Editor's Choice

Jean-Claude Carel and Ze'ev Hochberg

Linked to grow

Genetic linkage of human height is confirmed to 9q22 and Xq24

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Background: Human height is an important and heritable trait. The author's previous two genome-wide linkage studies using 630 (WG1 study) and an extended sample of 1,816 Caucasians (WG2 study) identified 9q22 [maximum LOD score (MLS) = 2.74 in the WG2 study] and preliminarily confirmed Xq24 (two-point LOD score = 1.91 in the WG1 study, 2.64 in the WG2 study) linked to height.

Methods: Here, with a much further extended large sample containing 3,726 Caucasians, a new genome-wide linkage scan was performed and confirmed, in high significance, the two regions' linkage to height.

Results: A MLS of 4.34 was detected on 9q22 and a two-point LOD score of 5.63 was attained for Xq24. In an independent sub-sample (i.e., the subjects not involved in the WG1 and WG2 studies), the two regions also achieved significant empirical p values (0.002 and 0.004, respectively) for 'region-wise' linkage confirmation. Importantly, the two regions were replicated on a genotyping platform different from the WG1 and WG2 studies (i.e., a different set of markers and different genotyping instruments). Interestingly, 9q22 harbors the ROR2 gene, which is required for growth plate development, and Xq24 was linked to short stature.

Conclusions: With the largest sample from a single population of the same ethnicity in the field of linkage studies for complex traits, the current study, together with the two previous ones, provided overwhelming evidence substantiating 9q22 and Xq24 for height variation. In particular, the three consecutive whole genome studies are uniquely valuable as they represent the first practical (rather than simulated) example of how significant increase in sample size may improve linkage detection for human complex traits.

Genetic studies using association or whole genome linkage approaches have suggested quite a few genes and genomic regions associated with or linked to human height. However, few such genes and genomic regions have been replicated across studies. This may be attributed to the relatively small sample sizes for most of the studies and the modest significance levels for the majority of the reported findings. As a result, genuine genetic effects could be missed or a reported linkage or association could be a false positive. The present study was performed on 3,726 subjects of the same ethnicity. With a MLS of 4.34, 9q22 can now be regarded to have a strong link to growth, and with a two-point LOD score of 5.63 Xq24 (didn't we expect Xp to be the major growth region?) is now open to hunt. Admittedly, Xp22 also achieved very significant linkage signals. Our growth plate investigators will be delighted to hear of 9q22-ROR2 gene, which is required for growth plate development. Linked to the Xq24 region were several syndromes characterized by short stature, including the mental retardation, x-linked, with short stature syndrome (MIM 300360), the mental retardation, x-linked, with short stature, small testes, muscle wasting, and tremor syndrome (MIM 300354) and the mental retardation, x-linked, with isolated growth hormone deficiency syndrome (MIM 300123). On a similar topic, the same group with the same cohort aimed to search for potential genomic regions that harbor interactive genes underlying human height [1]. Performing variance component linkage analyses of height based on a two-locus epistatic model, they found significant genetic interaction between 6p21 and 2q21. Interestingly, 6p21 contains a cluster of candidate genes for skeletal growth, suggesting a mechanism whereby 2q21 regulates height through 6p21.

Estrogen deficiency and bone loss: an inflammatory tale

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Background: Estrogen plays a fundamental role in skeletal growth and bone homeostasis in both men and women. Although remarkable progress has been made in our understanding of how estrogen deficiency causes bone loss, the mechanisms involved have proven to be complex and multifaceted.

New concepts involving the immune system: Although estrogen is established to have direct effects on bone cells, recent animal studies have identified additional unexpected regulatory effects of estrogen centered at the level of the adaptive immune response. Furthermore, a potential role for reactive oxygen species has now been identified in both humans and animals.

Challenge: The integration of a multitude of redundant pathways and cytokines, each apparently capable of playing a relevant role, into a comprehensive model of postmenopausal osteoporosis.

This review presents the current understanding of the process of estrogen deficiency-mediated bone destruction and explores some recent findings and hypotheses to explain estrogen action in bone. Due to the inherent difficulties associated with human investigation, many of the lessons learned have been in animal models. Consequently, many of these principles await further validation in humans. Estrogen receptors (ER) α and β are expressed in multiple organs, and the industry is in a race to develop a selective estrogen receptor modulator (SERM) that would activate the ER in bone but not in the breast. In 2002, a synthetic ligand, named estren, was reported to have increased bone mass and strength in gonadectomized mice but had no effects on uterine breast cancer cells [2]. The effect of estren was attributed to non-genomic activation of transcription factors by several kinase cascades. PubMed shows 96 entries for estrens. While everyone expected the results of their use in humans, a few reports have questioned the non-genomic mechanism of estrens. We now learn that while estrens were able to prevent gonadectomy-induced bone loss, they showed no bone anabolic effects when given at the same doses and in the same manner as originally reported. Moreover, they increased uterine weight and enhanced the proliferation of human breast cancer cells. The paper shows that estrens bind more strongly to androgen receptor than ER and suggests that they act more as androgens than estrogens.

Klotho converts canonical FGF receptor into a specific receptor for FGF23

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Context: FGF23 is a unique member of the fibroblast growth factor (FGF) family because it acts as a hormone that derives from bone and regulates kidney functions, whereas most other family members are thought to regulate various cell functions at a local level. The renotropic activity of circulating FGF23 indicates the possible presence of an FGF23-specific receptor in the kidney.

Results: A previously undescribed receptor conversion by Klotho, a senescence-related molecule, generates the FGF23 receptor. Using a renal homogenate, the authors found that Klotho binds to FGF23. Forced expression of Klotho enabled the high-affinity binding of FGF23 to the cell surface and restored the ability of a renal cell line to respond to FGF23 treatment. Moreover, FGF23 incompetence was induced by injecting wild-type mice with an anti-Klotho monoclonal antibody.

Conclusions: Klotho is essential for endogenous FGF23 function. Because Klotho alone seemed to be incapable of intracellular signaling, they searched for other components of the FGF23 receptor and found FGFR1(IIIc), which was directly converted by Klotho into the FGF23 receptor. Thus, the concerted action of Klotho and FGFR1(IIIc) reconstitutes the FGF23 receptor. These findings provide insights into the diversity and specificity of interactions between FGF and FGF receptors.

Last year, the *Yearbook* described the new hormone Klotho (daughter of Zeus and Themis, etc.), and how it suppresses tyrosine phosphorylation of insulin and IGF-1 receptors, which result in reduced activity of IRS proteins and their association with PI₃-kinase, thereby inhibiting insulin and IGF-1 signaling, and suppressing aging [3]. This year, Klotho gets closer to pediatric endocrinology. It converts FGF receptor-1, a canonical receptor for various FGFs, into a specific receptor for FGF23, the bone-derived phosphaturic hormone that has been implicated in three different varieties of hypophosphatemic rickets [4].

Eflornithine cream combined with laser therapy in the management of unwanted facial hair growth in women: a randomized trial

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Background: Eflornithine cream is approved for the reduction of unwanted facial hair in women. The mechanism of action for eflornithine is reduction in follicular cell growth rate, while laser photoepilation heats hair and adjacent tissues to suspend growth. The objective was to assess the efficacy and safety of eflornithine or vehicle with laser therapy in the treatment of unwanted facial hair in women.

Methods: Subjects were randomized to treatment with eflornithine on one side of the face and vehicle on the contralateral side for 34 weeks. Subjects received Nd:YAG or alexandrite laser therapy to both sides of the face at weeks 2 and 10. Blinded evaluations included left to right comparisons and appearance relative to baseline.

Results: Fifty-four women completed the trial. From weeks 6 through 22, eflornithine-treated sides showed significant reduction in hair growth. By week 34, no significant differences were seen. Subject grading showed significant and persistent hair reduction through week 34 for eflornithine-treated sides. The safety profile for combination therapy is similar to eflornithine alone.

Conclusion: Eflornithine is safely used in conjunction with laser hair removal treatments and promotes more rapid hair removal when combined with laser treatment. Patients demonstrate a clear preference for treatment with laser and eflornithine.

You will recognize that this isn't one of the journals we usually cite, but it seems that this new drug went unnoticed by the pediatric endocrine community, and its story is really fascinating. African sleeping sickness affects 300,000 new cases every year, and is caused by *Trypanosoma brucei*, which is transmitted by the bite of an infected tsetse fly. Eflornithine (difluoromethylornithine, DFMO, 1970s), which was developed as a potential anticancer drug by inhibiting ornithine decarboxylase (polyamine biosynthesis) and impairing cellular division, was evaluated for its efficacy in a mouse model of trypanosomiasis. Following dramatic results in mice, clinical trials showed revitalization of comatose sleeping sickness, and was named 'the resurrection drug'. The problem was that with parenteral administration, and costing USD 600/year, it wasn't practical or profitable for the industry to pursue and it was decided to discontinue production. At the end of 2000, the remaining vials were 1 year past expiration; the entire stockpile was projected to be depleted in another 6–9 months. Clinical observations in sleeping sickness showed for eflornithine side effects of hair loss and hearing loss (loss of hair cells in organ of Corti). It was tested and shown that when topically applied, DFMO inhibits DNA synthesis and hair follicular function. The topical formulation received FDA approval in July 2005. The happy end: Bristol-Myers Squibb, Dow Chemical Co., Akorn Manufacturing Inc., and Aventis, who are to make a fortune, have agreed to supply WHO with adequate supplies of parenteral eflornithine to last for the next 3 years.

Identification of a hormonal basis for gallbladder filling

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Context: The cycle of gallbladder filling and emptying controls the flow of bile into the intestine for digestion.

Results: Here the authors show that fibroblast growth factor-15, a hormone made by the distal small intestine in response to bile acids, is required for gallbladder filling.

Conclusions: These studies demonstrate that gallbladder filling is actively regulated by an endocrine pathway and suggest a postprandial timing mechanism that controls gallbladder motility.

The hormone cholecystokinin (CCK), which is secreted from the proximal duodenum, is the major determinant of gallbladder emptying. Fibroblast growth factor-19 (FGF15 in the mouse) is a hormone that has a central role in the feedback regulation of bile acid synthesis in the liver. It is made by the ileum, where its expression is induced by bile acids acting through the farnesoid X receptor. Mice lacking FGF15 have gallbladders that are almost devoid of bile and providing this new hormone stimulates gallbladder filling. Interestingly, all four FGF receptor mRNAs were detected in the gallbladder, with *Fgfr3* mRNA levels being the highest, suggesting a role for *Fgfr3*. Here is how this new endocrine loop works in the temporal regulation of gallbladder motility. During feeding, CCK secreted from the duodenum causes gallbladder contraction and the release of bile into the duodenum, which facilitates digestion and suppresses further CCK secretion. After traversing the small intestine, bile acids induce FGF19 synthesis in the ileum, which stimulates gallbladder refilling. It remains to be seen whether FGF19 will inhibit the effects of CCK on other physiological processes including gastric emptying, pancreatic enzyme secretion and food intake.

Family composition and menarcheal age: anti-inbreeding strategies

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Background: Family composition (e.g., the absence of a father) is associated with pubertal timing in women, although the socioendocrinology of the human primate is poorly understood.

Methods: To better understand social influences on sexual maturation, retrospective data were collected on menarcheal age and family composition from a sample of approximately 1,938 participants from a college population.

Results: Absence of a biological father, the presence of half- and stepbrothers, and living in an urban environment were associated with earlier menarche. The presence of sisters in the household while growing up, especially older sisters, was associated with delayed menarche. Menarcheal age was not affected by the number of brothers in the household, nor was there an effect of birth order. Body weight and race were also associated with menarche.

Conclusions: The present findings advance the literature as they are suggestive of putative human pheromones that modulate sexual maturation to promote gene survival and prevent inbreeding, as occurs in rodents and non-human primates.

Prevention of inbreeding is so crucial to successfully spread healthy genes that anti-inbreeding strategies are conserved across species. As part of an evolutionary strategy to prevent inbreeding, chemical cues from fathers may be delaying the onset of sexual maturity in daughters. If the biological father is removed from rodent families, the daughters tend to mature faster. In the human olfactory system a little-known pheromone receptor gene may link the role of pheromones to menarche. Menarcheal data collected from 1,938 college students indicate that girls without fathers matured

approximately 3 months before girls whose fathers were present; the earlier the absence, the earlier the menarche. The presence of half- and stepbrothers was also linked to earlier menarche. Girls living in an urban environment also had earlier menarche compared to girls in a rural environment, even when fathers were present for both groups, and had similar levels of education. The authors speculate that urban environments provide greater opportunities to get away from parents' inhibitory pheromones, and encounter attracting pheromones from unrelated members of the opposite sex.

Evolution of hormone-receptor complexity by molecular exploitation

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Background: According to Darwinian theory, complexity evolves by a stepwise process of elaboration and optimization under natural selection. Biological systems composed of tightly integrated parts seem to challenge this view, because it is not obvious how any element's function can be selected for unless the partners with which it interacts are already present.

Aim: To demonstrate how an integrated molecular system, the specific functional interaction between the steroid hormone aldosterone and its partner the mineralocorticoid receptor evolved by a stepwise Darwinian process.

Results: Using ancestral gene resurrection, this paper shows that, long before the hormone evolved, the receptor's affinity for aldosterone was present as a structural by-product of its partnership with chemically similar, more ancient ligands. Introducing two amino acid changes into the ancestral sequence recapitulates the evolution of present-day receptor specificity.

Conclusions: Tight interactions can evolve by molecular exploitation-recruitment of an older molecule, previously constrained for a different role, into a new functional complex.

Were hormones constructed to fit given receptors? Or did the latter evolve to fit existing ligands? Is it possible that the pair evolved together? The existence of orphan receptors suggested that receptors came first. This paper takes a closer look at this mystery and discovers a different answer in the molecular evolution of hormone-receptor interactions. Charles Darwin famously remarked that 'if it could be demonstrated that any complex organ existed which could not possibly have been formed by numerous successive slight modifications, my theory would absolutely break down'. I will not keep you suspended; there is no 'intelligent design' behind the endocrine pair. Luckily, it is now possible to reconstruct the ancestral genes of an existing species so that we can now study a gene's evolution. You recall that cortisol activates not only the glucocorticoid receptor but also the mineralocorticoid receptor if it wasn't for 11 β -HSD-2. Phylogeny tells us that an ancestral corticoid receptor gave rise to the glucocorticoid receptor and the mineralocorticoid receptor in a gene-duplication event more than 450 million years ago. Aldosterone evolved much later. Without aldosterone present, how could the mineralocorticoid receptor evolve to be activated by it? What the authors find is a surprise: Not only is the ancestral corticoid receptor sensitive to cortisol as expected, it is also activated by 11-deoxycorticosterone (DOC) and aldosterone. Because aldosterone was not present at the time, this sensitivity must be a by-product of sensitivity to another steroid, a promiscuity that has been exploited by evolution.

Effects of psychosocial stimulation and dietary supplementation in early childhood on psychosocial functioning in late adolescence: follow-up of randomized controlled trial

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Background: To determine whether dietary supplementation or psychosocial stimulation given to growth-retarded (stunted) children aged 9–24 months has long-term benefits for their psychosocial functioning in late adolescence.

Design: Sixteen-year follow-up study of a randomized controlled trial.

Methods: The study was set in poor neighborhoods in Kingston, Jamaica. The participants were 129 stunted children identified at age 9–24 months of whom 103 adolescents aged 17–18 were followed up. Intervention consisted in supplementation with 1 kg milk-based formula each week or psychosocial stimulation (weekly play sessions with mother and child), or both, for 2 years. The outcome measures included anxiety, depression, self-esteem, and antisocial behavior assessed by questionnaires administered by interviewers; attention deficit, hyperactivity, and oppositional behavior assessed by interviews with parents.

Results: Primary analysis indicated that participants who received stimulation had significantly different overall scores from those who did not ($F = 2.047$, $p = 0.049$). Supplementation had no significant effect ($F = 1.505$, $p = 0.17$). Participants who received stimulation reported less anxiety (mean difference -2.81), less depression (-0.43), and higher self-esteem (1.55) and parents reported fewer attention problems (-3.34). These differences are equivalent to effect sizes of 0.40–0.49 standard deviations.

Conclusions: Stimulation in early childhood has sustained benefits to stunted children's emotional outcomes and attention.

The reactive response to stunted growth in a poor neighborhood is to provide food supplementation. Whereas the importance of food cannot be overestimated, this paper shows that in the long run, psychosocial stimulation was a better investment than food was. Walker and colleagues show that in this group of poor Jamaican growth-retarded (stunted) children, aged 9–24 months, interventions to stimulate children and expose them to more positive parenting reduce the risks of antisocial behavior, pregnancy, substance misuse, delinquency, and emotional and behavioral disorders in adolescence, whereas food supplementation had no sustained effect on any of these crucial parameters. For us, in the field of growth promotion of stunted children, it may be high time to shift emphasis from reactive intervention to prevention and health promotion.

Toward a therapy for Marfan syndrome

Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome

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Background: Aortic aneurysm and dissection are manifestations of Marfan syndrome, a disorder caused by mutations in the gene that encodes fibrillin-1. Selected manifestations of Marfan syndrome reflect excessive signaling by the transforming growth factor- β (TGF- β) family of cytokines.

Results: In a mouse model of Marfan syndrome, aortic aneurysm is associated with increased TGF- β signaling and can be prevented by TGF- β antagonists such as TGF- β -neutralizing antibody or the angiotensin II type 1 receptor (AT1) blocker, losartan. AT1 antagonism also partially reversed non-cardiovascular manifestations of Marfan syndrome, including impaired alveolar septation.

Conclusions: These data suggest that losartan, a drug already in clinical use for hypertension, merits investigation as a therapeutic strategy for patients with Marfan syndrome and has the potential to prevent the major life-threatening manifestation of this disorder.

Marfan syndrome is caused by mutations in the gene encoding fibrillin-1, a component of the extracellular matrix that regulates TGF- β . This paper reports that losartan, an FDA-approved angiotensinogen II type 1 receptor antagonist that also antagonizes TGF- β , prevents aortic aneurysm in a mouse model of Marfan syndrome. Treatment of pregnant mice with losartan in the drinking water, or of postnatal mice by intraperitoneal injection, either prevented or resulted in a complete reversion of these major, life-threatening manifestations of the syndrome. Treated mice also showed

a reduction in distal airspace of the lung. A clinical trial is planned to test the drug in individuals with Marfan syndrome.

Repression of smoothened by patched-dependent (pro-)vitamin D₃ secretion

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Context: The developmentally important hedgehog pathway is activated by binding of hedgehog to patched (Ptch1), releasing smoothened (Smo) and the downstream transcription factor glioma associated (Gli) from inhibition. The mechanism behind Ptch1-dependent Smo inhibition remains unresolved.

Results: The authors now show that by mixing Ptch1-transfected and Ptch1 small interfering RNA-transfected cells with Gli reporter cells, Ptch1 is capable of non-cell autonomous repression of Smo. The magnitude of this non-cell autonomous repression of Smo activity was comparable to the fusion of Ptch1-transfected cell lines and Gli reporter cell lines, suggesting that it is the predominant mode of action. CHOD-PAP analysis of medium conditioned by Ptch1-transfected cells showed an elevated 3 β -hydroxysteroid content, which was hypothesized to mediate the Smo inhibition. Indeed, the inhibition of 3 β -hydroxysteroid synthesis impaired Ptch1 action on Smo, whereas adding the 3 β -hydroxysteroid (pro-)vitamin D₃ to the medium effectively inhibited Gli activity. Vitamin D₃ bound to Smo with high affinity in a cyclopamine-sensitive manner. Treating zebrafish embryos with vitamin D₃ mimicked the smo(–/–) phenotype, confirming the inhibitory action in vivo. Hh activates its signaling cascade by inhibiting Ptch1-dependent secretion of the 3 β -hydroxysteroid (pro-)vitamin D₃.

Conclusions: This action not only explains the seemingly contradictory cause of Smith-Lemli-Opitz syndrome, but also establishes hedgehog as a unique morphogen, because binding of Hh on one cell is capable of activating hedgehog-dependent signaling cascades on other cells.

We are used to think of vitamin D in terms of calcium metabolism, but a large volume of recent evidence links it to cancer. This paper makes an interesting connection through the hedgehog (Hh) pathway whose signaling mechanism is poorly understood. It suggests that vitamin D₃ (or its precursor) keeps the pathway silenced in the absence of the hedgehog ligand. Individuals who suffer from decreased hedgehog signaling have high levels of 7-dehydrocholesterol (provitamin D₃ or 7-DHC which accumulates in Smith-Lemli-Opitz syndrome). 7-DHC inhibits the Hh receptor SMO, and the other Hh receptor PTCH1 normally pumps out vitamin D₃ (or its precursor) from the cell, which then inhibits SMO in cells nearby. When hedgehog comes along and binds to PTCH1, the pump shuts down, and SMO is free to activate the intracellular hedgehog pathway.

Concepts revised: born to run (2)

Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study)

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Background: Atherosclerosis develops from early childhood; physical activity could positively affect this process. The aim of the study was to assess the associations of objectively measured physical activity with clustering of cardiovascular disease risk factors in children and derive guidelines on the basis of this analysis.

Methods: Cross-sectional study of 1,732 randomly selected 9- and 15-year-old schoolchildren from Denmark, Estonia, and Portugal. Risk factors included in the composite risk factor score (mean of

z scores) were systolic blood pressure, triglyceride, total cholesterol/HDL ratio, insulin resistance, sum of four skinfolds, and aerobic fitness. Individuals with a risk score >1 SD of the composite variable were defined as being at risk. Physical activity was assessed by accelerometry.

Results: Odds ratios for having clustered risk for ascending quintiles of physical activity (counts per min; cpm) were $3 \cdot 29$, $3 \cdot 13$, $2 \cdot 51$, and $2 \cdot 03$, respectively, compared with the most active quintile. The first to the third quintile of physical activity had a raised risk in all analyses. The mean time spent $>2,000$ cpm (equivalent to walking about 4 km/h) in the fourth quintile was 116 min/day in 9-year-old children and 88 min/day in 15-year-old children.

Conclusion: Physical activity levels should be higher than the current international guidelines of at least 1 h/day of physical activity of at least moderate intensity to prevent clustering of cardiovascular disease risk factors.

Objectively measured physical activity and fat mass in a large cohort of children

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Background: Previous studies have been unable to characterize the association between physical activity and obesity, possibly because most relied on inaccurate measures of physical activity and obesity.

Methods: A cross-sectional analysis on 5,500 12-year-old children enrolled in the Avon Longitudinal Study of Parents and Children was carried out. Total physical activity and minutes of moderate and vigorous physical activity were measured using the Actigraph accelerometer. Fat mass and obesity (defined as the top decile of fat mass) were measured using the Lunar Prodigy dual x-ray emission absorptiometry scanner.

Results: There were strong negative associations between moderate and vigorous physical activity and fat mass that were unaltered after adjustment for total physical activity. There was also a strong negative dose-response association between moderate and vigorous physical activity and obesity. The odds ratio for obesity in adjusted models between top and the bottom quintiles of minutes of moderate and vigorous physical activity was 0.03 (p value for trend <0.0001) in boys and 0.36 (p value for trend = 0.006) in girls.

Conclusions: There was a strong graded inverse association between physical activity and obesity that was stronger in boys. The data suggest that higher intensity physical activity may be more important than total activity.

Body weight is related to the balance of two essential components, food intake and energy expenditure, modulated by the genetic background and epigenetic influences. Physical activity is an essential component of energy expenditure and has received less attention than diet as the cause of the obesity epidemic. One of the reasons for this might have been the difficulty in measuring physical activity which was so far mostly based on questionnaires. The use of accelerometers has allowed a relatively precise evaluation of physical activity and its importance is illustrated in these two studies, evaluating the components of fat mass or cardiovascular risks in community-based samples of European children. In the study by Andersen et al., there was a more than 3-fold increased risk of having a cardiovascular risk profile in children with the lowest vs. highest activity. In the Avon Longitudinal Study, the relationship was demonstrated with fat mass, with special effect of moderate and vigorous physical activity. These studies remind us that during the past half century, the drastic change in food intake that occurred in developed countries was associated with a similarly drastic change in daily physical activities. *Homo sapiens* was selected to be the best endurance runner of all mammals [5]. These new data remind us that we have to pay a tribute to this selection process in the form of regular exercise. From a clinical point of view, it is likely that accelerometers will become a useful tool to evaluate obese patients in the clinical setting.

Awakening from sleep and hypoglycemia in type 1 diabetes mellitus

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Background: Nocturnal hypoglycemia frequently occurs in patients with type 1 diabetes mellitus. It can be fatal and is believed to promote the development of the hypoglycemia unawareness syndrome. Whether hypoglycemia normally provokes awakening from sleep in individuals who do not have diabetes, and whether this awakening response is impaired in type 1 diabetic patients, is unknown.

Methods: Two groups of 16 type 1 diabetic patients and 16 healthy control participants were tested. They had comparable distributions of gender, age, and body mass index. In one night, a linear fall in plasma glucose to nadir levels of 2.2 mmol/l was induced by infusing insulin over a 1-hour period starting as soon as polysomnographic recordings indicated that stage 2 sleep had been reached. In another night (control), euglycemia was maintained.

Results: Only 1 of the 16 type 1 diabetic patients, as compared to 10 healthy control participants, awakened upon hypoglycemia ($p = 0.001$). In the control nights, none of the study participants in either of the two groups awakened during the corresponding time. Awakening during hypoglycemia was associated with increased hormonal counterregulation. In all the study participants (from both groups) who woke up, and in 5 of the study participants who did not awaken (3 type 1 diabetic patients and 2 healthy control participants), plasma epinephrine concentration increased with hypoglycemia by at least 100% ($p < 0.001$). A temporal pattern was revealed such that increases in epinephrine in all participants who awakened started always before polysomnographic signs of wakefulness (7.5 ± 1.6 min).

Conclusions: A fall in plasma glucose to 2.2 mmol/l provokes an awakening response in most healthy control participants, but this response is impaired in type 1 diabetic patients. The counterregulatory increase in plasma epinephrine that we observed to precede awakening suggests that awakening forms part of a central nervous system response launched in parallel with hormonal counterregulation. Failure to awaken increases the risk for type 1 diabetic patients to suffer prolonged and potentially fatal hypoglycemia.

Corticotrophin-releasing factor receptors within the ventromedial hypothalamus regulate hypoglycemia-induced hormonal counterregulation

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Background: Recurrent episodes of hypoglycemia impair sympathoadrenal counterregulatory responses to a subsequent episode of hypoglycemia. For individuals with type 1 diabetes, this markedly increases (by 25-fold) the risk of severe hypoglycemia and is a major limitation to optimal insulin therapy. The mechanisms through which this maladaptive response occurs remain unknown. The corticotrophin-releasing factor (CRF) family of neuropeptides and their receptors (CRFR1 and CRFR2) play a critical role in regulating the neuroendocrine stress response.

Methods: Direct in vivo application to the ventromedial hypothalamus (VMH) of Sprague-Dawley rats, a key glucose-sensing region.

Results: Urocortin I, an endogenous CRFR2 agonist, suppressed (~ 55 – 60%), whereas CRF, a predominantly CRFR1 agonist, amplified (~ 50 – 70%) counterregulatory responses to hypoglycemia. Urocortin I was shown to directly alter the glucose sensitivity of VMH glucose-sensing neurons in whole cell current clamp recordings in brain slices. The suppressive effect of urocortin I-mediated CRFR2 activation persisted for at least 24 h after in vivo VMH microinjection.

Conclusion: The data suggest that regulation of the CRR is largely determined by the interaction between CRFR2-mediated suppression and CRFR1-mediated activation in the VMH.

Hypoglycemia is one of the main limiting factors to metabolic control in diabetic patients. Several decades of clinical research initiated by Phil Cryer who writes a thoughtful editorial with the *JCI* paper of McCrimmon et al. [6] who have evaluated its clinical importance and mechanisms. In the first study selected this year, diabetic patients submitted to a graded nocturnal hypoglycemia did not wake up, as compared to unaffected controls. This was associated with a decreased epinephrine, nor-epinephrine, ACTH and cortisol secretion in response to hypoglycemia in diabetic patients and with altered sleep structure during hypoglycemia. The patients had a wide range of HbA1c and the correlation between awakening response and HbA1c was not evaluated but it is likely that the pattern of defective awakening response to hypoglycemia is more pronounced in those with lower HbA1c (and therefore higher risk of previous hypoglycemia). The second paper tackles the same issue from a very different approach and analyses the influence of hypothalamic CRF signaling on counterregulatory hormones' response to hypoglycemia, hypoglycemia, a topic which we discussed previously [7]. CRF signals through two receptors that play important roles not only in the regulation of the corticotrophic axis but also in the behavioral response to stress. The two types of receptors have specific ligands and can be manipulated separately and allowed to dissect their respective roles in hypoglycemia. Urocortin I, a CRF type 2 receptor agonist, suppressed the response to hypoglycemia with a persistent action. These observations provide a mechanistic substratum for hypoglycemia-associated autonomic failure in type 1 diabetes, by suggesting that hypoglycemia elicits urocortin I release during antecedent hypoglycemia that could explain a decreased sympathoadrenal response to subsequent hypoglycemia. However, that causal connection was not demonstrated and, therefore, remains a possibility.

Clinical trials. The missing hormone: androgens in hypopituitary women

Effects of testosterone therapy on cardiovascular risk markers in androgen-deficient women with hypopituitarism

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Background: Hypopituitarism in women is characterized by profound androgen deficiency due to a loss of adrenal and/or ovarian function. The effects of testosterone replacement in this population have not been reported. The objective of the study was to determine whether physiologic testosterone replacement improves bone density, body composition, and/or neurobehavioral function in women with severe androgen deficiency secondary to hypopituitarism.

Methods: This was a 12-month randomized, placebo-controlled study, conducted at a general clinical research center. 51 women of reproductive age with androgen deficiency due to hypopituitarism participated. Physiologic testosterone administration using a patch that delivers 300 µg daily or placebo was administered. Bone density, fat-free mass, and fat mass were measured by dual x-ray absorptiometry. Thigh muscle and abdominal cross-sectional area were measured by computed tomography scan. Mood, sexual function, quality of life, and cognitive function were assessed using self-administered questionnaires.

Results: Mean free testosterone increased into the normal range during testosterone administration. Mean hip ($p = 0.023$) and radius ($p = 0.007$), but not posteroanterior spine, bone mineral density increased in the group receiving testosterone, compared with placebo, as did mean fat-free mass ($p = 0.040$) and thigh muscle area ($p = 0.038$), but there was no change in fat mass. Mood ($p = 0.029$) and sexual function ($p = 0.044$) improved, as did some aspects of quality of life, but not cognitive function. Testosterone at physiologic replacement levels was well tolerated, with few side effects.

Conclusions: This is the first randomized, double-blind, placebo-controlled study to show a positive effect of testosterone on bone density, body composition, and neurobehavioral function in women with severe androgen deficiency due to hypopituitarism.

Clinical endocrinology is based on the principle of exogenously restoring a defective hormonal milieu and all clinical endocrinologists know that this is better said than done. Hypopituitarism is one of the most challenging endocrine disorders due to the multiplicity of interacting hormonal systems that need replacement. Androgen deficiency in women has been associated with decreased bone mass, lean mass and sexual function. This is the first study to address the question in women with hypopituitarism and confirms a positive influence of testosterone on bone, mood, quality of life and sexual function. With a dose of 300 μ g daily, the treatment was well tolerated and did not induce androgenic signs. A similar study was published also this year with similar findings using DHEA which is converted to testosterone [8] and an ESPE 2007 abstract suggests a beneficial effect of testosterone in Turner syndrome [9]. These studies performed in adult women raise the question of the replacement of adrenarche and of testosterone secretion during pubertal development in female. Innovative clinical trials with relevant endpoint should be organized to address these questions in adolescents with hypopituitarism. A recent consensus summary suggested that androgen therapy in women remains investigational [10].

New concerns

Growth hormone treatment and risk of second neoplasms in the childhood cancer survivor

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Background: GH deficiency is common in childhood cancer survivors. In a previous report, although no increase in the risk of disease recurrence in survivors treated with GH was detected, GH-treated survivors did have an increased risk of developing a second neoplasm (rate ratio 3.21).

Methods: In this analysis, the risk of GH-treated survivors developing a second neoplasm after an additional 32 months of follow-up was reassessed. This was a retrospective cohort multicenter study. Among a total of 14,108 survivors who were enrolled in the Childhood Cancer Survivor Study, a retrospective cohort of 5-year survivors of childhood cancer, 361 who were treated with GH were identified. The risk of developing a second neoplasm was assessed.

Results: During the extended follow-up, 5 new second neoplasms developed in survivors treated with GH, for a total of 20 second neoplasms, all solid tumors. Using a time-dependent Cox model, the rate ratio of GH-treated survivors developing a second neoplasm, compared with non-GH-treated survivors, was 2.15 (95% confidence interval, 1.3–3.5; $p = 0.002$). Meningiomas were the most common second neoplasm ($n = 9$) among the GH-treated group.

Conclusion: Although cancer survivors treated with GH appear to have an increased risk of developing a second neoplasm compared with survivors not so treated, the elevation of risk due to GH use appears to diminish with increasing length of follow-up. Continued surveillance is essential.

The question of long-term risks of any chronic treatment is always very difficult to address given the fact that small increases of rare adverse events are very difficult to detect. Several reviews have been written on GH treatment and the risk of cancer including the very thoughtful paper by the team of Steve Shalet [11]. Cancer survivors can become GH-deficient as a result of their neoplasm or its treatment raising the question whether GH treatment increases the risk of a second neoplasm in this population. Cancer survivors are a particularly difficult group where to assess this risk, given that, as a group, they are at increased risk of developing a second neoplasm. Large observational databases comprising GH-exposed and non-exposed individuals can help answer this question, although multivariate analysis after adjustment on covariates always raises the question of undetected confounding factors. The very powerful Childhood Cancer Survivor Study comprises $\approx 14,000$ patients of whom 361 had received GH. After adjustment on covariates, a significant 2.1-fold increase of the risk of developing a second neoplasm was detected in GH-treated patients. A second neoplasm occurred in 20/361 GH-treated patients, on average 6.3 ± 4.1 years after initiation of GH treatment. Meningiomas,

associated with radiotherapy, were the most common second neoplasm. These worrisome data raise several important points. First they call for an assessment of the risk-benefit balance in this population of patients, who often have severe GH deficiency, with its intrinsic morbidity. Second they put the pediatric endocrinologist in a difficult position where detailed information is needed on a particularly sensitive issue in families who have already gone through the ordeal of oncology treatments. Last, they need to be replicated, to have a more definitive answer on this particularly sensitive issue.

New mechanisms: Gnas regulation unraveled

Identification of an imprinting control region affecting the expression of all transcripts in the Gnas cluster

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Background: Genomic imprinting results in allele-specific silencing according to parental origin. Silencing is brought about by imprinting control regions that are differentially marked in gametogenesis. The group of imprinted transcripts in the mouse Gnas cluster (Nesp, Nespas, Gnasxl, Exon 1A and Gnas) provides a model for analyzing the mechanisms of imprint regulation. The authors have previously identified an imprinting control region that specifically regulates the tissue-specific imprinted expression of the Gnas gene.

Results: A second imprinting control region at the Gnas cluster is identified here. A paternally derived targeted deletion of the germline differentially methylated region (DMR) associated with the antisense Nespas transcript unexpectedly affects both the expression of all transcripts in the cluster and methylation of two imprinting control regions.

Conclusions: These results establish that the Nespas DMR is the principal imprinting control region at the Gnas cluster and functions bidirectionally as a switch for modulating expression of the antagonistically acting genes Gnasxl and Gnas. Uniquely, the Nespas DMR acts on the downstream imprinting control region at exon 1A to regulate tissue-specific imprinting of the Gnas gene.

The Gnas gene is of interest to pediatric endocrinologists for its involvement in several diseases including pseudohypoparathyroidism type Ia and Ib, acromegaly and McCune-Albright syndrome. Old textbooks clearly had trouble defining the mode of transmission of pseudohypoparathyroidism quoting it as dominant, recessive or sex-linked. Indeed, Mendel had not foreseen imprinting mechanisms and we now know that the Gnas gene is imprinted and that parental origin affects the clinical expression of these diseases. However, the mechanisms behind the differential expression of paternal and maternal alleles of this particularly complex gene are still being worked up. Here, in a mouse model, several lines were created with deletions of promoters within the Gnas gene, associated with point mutations in the Gnas coding sequence, similar to those of pseudohypoparathyroidism type Ia. An imprinting control region is identified that affects the methylation of other control regions in the gene and the expression of key transcripts from the gene. Deletion of this imprinting control region rescues the phenotype due to a point mutation in the Gnas transcript (a therapeutic approach for pseudohypoparathyroidism type Ia?). Importantly, this imprinting control region behaves in accordance with the parental-conflict theory where it is the interest of paternal genes in the offspring to acquire resources from the mother and the interest of maternal genes to spare these resources. As Gnas transcript Xl α is involved in the postnatal adaptation to breast feeding [12], it is of the 'interest' of the father to promote its expression. Since Xl α and Gs α act antagonistically, it is also of the 'interest' of the father to repress Gnas. Altogether, the complex puzzle of the regulation of the Gnas gene is progressively solved and shows the complexity of gene regulation and its implication in human diseases.

A single IGF1 allele is a major determinant of small size in dogs

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The domestic dog exhibits greater diversity in body size than any other terrestrial vertebrate. We used a strategy that exploits the breed structure of dogs to investigate the genetic basis of size. First, through a genome-wide scan, we identified a major quantitative trait locus (QTL) on chromosome 15 influencing size variation within a single breed. Second, we examined genetic variation in the 15-megabase interval surrounding the QTL in small and giant breeds and found marked evidence for a selective sweep spanning a single gene (*IGF1*), encoding insulin-like growth factor 1. A single *IGF1* single-nucleotide polymorphism haplotype is common to all small breeds and nearly absent from giant breeds, suggesting that the same causal sequence variant is a major contributor to body size in all small dogs.

The evolution of dogs is mostly man-made, and the vast majority of dog breeds originate from enforced breeding over the past few hundred years. This resulted in size variation in the domestic dog that is extreme and surpasses that of most other living and extinct species. These authors show that sequence variation in the *IGF-1* gene plays a causal role in dog size: a single allele of the (*IGF-1*) gene is shared by all small dog breeds that have significantly lower serum levels of IGF-1, but is nearly absent from giant dog breeds, which carry one or both of two distinct haplotypes. This explanation is different from the dogma of phenotypic diversity due to elevated mutation or recombination rates. Rather, intense artificial selection has left a signature in the proximity of IGF-1. The ability to identify a gene contributing to morphology without doing a genetic cross, provides a precedent for future studies aimed at identifying the genetic basis for complex traits in dogs and other species with small populations that have experienced strong artificial or natural selection.

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