related to satiety responsiveness (β coefficient −0.060; 95% CI −0.019 to −0.101) and positively related to adiposity (β coefficient 0.177; 95% CI 0.136–0.218 for body mass index SD scores and β coefficient 0.167; 95% CI 0.126–0.208 for waist SD scores). More children in the top 25% of the PRS were overweight than in the lowest 25% (18.5 vs. 7.2%; odds ratio 2.90; 95% CI 1.98–4.25). Associations between the PRS and adiposity were significantly mediated by satiety responsiveness (p = 0.006 for body mass index SD scores and p = 0.005 for waist SD scores).

Conclusions: These results support the hypothesis that low satiety responsiveness is one of the mechanisms through which genetic predisposition leads to weight gain in an environment rich with food. Strategies to enhance satiety responsiveness could help prevent weight gain in genetically at-risk children.

Sibling design (data from same-sex, dizygotic twin pairs) is a powerful tool to test assumptions of genetic components in population studies. As we well know, even children raised together in the same family may experience diverging trajectories of body mass. The fact that children confronted with similar environments experience disparate outcomes has been attributed to genetic factors. A cohort of such siblings, who were discordant for obesity, was used here to evaluate early markers for a genetic tendency to develop obesity. These two studies show that appetite during the first 3 months of life predicted overweight at age 6 and 15 months – and appetite therefore seems to be an inherent trait. Moreover, appetite is related to genetically-determined low satiety responsiveness. Early craving could be a focused target for preventive intervention, though we have no evidence as yet that such intervention would be effective.

No sweet memories

Higher glucose levels associated with lower memory and reduced hippocampal microstructure

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Neurology 2013;81:1746–1752

Objectives: For this cross-sectional study, the authors aimed to elucidate whether higher glycosylated hemoglobin (HbA1c) and glucose levels exert a negative impact on memory performance and hippocampal volume and microstructure in a cohort of healthy, older, non-diabetic individuals without dementia.

Methods: In 141 individuals (72 women, mean age 63.1 years ± 6.9 SD), memory was tested using the Rey Auditory Verbal Learning Test. Peripheral levels of fasting HbA1c, glucose, and insulin and 3-tesla MRI scans were acquired to assess hippocampal volume and microstructure, as indicated by gray matter barrier density. Linear regression and simple mediation models were calculated to examine associations among memory, glucose metabolism, and hippocampal parameters.

Results: Lower HbA1c and glucose levels were associated with better scores in delayed recall, learning ability, and memory consolidation. In multiple regression models, HbA1c remained strongly associated with memory performance. Moreover, mediation analyses indicated that beneficial effects of lower HbA1c on memory are in part mediated by hippocampal volume and microstructure.

Conclusions: The results indicate that even in the absence of manifest type 2 diabetes mellitus or impaired glucose tolerance, chronically higher blood glucose levels exert a negative influence on cognition, possibly mediated by structural changes in learning-relevant brain areas. Therefore, strategies aimed at lowering glucose levels even in the normal range may beneficially influence cognition in the older population, a hypothesis to be examined in future interventional trials.

Deleterious effects of diabetic glucose levels on brain structure, particularly the hippocampus, have been reported in both animal and human studies; impaired glucose tolerance and T2DM are associated with lower cognitive function and higher incidence of dementia, including Alzheimer disease and vascular dementia. But even in individuals without T2DM, higher glucose levels may exert nega-
tive effects on memory performance and the volume of the hippocampus. This study assessed the association between peripheral short- and long-term glucose metabolism and memory performance in non-diabetic adults without IGT, by analyzing hippocampal volume and microstructure including MRI-based volume and MD. The results are eye-opening: lower HbA1c and glucose levels were associated with better memory and mental scores in most parameters, in part mediated by hippocampal volume and microstructure. Lifestyle strategies aimed at long-term improvement of glucose control by lower dietary caloric intake and physical activity may be a promising strategy to prevent cognitive decline in aging even in non-diabetic subjects.

**Gut microbiome and other phenotypes – two articles**

**Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment**

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Science 2013;342:967–970

**Context:**  
The gut microbiota influences both local and systemic inflammation. Inflammation contributes to development, progression, and treatment of cancer, but it remains unclear whether commensal bacteria affect inflammation in the sterile tumor microenvironment.

**Methods and Results:**  
The paper shows that disruption of the microbiota impairs the response of subcutaneous tumors to CpG-oligonucleotide immunotherapy and platinum chemotherapy. In antibiotics-treated or germ-free mice, tumor-infiltrating myeloid-derived cells responded poorly to therapy, resulting in lower cytokine production and tumor necrosis after CpG-oligonucleotide treatment and deficient production of reactive oxygen species and cytotoxicity after chemotherapy.

**Conclusions:**  
Optimal responses to cancer therapy require an intact commensal microbiota that mediates its effects by modulating myeloid-derived cell functions in the tumor microenvironment. These findings underscore the importance of the microbiota in the outcome of disease treatment.

**Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity**

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Proc Natl Acad Sci USA 2013;110:9066–9071

**Context:**  
Obesity and type-2 diabetes are characterized by altered gut microbiota, inflammation, and gut barrier disruption. Microbial composition and the mechanisms of interaction with the host that affect gut barrier function during obesity and type 2 diabetes have not been elucidated. We recently isolated *Akkermansia muciniphila*, which is a mucin-degrading bacterium that resides in the mucus layer. The presence of this bacterium inversely correlates with body weight in rodents and humans. However, the precise physiological roles played by this bacterium during obesity and metabolic disorders are unknown.

**Methods and Results:**  
This study demonstrated that the abundance of *A. muciniphila* decreased in obese and type 2 diabetic mice. It also observed that prebiotic feeding normalized *A. muciniphila* abundance, which correlated with an improved metabolic profile. In addition, it demonstrates that *A. muciniphila* treatment reversed high-fat diet-induced metabolic disorders, including fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance. *A. muciniphila* administration increased the intestinal levels of endocannabinoids that control inflammation, the gut barrier, and gut peptide secretion. Finally, it demonstrates that all these effects required viable *A. muciniphila* because treatment with heat-killed cells did not improve the metabolic profile or the mucus layer thickness.
Conclusion: This study provides substantial insight into the intricate mechanisms of bacterial (i.e. *A. muciniphila*) regulation of the cross-talk between the host and gut microbiota. These results also provide a rationale for the development of a treatment that uses this human mucus colonizer for the prevention or treatment of obesity and its associated metabolic disorders.

In previous years, the *Yearbook* has cited several reports on a role for the microbiome in body composition. This year, several additional links were made between gut microbes and obesity, but also cancer. Anticancer therapy is now shown to need gut bacteria to be effective; the bacteria help to prime the immune system to respond to drug treatment. The mouse study showed increasing the amount of the mucus-eating gut bacteria, the animals lost weight and had better insulin control, even on a high-fat diet. Obese mice, as well as obese people and people with type 2 diabetes, typically have reduced numbers of these bacteria. Interestingly, the same bacterium also seems to play a role in the weight loss that accompanies gastric bypass surgery. Each one of us has a unique microbiome, which calls for personalized medicine for gut microbial therapy to be most effective. But can we transplant feces from a healthy to a diseased subject? This has been done for many years, and made headlines in 2013 when the FDA lifted the restriction of fecal transplantation as an experimental drug that required permit to perform the procedure.

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**Thyroxine and the heart**

**Subclinical hypothyroidism and left ventricular mechanics: a three-dimensional speckle tracking study**

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*J Clin Endocrinol Metab* 2014;99:307–314

**Context:** Subclinical hypothyroidism (SHT) is associated with left ventricular (LV) remodeling. The LV mechanics has not been previously assessed by two- and three-dimensional (2DE and 3DE) speckle tracking imaging in the SHT patients.

**Objectives:** The objective of the study was to investigate LV mechanics by 2DE and 3DE speckle tracking in the SHT patients and evaluate the influence of levothyroxine therapy on LV remodeling.

**Design:** In a prospective study, all SHT patients received levothyroxine therapy and were followed up for 1 year after the euthyroid state had been achieved. It included 54 untreated women with SHT and 40 healthy control women who were of similar age. The 2DE strain and strain rates, 3DE volumes, 3DE strain, and thyroid hormones levels were assessed.

**Results:** The 2DE LV longitudinal and circumferential strain and systolic and early diastolic strain rates were decreased in the SHT patients before therapy in comparison with the controls or the SHT patients after therapy. The 3DE LV cardiac output and ejection fraction were reduced in the SHT patients at baseline compared with the controls or patients after 1 year of treatment. The 3DE LV longitudinal and radial strains were lower in the SHT group before treatment in comparison with the controls or patients after therapy, whereas the 3DE LV circumferential and area strains gradually increased from untreated SHT patients, among the treated SHT patients, to the controls.

**Conclusion:** SHT significantly affects LV deformation assessed by 2DE and 3DE speckle tracking. The improvement of LV mechanics after 1 year of levothyroxine treatment is significant but incomplete.

Subclinical hypothyroidism (SHT) is common, its prevalence ranging from 4% to even 17% in different populations. Current data show that SHT patients are at risk of adverse cardiovascular events, which is often explained by unfavorable effects of SHT on atherosclerosis development due to high cholesterol levels and endothelial dysfunction. The effect of replacement therapy is controversial. This study evaluated LV in SHT patients in relation to TSH level and evaluated the influence of levothyroxine therapy on LV function. It included 54 untreated women with SHT and 40 healthy controls, and found that LV longitudinal and circumferential strain, systolic and early diastolic strain rates and
cardiac output were decreased in the SHT patients before therapy and normalized after therapy. Importantly, the improvement of LV mechanics is not complete after 1 year of therapy, which implies that the achievement of euthyroid status and the restoration of LV mass do not necessarily denote the normalization of LV function and mechanics. It could also mean that longer maintenance of euthyroid status with a normal TSH value is needed for the normalization of LV function and deformation.

Sensation-seeking and substance use in adolescence – two articles

Pubertal development, personality, and substance use: a 10-year longitudinal study from childhood to adolescence

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J Abnorm Psychol 2013;122:782–796

Context: Most research linking early pubertal development to substance use has focused on the effects of pubertal timing (age at which a certain stage of pubertal development is reached or pubertal status at a particular age; the maturation disparity hypothesis), but little research has focused on pubertal tempo (rate of growth through pubertal stages; the maturation compression hypothesis). However, both timing and tempo have not only been identified as important components of pubertal development, with different predictors, but have also been shown to be independently associated with other adolescent psychopathologies.

Methods: Using latent growth-curve modeling, this study examined how pubertal status at age 12 and pubertal tempo (between 11 and 13 years) related to substance use from 15 to 16 years in boys from low socioeconomic backgrounds (n = 871).

Results: Both pubertal status at age 12 and tempo were significant predictors of increased levels of substance use and problems in mid to late adolescence. In an attempt to identify mechanisms that may explain the association between pubertal development and substance use, it was found that sensation-seeking partially mediated the association between pubertal status at age 12 and substance-use behaviors. Impulse control was found to moderate the association sensation-seeking had with marijuana-use frequency, with high sensation-seeking scores predicting higher marijuana-use frequency only at low levels of impulse control.

Conclusions: These findings highlight the importance of considering multiple sources of individual variability in the pubertal development of boys and provide support for both the maturational disparity and compression hypotheses.

Pubertal status, sensation-seeking, impulsivity, and substance use in high school-aged boys and girls

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J Addict Med 2013;7:116–121

Objective: To examine how factors such as pubertal status, sensation-seeking, and impulsivity are related to substance use (cigarettes, alcohol, and marijuana) in high school students and examine these associations by gender.

Methods: Ten public high schools in Connecticut participated in a survey of high-risk behaviors. Adolescents from grades 9 to 12 (n = 3,068) completed measures of physical development (Pubertal Development Scale), perceived pubertal timing, impulsivity and sensation-seeking (Zuckerman-Kuhlman Personality Questionnaire-Form III), and cigarette, marijuana, and alcohol use in the past 30 days.

Results: Logistic regression analyses modeling each substance use (cigarettes, marijuana, and alcohol) and gender separately showed that (1) early perceived pubertal timing was associated with cigarette use...
Adolescence is an important developmental period for the onset of substance use and misuse; in the USA up to 50% of high school students report binge drinking, and around 25% report having tried an illicit substance. Several reports suggested an important role of early pubertal development. Indeed, puberty is a sensitive period that is associated with emotional and behavioral problems, including depression, externalizing behaviors such as aggression and delinquency, with much of the work having been conducted in girls.

The first study examined how pubertal status and tempo were related to substance use in boys from low socioeconomic backgrounds. Both pubertal status at age 12 and tempo were significant predictors of increased levels of substance use and problems in mid-to-late adolescence. It was found that sensation-seeking partially mediated the association. Impulse control was found to moderate the association sensation-seeking had with marijuana-use frequency, with high sensation-seeking scores predicting higher marijuana-use frequency only at low levels of impulse control. The second study aimed to clarify the roles of pubertal status and tempo in the development of substance use in adolescent boys and girls, and finds that early perceived pubertal timing was associated with cigarette use but Pubertal Development Scale was not associated with any substance use, that sensation-seeking was associated with use of abusive substances in girls but not in boys.

Selective approaches targeting pubertal and personality liability factors in childhood or early adolescence rather than the behavior or problems later in adolescence have shown very promising results. For example, one approach that targets disruptive behaviors and impulse control in childhood has been shown to have long-term effects on adolescent substance use. Clinical interventions implemented prior to initial exposure to drugs prevents or reduces the adverse impact from substance use on the developing brain and other potential harms, as well as reduces some of the huge financial costs of addiction treatment in adulthood. However, there is no evidence that delaying the onset of puberty would affect substance use.

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**Lactational anovulation in mice results from a selective loss of kisspeptin input to GnRH neurons**

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Endocrinology 2014;155:193–203

**Context:** In mammals, lactation is associated with a period of infertility characterized by the loss of pulsatile secretion of gonadotropin-releasing hormone (GnRH) and cessation of ovulatory cycles. Despite the importance of lactational infertility in determining overall fecundity of a species, the mechanisms by which the suckling stimulus suppresses GnRH secretion remain unclear. As kisspeptin neurons are critical for fertility, the aim of this study was to test the hypothesis that reduced kisspeptin expression might mediate the lactation-induced suppression of fertility, using mouse models.

**Methods and Results:** In the rostral periventricular area of the third ventricle (RP3V), a progressive decrease in RP3V Kiss1 mRNA levels was observed during pregnancy culminating in a tenfold reduction during lactation compared with diestrous controls. This was associated with 60% reduction in the numbers of kisspeptin-immunoreactive neurons in the RP3V detected during lactation. Similarly, in the arcuate nucleus there was also a decrease in Kiss1 mRNA levels during late preg
nancy and mid-lactation, and a notable decrease in kisspeptin fiber density during lactation. The functional characteristics of the RP3V kisspeptin input to GnRH neurons were assessed using electrophysiological approaches in an acute brain slice preparation. Although endogenous RP3V kisspeptin neurons were found to activate GnRH neurons in diestrous mice, this was never observed during lactation. This did not result from an absence of kisspeptin receptors as GnRH neurons responded normally to 100 nM exogenous kisspeptin during lactation. The kisspeptin deficit in lactating mice was selective, as GnRH neurons responded normally to RP3V GABA inputs during lactation.

Conclusions: These data demonstrate that a selective loss of RP3V kisspeptin inputs to GnRH neurons during lactation is the likely mechanism causing lactational anovulation in the mouse.

Lactation is associated with a period of infertility in most mammalian females, including humans. This is an important adaptive response, allowing the mother to focus energy on feeding her offspring, before investing resources in a further pregnancy. In humans, this function serves as a critical determining factor defining infancy and a regulator of growth; children with delayed infancy-childhood transition (DICT) will grow to be shorter. The mechanisms mediating lactational infertility remains only partly understood. It includes the hyperprolactinemia of suckling and the energetic drainage on breastfeeding mother in societies where energy availability is borderline. Previous studies have identified that Kiss1 mRNA and protein levels are reduced in the arcuate nucleus of lactating rats associated with the suppression of pulsatile GnRH secretion during lactation. Here, the authors tested the hypothesis in mice that the anovulatory aspects of lactation may result from a deficit in the kisspeptin input to GnRH neurons, and show that kisspeptin expression is reduced and that the functional connection between kisspeptin neurons and the GnRH neurons is totally abolished.

An in-frame deletion at the polymerase-active site of POLD1 causes a multisystem disorder with lipodystrophy

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Nat Genet 2013;45:947–950

Background: DNA polymerase delta, whose catalytic subunit is encoded by POLD1, is responsible for lagging-strand DNA synthesis during DNA replication. It carries out this synthesis with high fidelity owing to its intrinsic 3’- to 5’-exonuclease activity, which confers proofreading ability. Missense mutations affecting the exonuclease domain of POLD1 have recently been shown to predispose to colorectal and endometrial cancers.

Methods: The authors performed exome sequencing on two probands with MDP syndrome (mandibular hypoplasia, deafness and progeroid features) and their unaffected parents to identify de novo disease-causing mutations.

Results: A recurring heterozygous single-codon deletion in POLD1 was identified, which affects the polymerase-active site that abolishes DNA polymerase activity but only mildly impairs 3’- to 5’-exonuclease activity. This mutation causes a distinct multisystem disorder that includes subcutaneous lipodystrophy, deafness, mandibular hypoplasia and hypogonadism in males.

Conclusions: Disrupted function of the ubiquitously expressed POLD1 polymerase has unexpected tissue-specific effects in humans. These findings support an important role for POLD1 function in adipose tissue homeostasis.

Genetic causes of partial lipodystrophy, a condition characterized by severe insulin resistance and elevated type 2 diabetes risk, have identified important mechanisms in adipogenesis and insulin sig-
nalling, for example mutations in PPARG, PLIN1, LMNA and CIDEC. This paper reports a new genetic cause of lipodystrophy, de novo mutations in POLD1, which encodes DNA polymerase delta. Missense mutations affecting the proofreading domain of POLD1 predispose to cancer. The current mutation, a single-codon deletion, disrupted the polymerase-active site but with little loss of exonuclease capacity, which the authors claim explains the markedly different phenotype including lipodystrophy. Surprisingly, disruption of this key polymerase activity with widespread critical cellular functions, had only tissue-specific effects in humans. Subcutaneous adipose tissue was particularly affected and showed increased fibrosis, which has been previous correlated with adipose tissue dysfunction and insulin resistance in other lipodystrophies and also in common obesity. Similar to other forms of lipodystrophy, the affected probands had normal birth weight but from early childhood they developed marked lack of subcutaneous adipose tissue at all sites. Their very low BMIs contrasted with their marked increase in visceral adipose tissue. Remarkably, one of the probands is a National Paracycling Champion. He has type 2 diabetes, which is managed using metformin and a carefully controlled diet. However, when training intensely, he is able to reduce his use of metformin. Other forms of lipodystrophy also exhibit marked susceptibility to changes in diet and lifestyle.

Metformin-sensitive monogenic obesity

**KSR2 mutations are associated with obesity, insulin resistance, and impaired cellular fuel oxidation**


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*Cell 2013;155:765–777*

**Background:** Kinase suppressor of Ras 2 (KSR2) is an intracellular scaffolding protein involved in multiple signaling pathways. Targeted deletion of Ksr2 leads to obesity in mice, suggesting a role in energy homeostasis.

**Methods:** The authors explored the role of KSR2 in humans by sequencing its coding region and intron/exon boundaries of 2,101 individuals with severe early-onset obesity and 1,536 controls.

**Results:** Multiple rare variants were identified in KSR2 that disrupt signaling through the Raf-MEK-ERK pathway and impair cellular fatty acid oxidation and glucose oxidation in transfected cells. These effects were ameliorated by the insulin sensitizing drug, metformin. Mutation carriers exhibit hyperphagia in childhood, low heart rate, reduced basal metabolic rate and severe insulin resistance.

**Conclusions:** These findings establish KSR2 as an important regulator of energy intake, energy expenditure, and substrate utilization in humans. Modulation of KSR2-mediated effects may represent a novel therapeutic strategy for obesity and type 2 diabetes.

The Ras-Raf-MEK signaling pathway links the nutritional status of an organism to cellular proliferation and differentiation. Here, the authors identified 27 different rare variants in the K2R2 gene, which encodes a regulator of MEK activation and promotes AMPK signaling, in 45/2,101 (2.1%) unrelated severely obese individuals. Compared to other monogenic forms of obesity, which predominantly cause hyperphagia, individuals affected by loss-of-function variants in KSR2 have more complex phenotypes, exhibiting both hyperphagia (which becomes less prominent with age) and low basal metabolic rate as mechanisms contributing to positive energy imbalance. So maybe some of our patients really do become obese despite non-excessive levels of food intake.

The loss-of-function variants in KSR2 did not consistently co-segregate with severe obesity in families, indicating that the manifestation of obesity may depend on interactions with other genetic and/or environmental factors. Notably, in their cell models, addition of the common anti-diabetic drug met-
formin, which can stimulate AMPK activation, completely rescued the KSR2 mutation-associated defects in fatty acid oxidation. Furthermore, some KSR2 mutation carriers reported marked weight loss in childhood when prescribed metformin for their severe insulin resistance; whether this is a consistent effect remains to be confirmed.

Towards a long and healthy lifespan

**Metformin improves healthspan and lifespan in mice**


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*Nat Commun* 2013;4:2192

**Background:** Metformin is a drug commonly prescribed to treat patients with type 2 diabetes.

**Methods:** Cohorts of middle-aged male C57BL/6 and B6C3F1 mice were provided with either a standard diet (SD) or SD supplemented with 0.1% (w/w) or 1% (w/w) metformin for the remainder of their lives.

**Results:** 0.1% metformin starting at middle age leads to healthier and longer lives in male mice. In contrast, the higher dose (1% w/w) is toxic. Treatment with metformin mimics some of the benefits of calorie restriction, such as improved physical performance, increased insulin sensitivity, and reduced low-density lipoprotein and cholesterol levels without a decrease in caloric intake. At a molecular level, metformin increases AMP-activated protein kinase activity and increases antioxidant protection, resulting in reductions in both oxidative damage accumulation and chronic inflammation.

**Conclusions:** These findings identify mechanisms that may contribute to the beneficial effects of metformin on health and lifespan. These findings are in agreement with current epidemiological data and raise the possibility of metformin-based interventions to promote healthy aging.

Metformin is a biguanide used since the 1960s to treat type 2 diabetes and the metabolic syndrome. It had previously been reported to extend lifespan in in the nematode worm *Caenorhabditis elegans* and the current authors noted that metformin induces a gene expression profile similar to that seen in calorie restriction, a recognized intervention to prolong lifespan in animals. They found that diet supplementation with 0.1% metformin extended lifespan by 4.15–5.83% across different strains. Furthermore, mouse ‘healthspan’, measured across a range of parameters, was generally improved. In contrast, the higher dose shortened lifespan by 14.4% due to renal failure. Yet there remains debate regarding metformin’s mechanism of action, which limits the development of more potent and safe agents. Metformin is known to activate AMP-activated protein kinase and reduce ATP production in mitochondria. The current authors suggested that the consequent reduction in ATP/AMP ratio, a marker of energy depletion, is similar to that in calorie restriction. In contrast, an alternative mechanism, by which it suppresses gluconeogenesis in the liver, was recently described in May 2014 by Gerald Shulman’s group in Yale [1]. They found that acute activation of AMP-activated protein kinase was not sufficient to reduce endogenous glucose production, but rather chronic metformin treatment was associated with an increase in the plasma and cytosolic redox state and a decrease in the mitochondrial redox state. Specifically, metformin inhibits mitochondrial respiration from glycerol-3-phosphate. The pleiotropic effects of metformin present challenges to identify the specific mechanisms that underlie its observed benefits on health and aging.
Stem cells remember the fetal environment

Fetal programming of adult Leydig cell function by androgenic effects on stem/progenitor cells

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 Proc Natl Acad Sci USA 2014;111:E1924–E1932

Background: Long-term links between fetal growth or fetal androgen exposure and testosterone levels in men are puzzling, because the adult Leydig cells (ALCs) that produce testosterone do not differentiate until puberty. The authors hypothesized that stem cells for ALCs are present in the fetal testis and might be susceptible to programming by fetal androgen exposure during masculinization.

Methods: To address this hypothesis, the authors studied a rat model used ALC ablation/regeneration techniques.

Results: ALCs were identified to derive from stem/progenitor cells, which are abundant in the fetal testis of humans and rodents. The stem cells also express androgen receptors (ARs) and reduction in fetal androgen action through AR KO in mice or dibutyl phthalate (DBP)-induced reduction in intratesticular testosterone in rats reduced ALC stem cell number by approximately 40% at birth to adulthood and induced compensated ALC failure (low/normal testosterone and elevated luteinizing hormone). In DBP-exposed males, this failure was probably explained by reduced testicular steroidogenic acute regulatory protein expression, which is associated with increased histone methylation (H3K27me3) in the proximal promoter. Accordingly, ALCs and ALC stem cells immunoexpressed increased H3K27me3, a change that was also evident in ALC stem cells in fetal testes.

Conclusions: These findings show that a key component of male reproductive development can fundamentally reprogram adult hormone production through an epigenetic change, with potential consequences for lifetime disease risk.

These and other authors have previously reported that indirect measures of fetal androgen exposure, such as anogenital distance or digit length ratio, are associated with adult testosterone levels, thus indicating a long-term programming of male reproductive function [2]. However, the mechanisms had not been identified and the late appearance only from puberty of adult Leydig cells, which are the source of testosterone in men, questioned the validity of fetal programming of this endocrine axis. Remarkably, this study shows for the first time that testosterone levels during fetal masculinization can (re)program adult testosterone levels through effects on stem cells, which are numerous in fetal testes of humans and animals, and develop into adult Leydig cells after puberty. Crucially, these stem cells are themselves androgen targets, and disrupted androgen exposure in fetal life may cause long-term defects through altered chromatin epigenetic changes in the steroidogenic acute regulatory protein (StAR) gene promoter. The authors also highlight the links between lowered testosterone levels in adult men and higher risks of cardiometabolic diseases. Therefore, disrupted fetal androgen exposure might have pervasive importance not only for later masculinity but also for health and ageing.
Pituitary control of adrenarche

Absent adrenarche in children with hypopituitarism: a study based on urinary steroid metabolomics

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Background: The regulation of adrenarche remains unknown although ACTH has been suggested to play an important role.

Methods: The authors used urinary steroid profiling by gas chromatography-mass spectrometry to study non-invasively the adrenarchal steroid metabolome in 13 children aged 6–16 years with partial or complete hypopituitarism (HP) whose ACTH/cortisol axis was affected and compared it with 24 healthy age-matched controls. The sum of DHEA, 16α-hydroxy-DHEA and 3β,16α,17β-androstenetriol served as markers for adrenarche parameters (AP). The excretion rates of major urinary cortisol metabolites were also determined.

Results: AP excretion rates were substantially lower in HP children than in controls (p < 0.001). Both hydrocortisone (HC)-treated and HC-untreated HP subgroups showed lower AP excretion rates (p < 0.001 and p = 0.045, respectively) than controls.

Conclusion: These findings indicate a significant contribution of ACTH to the regulation of adrenarche. Furthermore, absent adrenarche may be indicative of ACTH deficiency.

Adrenarche is triggered by the development of the adrenal zona reticularis starting as early as age 3 years, is accompanied by decreasing expression of 3α-hydroxysteroid dehydrogenase and increasing 17,20-lyase activity of P450c17, and results in a continuous age-related rise in adrenal secretion of the C19 steroids, dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Well-established early life correlates of adrenarche, both in patient cohorts and population-based studies, include low birth weight, rapid infancy growth and weight gain, and childhood overweight and obesity. However, the regulation of adrenarche has remained a mystery.

The current study provides strong evidence for a key central regulation of adrenarche. Both hypopituitarism (HP) subgroups, whether cortisol treated or untreated, showed low urinary excretion of adrenarche parameters (AP) compared to controls, indicating that low AP excretion rates was not simply due to adrenal suppression by cortisol therapy. The differences in AP excretion rates were striking; the median in the cortisol-treated HP subgroup was 0 μg/day/m² (interquartile range 0–10, n = 9) was similar to that in the HC-untreated HP subgroup 10 μg/day/m² (2–359, n = 4), compared to 268 μg/day/m² (142–521) in the controls. Absent adrenarche, measured by a low urine AP excretion rate, could be a novel indicator of ACTH deficiency.

Testing the fetus in mother’s blood

DNA sequencing versus standard prenatal aneuploidy screening

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Background: This study aimed to test the performance to detect fetal autosomal aneuploidy in low-risk women of non-invasive prenatal testing with massively parallel sequencing of maternal plasma cell-free DNA (cfDNA testing).

Methods: The authors collected blood samples from women with singleton pregnancies undergoing standard aneuploidy screening (using serum biochemical assays with or without measurement of nuchal
translucency) at 21 centers in the USA. Massively parallel sequencing was performed in a blinded fashion to determine the chromosome dosage in each sample. The primary end-point was the false positive rates of detection of fetal trisomies 21 and 18. cfDNA testing was compared to standard screening, with birth outcomes or karyotypes as the reference standard.

Results: The eligible sample for primary outcome was 1,914 women (mean age 29.6 years), who had a singleton fetus without aneuploidy, results from cfDNA testing, and a risk classification based on standard screening. For trisomies 21 and 18, the false positive rates with cfDNA testing were lower than those with standard screening (0.3 vs. 3.6% for trisomy 21, p < 0.001, and 0.2 vs. 0.6% for trisomy 18, p = 0.03). cfDNA testing detected all cases of aneuploidy (5 cases of trisomy 21, 2 cases of trisomy 18, and 1 case of trisomy 13; negative predictive value, 100% (95% CI 99.8–100)). The positive predictive values for cfDNA testing versus standard screening were 45.5 vs. 4.2% for trisomy 21 and 40.0 vs. 8.3% for trisomy 18.

Conclusions: In a general obstetrical population, compared to standard screening, prenatal testing using cfDNA resulted in lower false positive rates and higher positive predictive values for detection of trisomies 21 and 18.

The detection of cell-free fetal DNA in mother’s blood during pregnancy holds great promise for better antenatal diagnosis and management of congenital disease. Cell-free fetal DNA is derived from placental trophoblasts as they undergo cellular apoptosis and necrosis, it comprises around 10% of the cell-free DNA in mother’s blood during the first half of pregnancy, and this proportion increases as pregnancy progresses.

This study, funded by Illumina, reports a head-to-head comparison in the general population of pregnant women of the use of next-generation DNA sequencing of cell-free fetal DNA using an Illumina HiSeq 2000 instrument versus standard screening algorithms using current biochemical markers (e.g. PAPP-A, hCG, AFP, unconjugated estriol, and inhibin A) and ultrasound. Both DNA sequencing and standard screening correctly picked up all 8 cases of aneuploidy (100% sensitivity). But, DNA sequencing clearly performed better with regard to specificity: for trisomies 21 and 18 combined, the false positive rate was only 0.5% for DNA sequencing compared to 4.2% for standard screening. Positive results on pregnancy screening tests are a huge cause of distress and are usually followed by invasive confirmatory tests. Widespread use of cell-free fetal DNA could reduce this burden by tenfold and may also be cost-effective (although this was not assessed in the study).

A couple of limitations should be noted. Firstly, DNA sequencing of cell-free fetal DNA is still only a screening tool for fetal aneuploidy (rather than diagnostic); because the positive predictive values are less than 50%, confirmatory tests are still needed. The reasons for false positivity are not known. Secondly, the test did not provide a valid result in 18 of 2,042 samples (0.9%); failures occurred at DNA extraction and sequencing stages.

Public and professional prejudice

Abolishing mammography screening programs? A view from the Swiss Medical Board

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Background: In February 2014, the Swiss Medical Board, an independent health technology assessment initiative under the auspices of the Conference of Health Ministers of the Swiss Cantons, the Swiss Medical Association, and the Swiss Academy of Medical Sciences, published their report on mammography screening. Based on a review of the evidence, the Board concluded that systematic mammography screening might prevent about 1 death from breast cancer for every 1,000 women screened, but no effect on overall mortality was evident. Conversely, the risk of overdiagnosis and numbers of false positive test results substantially outnumbered the breast-cancer deaths prevented. The Board recom
Assessing the phenotypic effects in the general population of rare variants in genes for a dominant mendelian form of diabetes
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**Background:** Genome sequencing can identify individuals in the general population who harbor rare coding variants in genes for mendelian disorders and who may consequently have increased disease risk. Previous studies of rare variants in phenotypically extreme individuals display ascertainment bias and may demonstrate inflated effect-size estimates.

**Methods:** The authors sequenced seven genes for maturity-onset diabetes of the young (MODY) in well-phenotyped population samples (n = 4,003) from the Framingham and Jackson Heart Studies. Rare variants were categorized according to two different prediction criteria.

**Results:** Approximately 1.5% of randomly selected individuals carry rare variants that have been reported previously in MODY, and 0.5% carry variants that satisfy stringent de novo mutation thresholds (i.e. they are rare, conserved and protein damaging). However, the vast majority of carriers remain euglycemic through middle age.

**Conclusions:** Accurate estimates of variant effect sizes from large population-based sequencing are needed to avoid falsely predicting a substantial fraction of individuals as being at high risk for mendelian diseases.

With increasing clinical and ‘direct-to-consumer’ use of genetic tests, what should we do with those patients with incidentally-identified rare ‘disease-causing’ mutations? Here, the authors tested the predictive value in the general population of finding deleterious mutations in 7 genes for maturity-onset diabetes.
onset diabetes of the young (MODY). If we had found any of these mutations in patients from our diabetes clinic, we would label them as having MODY. In contrast, in the general population, 3 in 200 individuals carry a variant that has been reported previously to cause MODY and yet show completely normal glucose status as adults. Why the difference?

The reasons are twofold. The first is ascertainment bias. Mendelian genetic research is naturally performed in cases with very high risk of having the diagnosis in question, and the resulting estimates of effect sizes (or ‘penetrance’) are relevant only to future patients who present with similar high risk phenotypes. Second is the concept that, even if the calculated specificity is correct, the positive predictive value of any test is directly related to the prevalence of the outcome in the population being tested. So the answer to the clinical scenario posed above is – we don’t know. To answer such questions, we will need data on high-depth whole genome sequencing in very large populations. Alternatively, the question should not be posed in the first place. (Except where screening has been shown to be valid) we, and the public, should not test for conditions that we don’t suspect.

References


