Growth and Growth Factors

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Whereas in previous years many studies were focused on new potential indications for GH therapy, in the last 12 months, the attention of researchers has been focusing on possible long-term adverse effects of such treatment. Two preliminary reports from the SAGhE European study, which represents the first effort to assess long-term safety in children receiving GH, have been published generating concern and controversy as they apparently lead to opposite conclusions. The publication of the final and comprehensive results of this study is needed before drawing any conclusions. Another interesting novelty is the evidence for a role of IGF-II in postnatal metabolism. IGF-II has always been associated with brain and pancreas development in fetus and cancer growth in postnatal life, now it seems to be living a second life as a metabolic regulator. Finally, in basic science research, the attention has been focusing on the interplay between GH receptor signaling in specific organs and whole-body glucose metabolism. Although the results have been obtained in animal models carrying selective gene deletions, potential implications for a better understanding of physiological factors regulating glucose homeostasis and for therapeutic fallout can be envisaged.

New Concerns

Meta-analysis and dose-response meta-regression: circulating insulin-like growth factor I (IGF-I) and mortality

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Background: Epidemiological studies have shown that circulating IGF-I concentrations are positively associated with cancer risk and inversely with risk of cardiovascular disease. The aim of this study was to perform a meta-analysis and meta-regression of population-based reports to quantify the robustness of these relationships.

Methods: The search was performed in PubMed, EMBASE, Web of Science, and Cochrane Library from 1985 to September 2010 to identify the population-based cohort studies and (nested) case-control studies reporting on the relation between circulating IGF-I and mortality.

Results: Twelve studies, with 14,906 participants, were selected. All-cause mortality was significantly increased in subjects with low as well as high IGF-I, with a hazard ratio (HR) of 1.27 (95% CI 1.08–1.49) and HR of 1.18 (95% CI 1.04–1.34), respectively. Dose-response meta-regression showed a U-shaped relation of IGF-I and all-cause mortality ($p = 0.003$). The predicted HR for the increase in mortality comparing the 10th with the 50th percentile was 1.56 (95% CI 1.31–1.86); the predicted HR comparing the 90th with the 50th percentile was 1.29 (95% CI 1.06–1.58). Four studies also reported the association of IGFBP-3 levels with all-cause mortality. This meta-analysis showed a higher mortality for subjects in the lowest category of IGFBP-3 compared with the middle category with a HR of 1.40 (95% CI 1.17–1.68).

Conclusions: IGF-I and IGFBP-3 are associated with mortality. Both low and high IGF-I concentrations are associated with increased cancer and cardiovascular disease mortality, whereas only low IGFBP-3 is associated with increased mortality.

The IGFs are increasingly recognized to be important growth factors in many tumor types and virtually all human cancer cells express IGF receptors and produce IGFs, IGFBPs, and IGFBP proteases. Type I IGF receptor is actively involved in the control of cell growth and differentiation, and mutations of IGF-I receptor result in a tumorigenic or mitogenic effect of IGF-I [1]. Preliminary short-term clinical trials aimed at testing the efficacy of anti-IGF-IR monoclonal antibodies in patients with sarcomas have shown

promising results [2, 3]. In the 1990s, a series of papers based on data from prospective cohorts showed that circulating IGF-I levels were positively correlated with the risk of many types of cancers, including colorectal, breast and prostate cancer, whereas circulating IGFBP-3 concentrations were inversely correlated with the risk of these cancers. These observations were consistent with the mitogenic and anti-apoptotic effects of IGF-I and the proapoptotic effects of IGFBP-3. A recent review summarizes the evidence from epidemiological studies that support the association between raised circulating levels of IGF-I and the risk of certain cancers [4]. We used to think of high IGF1 as a risk factor for cancer and low IGF1 – for cardiovascular morbidity. The current study reveals, for the first time, a U-shaped curve relating IGF-I levels to both cancer and cardiovascular disease mortality. According to this model, low as well as high IGF-I concentrations would be associated with increased mortality risk. Transferring this finding to clinical practice, the goal of GH replacement therapy should be to maintain the IGF-I levels around the average. However, we should be cautious in interpreting these results as they could be affected by different IGF-I and IGFBP-3 assays, by confounding factors such as nutritional status, and by genetic background which alone may account for up to 40% of the variability of IGF-I concentrations.

Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study

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Background: Few independent studies have addressed the issue of long-term safety of GH therapy in childhood. The aim of this study, which represents the first report from the SAGhE (Safety and Appropriateness of Growth hormone treatments in Europe) project, was to determine the long-term mortality of patients treated with recombinant GH in childhood in France.

Methods: This was a population-based cohort study. 6,928 children with idiopathic isolated GH deficiency ($n = 5,162$), neurosecretory dysfunction ($n = 534$), idiopathic short stature ($n = 871$), or born short for gestational age ($n = 335$) who started treatment between 1985 and 1996 were enrolled in the study. Follow-up data on vital status were available in September 2009 for 94.7% of the patients.

Results: All-cause mortality was increased in treated subjects (standardized mortality ratio (SMR) 1.33, 95% confidence interval (CI) 1.08–1.64). The use of GH doses >50 $\mu\text{g/kg/day}$ was associated with increased mortality rates (SMR 2.94, 95% CI 1.22–7.07, hazard ratio 2.79, 95% CI 1.14–6.82). Whereas all type cancer-related mortality was not increased, bone tumor-related mortality resulted in being significantly increased (SMR 5.00, 95% CI 1.01–14.63). Data analysis also showed an increase in mortality due to cardiovascular disease (SMR 3.07, 95% CI 1.40–5.83) and cerebrovascular events (SMR 6.66, 95% CI 1.79–17.05).

Conclusions: Subjects treated with GH during childhood showed increased mortality rate which was associated with the use of higher GH doses. Bone tumors and cerebrovascular diseases resulted to be the main causes of death. These results highlight the need for further studies on long-term morbidity and mortality after GH treatment in childhood.

Long-term mortality and causes of death in isolated GHD, ISS, and SGA patients treated with recombinant growth hormone during childhood in Belgium, the Netherlands, and Sweden: preliminary report of three countries participating in the EU SAGhE study

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Background: The preliminary data from SAGhE (Safety and Appropriateness of Growth hormone treatments in Europe) consortium, released from the French Unit, have shown an increased mortality rate in

adults treated with GH during childhood. The aim of this second paper from SAGhE consortium was to report the preliminary data on long-term mortality in the same diagnostic groups of the French study, in Belgium, the Netherlands, and Sweden.

Methods: Data were retrieved from national registries of GH-treated patients and vital status from National Population Registries. Causes of death were retrieved from a National Cause of Death Register (Sweden), Federal and Regional Death Registries (Belgium), or individual patient records (the Netherlands). Patients with isolated GH deficiency or idiopathic short stature or born small for gestational age, being started on recombinant GH treatment during childhood between 1985 and 1997, irrespective of treatment duration, and who had attained 18 years of age by the end of year 2009 (the Netherlands) or the end of year 2010 (Belgium and Sweden) were included. Vital status was available for approximately 98% of these 2,543 patients, corresponding to 46,556 person-years of observation. The standardized mortality ratios were not calculated.

Results: 21 deaths were identified, the majority being caused by accidents and suicides (76%). Not a single case of death caused by cancer or cardiovascular or cerebrovascular diseases was observed.

Conclusions: These results, strikingly in contrast with the French data, highlight the need of exercising extreme caution in drawing conclusions based on preliminary data from a single country and the importance to wait for the final results of the whole EU SAGhE consortium.

After many years of the industry dominance as gatekeepers of GH therapy, we now have two reports generated by public funding. Both Carel's and Säwendahl's reports stem from the same EU SAGhE project which was started in 2009 in eight countries (France, Sweden, Belgium, the Netherlands, Switzerland, Germany, United Kingdom and Italy). The French study began 3 years before the European consortium and that is the reason why they could finish data collection and analysis earlier than the other countries. Sweden, Belgium and the Netherlands, taking advantage of the existence of national registries of GH-treated patients, could achieve the endpoint of mortality rates well in advance the end of the project which is scheduled for November 2012. The fact that these two preliminary reports show opposite results in the long-term mortality of subjects treated with GH during childhood is both interesting and puzzling. Whereas the French data raise concern on the risk of cancer and cerebrovascular diseases, in the other three countries not a single case of death from cancer or cerebrovascular disease was observed. It is hard to prove whether differences in genetics, environment, or, more simply, confounding factors might account for this discrepancy. This kind of study is certainly worthwhile and these two preliminary reports stress the importance to replicate these surveys in different contexts. An editorial by Rosenfeld et al. [5] underscores the need of a joint effort involving all the stakeholders (physicians, regulatory agencies, pharmaceutical companies, patients and their families), to invest resources and expertise in setting up an international task force aimed at addressing the still controversial issue of long-term GH safety.

New Paradigms

Insulin-like growth factor 2/H19 methylation at birth and risk of overweight and obesity in children

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Background: Childhood obesity may be the expression of an early adaptive response which can be driven by epigenetic mechanisms driving the expression of genes involved in energy balance, ultimately leading to overweight and obesity. Higher circulating insulin-like growth factor 2 (IGF2) protein levels have been associated with obesity. The aim of this study was to determine whether altered methylation in differentially methylated regions (DMRs) regulating IGF2 expression at birth, is associated with the risk of overweight or obesity in early childhood.

Methods: Methylation analyses were performed in cord blood leukocytes of 204 newborns by using pyrosequencing. Anthropometric and feeding data were collected at age 1 year. Methylation differences were compared between children >85th percentile and children ≤85th percentile of weight-for-age at 1 year by adjusting for postnatal caloric intake, maternal cigarette smoking, and race/ethnicity.

Results: The methylation percentages at the H19 imprint center DMR was higher in infants with weight >85th percentile (62.7%; 95% CI 59.9–65.5%) than in infants with weight ≤85th percentile (59.3%; 95% CI 58.2–60.3%; $p = 0.02$).

Conclusions: The finding that children who were overweight or obese at age 1 year had higher methylation percentages at the H19 DMR at birth suggests that IGF2 methylation status may play a key role in the development of overweight or obesity in early childhood, and may represent a useful marker of obesity risk.

Epigenetic mechanisms are commonly associated with gene silencing, genomic imprinting and transcriptional regulation of tissue-specific gene expression during cellular differentiation. In humans, evidence that epigenetic changes predispose the organism to type 2 diabetes stems among others from studies on individuals who were prenatally exposed to famine during the Dutch Hunger Winter of 1944–45 that showed a higher risk for obesity and obesity-related chronic disease [6]. These individuals were at higher risk for cardiovascular and metabolic diseases in adulthood [7], and periconceptional exposure to famine was associated with lower methylation of the IGF-II gene six decades later [8]. In the present study, for the first time, an association between IGF2 methylation and risk of obesity has been observed in early childhood. Interestingly, the authors report that breastfeeding modified the magnitude of IGF2 methylation, thus suggesting an interplay between prenatal environment (nutrient transfer from mother to fetus) and early postnatal feeding behavior which could stabilize or change the epigenetic patterns acquired in utero. Although cause-and-effect could not be established in this epidemiological study, these results warrant further investigations aimed at clarifying the role of IGF2 epigenetic changes in obesity and its metabolic consequences. Methylation patterns may represent early markers of long-term metabolic risk, offering the possibility to identify high-risk individuals thus driving early targeted interventions.

Associations between paternally transmitted fetal IGF2 variants and maternal circulating glucose concentrations in pregnancy

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Background: Genomic variation in the fetal genome, in particular in fetal growth genes, could affect maternal metabolism in pregnancy and alter the mother's risk of developing gestational diabetes mellitus. The aim of this study was to determine whether polymorphisms in the paternally transmitted fetal IGF2 gene was associated with maternal glucose concentrations in the third trimester of pregnancy.

Methods: A total of 17 single nucleotide polymorphisms (SNPs) in the IGF2 gene region were genotyped in 1,160 mother/partner/offspring trios from the prospective Cambridge Baby Growth Study ($n = 845$ trios) and the retrospective Cambridge Wellbeing Study ($n = 315$ trios) (3,480 samples in total). Associations were tested between the inferred parent-of-origin fetal alleles, Z scores of maternal glucose concentrations 60 min after an oral glucose load performed at weeks 27–29 of pregnancy, and offspring birth weights.

Results: Paternally transmitted fetal rs6578987, rs680, rs10770125, and rs7924316 alleles were associated with increased maternal glucose concentrations in the third trimester of pregnancy and placental IGF-II contents at birth ($p = 0.03$). In contrast, no association was observed between maternally transmitted fetal IGF2 genotypes and maternal glucose concentrations.

Conclusions: The association between polymorphic variation in a paternally transmitted fetal gene, namely, IGF2, and maternal glucose concentrations in pregnancy is described for the first time. These findings support the hypothesis that variations in fetal imprinted genes regulate the maternal availability of nutrients.

Pregnant mice carrying pups with targeted disruption of 13 kb genetic region, including the imprinted H19 gene and IGF2 control element, show increased circulating glucose concentrations in late pregnancy [9]. This finding prompted the authors to test the hypothesis that functional variation in the

fetal IGF2 gene alters maternal glucose concentrations in human pregnancy. The results of the present study demonstrate that specific polymorphisms in paternally imprinted fetal IGF2 are associated with increased maternal glucose concentrations in pregnancy and could thus affect the risk of gestational diabetes in the mother. These data are consistent with SRS and BWS imprinting findings and with Haig's parental conflict hypothesis, which suggests that paternally expressed fetal imprinted genes will tend to increase fetal growth, whereas maternally expressed genes will tend to restrain it [10]. These effects are probably achieved through modifications in fetal and placental nutritional demand and supply, and alterations in maternal glucose concentrations. However, no association between any of the IGF2 fetal variants and offspring birth weight was observed. The authors speculate that probably the effect sizes on maternal glucose concentrations were too small to make a detectable difference in birth weight. Important limitations of this study are the lack of paternity testing and the unavailability of maternal BMI in the vast majority of the study cohort. Nevertheless, this report represents a major breakthrough in prenatal programming for adult health and disease research, showing that mother-fetus interaction is bidirectional, the developing host influencing maternal metabolism according to paternally transmitted genetic information.

Higher levels of IGF-I and adrenal androgens at age 8 years are associated with earlier age at menarche in girls

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Background: Earlier age at menarche has been associated with low birth weight, rapid weight gain during infancy, and childhood obesity. A role of intrauterine programming has been suggested but the endocrine mechanisms underlying these associations are still unclear. The aim of this study was to relate hormone levels at age 8 years with the timing of menarche.

Methods: A total of 329 girls from the Avon Longitudinal Study of Parents and Children (ALSPAC) prospective study provided blood samples at mean age 8.1 years (range 8.0–8.5) for hormone measurements, and were followed longitudinally to establish age at menarche. Age at menarche was reported by questionnaire and categorized as before 12.0, 12.0–13.0, or later than 13 years.

Results: Earlier menarche was associated with greater body weight, height, and body mass index at age 8 years. Among the endocrine variables, after adjustment for body mass index and height only IGF-I, androstenedione, and DHEAS remained significantly associated with earlier menarche.

Conclusions: These findings support direct roles of IGF-I and adrenal androgens on the regulation of pubertal maturation.

This study confirms earlier evidence that body size closely relates to the timing of pubertal development; girls with greater weight, height, BMI and waist circumference undergoing earlier puberty. This finding is consistent with a direct role of childhood obesity on the secular trends in pubertal timing previously suggested by the same research team [11]. An original finding is that IGF-I levels are an independent predictor of early menarche, suggesting a direct role of IGF-I in controlling pubertal timing in humans [12]. IGF-I may, in fact, act at multiple sites such as hypothalamus, pituitary, and ovaries to modulate the reproductive system [13]. The association between the concentrations of adrenal androgens and the timing of menarche is less surprising since adrenarche is a well-known preliminary stage heralding the onset of puberty. Low birth weight and rapid weight gain in infancy are associated with increased levels of IGF-I and adrenal androgens, suggesting that these hormones may mediate the effects exerted by intrauterine and early postnatal programming on childhood growth and pubertal development.

Three-year efficacy and safety of LB03002, a once-weekly sustained-release growth hormone (GH) preparation, in prepubertal children with GH deficiency (GHD)

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Background: The availability of a GH depot preparation might considerably improve the management of children on GH therapy. LB03002 is a novel once-weekly sustained-release formulation of recombinant human GH (rhGH) that could potentially offer patients and caregivers a suitable alternative to daily subcutaneous injections. The aim of this study was to explore the optimal LB03002 dose to stimulate long-term longitudinal growth and to establish the long-term safety profile of the compound.

Methods: GH-naïve prepubertal GH-deficient children were randomized to four groups who received: (1) 0.2 mg/kg/week LB03002 for 12 months, followed by 0.5 mg/kg/week for another 24 months ($n = 13$); (2) 0.5 mg/kg/week LB03002 for 36 months ($n = 13$); (3) 0.7 mg/kg/week LB03002 for 12 months, followed by 0.5 mg/kg/week for another 24 months ($n = 13$), or (4) conventional therapy with daily GH 0.03 mg/kg/day for 24 months, switched to 0.5 mg/kg/week LB03002 for 12 months ($n = 12$). Height measurements were taken at baseline and after 6, 12, 24, and 36 months, and height and height velocity were calculated.

Results: During the first 12 months of treatment, catch-up growth was observed in all treatment groups, with a dose-dependent pattern of response in the three LB03002 groups. Whereas patients treated with the lower dose of LB03002 showed a reduced growth response in comparison with those receiving daily GH injections, the growth responses for the 0.5 and 0.7 mg/kg/week LB03002 groups were comparable to the daily GH group during the first year. There were no significant differences in height SD score gain between any groups at 24 and 36 months. Bone maturation and IGF-I levels did not differ for any LB03002 dose compared with daily GH. No significant change in mean fasting glucose and glycosylated hemoglobin concentrations was observed in any treatment group at any time.

Conclusions: LB03002 treatment of prepubertal GHD children for up to 3 years resulted in an efficacy and safety profile that did not differ from that of conventional daily GH regimen. 0.5 mg/kg/week appeared to be the optimal dose for long-term treatment.

Long-term GH therapy in children is currently based on daily subcutaneous injections, which makes it cumbersome for patients and their caregivers, leading to a reduced adherence to treatment and potentially affecting the long-term growth response. Pharmaceutical research in this field has been focusing on the development of GH depot preparations to be given once or twice weekly. A previous depot GH preparation tested over a period of 1 year did not provide satisfactory results in comparison with the conventional daily therapy [14]. LB03002 is a novel once-weekly sustained-release formulation of recombinant human GH (rhGH), manufactured using the yeast *Saccharomyces cerevisiae* as the expression system, contained in sodium hyaluronate microparticles, which are suspended in medium-chain triglycerides before injection. This is the first study reporting on the efficacy and safety of LB03002 administered once per week over a period of 3 years. These preliminary data look promising, showing a growth response and safety profile comparable to conventional daily therapy. However, this study was designed as a dose-finding study, and thus, the overall number of treated patients was small, and follow-up limited to 36 months. Therefore, data on adult height and longer safety assessment are needed before drawing any conclusions.

Beneficial effects of growth hormone treatment on cognition in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study

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Background: Data on GH effect on mental development are few and conflicting. GH therapy has been suggested to improve mental development in conditions associated with mild or severe cognitive impairment such as children born small for gestational age or with Down syndrome. Short-term GH treatment significantly improved mental and motor development in infants with Prader-Willi syndrome (PWS), compared with randomized controls. The aim of this study was to investigate the effect of GH treatment on cognitive functioning in children with PWS followed over a period of 4 years.

Methods: 50 prepubertal children with PWS aged 3.5–14 years were studied in a randomized controlled GH trial. Cognitive functioning was measured biennially by short forms of the WPPSI-R or WISC-R, depending on age. Total IQ (TIQ) score was estimated based on two subtest scores.

Results: Whereas in GH-treated children mean SD scores of all subtests and mean TIQ score remained similar compared to baseline, in untreated controls mean subtest SD scores and mean TIQ score decreased with time. This decline was significant for the Similarities and Vocabulary subtests. After 4 years of GH treatment, mean SD scores on the Similarities and Block design subtests were significantly higher than at baseline.

Conclusions: GH treatment prevents deterioration of certain cognitive skills in children with PWS and improves abstract reasoning and visuospatial skills during 4 years of GH treatment. Children with a more severe mental impairment at baseline had more benefit from GH treatment.

The issue of a potential beneficial effect of GH therapy on mental development is under debate as the available data are conflicting [15]. The authors previously reported an improvement of mental and motor performance in infants with PWS undergoing 12 months of GH therapy [16]. In this study they show that children with PWS undergo a spontaneous deterioration of cognitive skills, which can be partially prevented by GH treatment. Another interesting finding was that children with a maternal uniparental disomy had a significantly lower score on the Block design subtest but a better response during GH treatment than children with a mutation, suggesting that the more is the mental impairment at baseline, the more is the benefit induced by GH treatment. It is noteworthy that these effects of GH therapy were independent of IGF-I circulating levels, suggesting either a paracrine/autocrine IGF-I action or a direct GH effect on the brain [17]. If these results will be confirmed by other trials in larger cohorts of patients, the goals of GH treatment in children with PWS will comprise not only the normalization of height and the improvement of body composition, but also the prevention of mental deterioration. However, these findings and conclusions should be taken with caution. The average age of children with PWS treated with GH was 7.4 years and, although the authors quote a review reporting an effect of GH on adult brain, it seems unlikely that the GH action on a mature brain may be so relevant to have an impact on function. The other explanation provided for explaining the improved cognitive skills during GH treatment was the improvement of sleep apnea in children with PWS induced by therapy. A significant positive effect of GH on sleep apnea has never been proved. On the contrary, GH therapy has been related to tonsillar and adenoid hypertrophy and consequent worsening of obstructive apnea [18].

Three-year growth hormone treatment in short children with X-linked hypophosphatemic rickets: effects on linear growth and body disproportion

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Background: Despite adequate replacement therapy, children with X-linked hypophosphatemic rickets (XLH) fail to achieve a normal adult height. They show progressive disproportional stunting characterized by reduced leg growth associated with normal trunk growth. Preliminary studies showed that GH therapy increases height velocity in short XLH patients during short-term trials. However, GH was also suggested to worsen preexisting body disproportions. The aim of this study was to determine the effects of 3 years of GH treatment on stature and body proportions in short children with XLH.

Methods: The study design was a 3-year randomized controlled open-label GH study in short prepubertal children with XLH (n = 16) on phosphate and calcitriol treatment. 76 XLH patients on conservative treatment represented the control group. Changes in SD scores (SDS) of stature and linear body segments were the main outcome measures.

Results: Growth of XLH patients at time of enrollment was severely impaired (–3.3 SDS), leg length (–3.8 SDS) being particularly affected by disease, whereas sitting height (–1.7 SDS) was preserved. GH therapy was effective in inducing a sustained increase in linear growth (stature +1.1 SDS; sitting height +1.3 SDS; leg length +0.8 SDS; arm length +1.1 SDS; each p < 0.05 vs. baseline). However, mean height SDS at the end of the 3-year trial did not significantly differ between treated and control group. Sitting height index remained stable in both the GH-treated patients and in study controls but increased further in the XLH-reference population. During the 3-year observation period, bone age tended to increase faster in GH-treated patients compared with controls.

Conclusions: Three-year GH treatment in short children with XLH resulted partly effective in improving linear growth without progression of body disproportion.

GH therapy has been proposed in various conditions associated with short stature even when GH secretion is not impaired. If proved beneficial XLH might represent a further indication for a pharmacologic rather than replacement treatment, with all the precautions discussed above about GH and IGF-1 potential untoward effects in the long run. Children with XLH, though undergoing specific therapy with large doses of oral phosphate and calcitriol, fail to achieve normal adult height and show progressive disproportional stunting mainly due to a continuously diminished leg growth during the prepubertal growth period [19]. Previous reports have shown that GH treatment increases growth velocity mildly and has a small effect on adult height in short XLH patients [20, 21], although some studies indicated that GH might aggravate the preexisting body disproportion in XLH patients [22]. The results of these preliminary reports looked promising though only few patients were enrolled in nonrandomized trials. The present study conducted over a period of 3 years on a relatively large cohort of XLH patients still offers conflicting results showing an acceleration of growth, with no detrimental effect on body proportions, but at the same time, an acceleration of bone maturation in GH-treated subjects. At the end of the treatment period, no significant difference in height between GH-treated and untreated children was observed. Let us wait until the completion of growth in these patients before we prescribe GH for unproven indications. Most of all, no long-term results also means no evidence for safety.

Light stimuli control neuronal migration by altering of insulin-like growth factor 1 (IGF-1) signaling

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Background: Environmental cues, such as temperature and light, intervene in brain development by controlling neuronal proliferation and migration. The aim of this study was to determine the role of light stimuli in neuronal cell migration.

Methods: Newborn mice were exposed to a standard light-dark cycle and the migration of cerebellar granule cells in the external granular layer (EGL) was determined at postnatal day 10. Serum and cerebellum IGF-I levels were assessed by ELISA. Microexplant cultures of postnatal day 3 (P3) mouse cerebella were used to test the effect of IGF-I on cell migration. The activity of IGF-1 receptors was specifically inhibited by the use of picropodophyllin (PPP).

Results: Granule cells migrated faster during light cycles and more slowly during dark cycles in early postnatal mouse cerebella. Cerebellar and serum IGF-1 levels of P10 mice depended on light stimuli: IGF-1 levels being high during light cycles, when granule cells migrated faster, and low during dark cycles, when granule cells migrated more slowly. The addition of IGF-I to the culture medium of mouse cerebella stimulated migration in a dose-dependent manner by enhancing PI3K- and PKC-related signaling pathways through the activation of IGF-1 receptors. The inhibition of IGF-1 receptors decelerated granule cell migration during light cycles (high IGF-1 levels) but did not alter migration during dark cycles (low IGF-1 levels).

Conclusions: These results suggest that during early postnatal development, light stimuli control granule cell migration by altering the activity of IGF-1 receptors through modification of cerebellar IGF-1 levels.

The mechanisms underlying the link between environmental stimuli and brain development are largely unknown. This study for the first time elegantly demonstrates that cerebellum development, particularly the migration of cerebellar granule cells in early postnatal life, is regulated by light stimuli. Even more interestingly, light acts through modulation of local (cerebellar) IGF-I levels; thus IGF-I representing the final mediator of light effects on early cerebellum maturation. Circulating IGF-1 crosses the blood-brain barrier easily [23], but is also synthesized in the developing cerebellum [24]. The potential clinical implications of these findings are the following: (1) our ancestors raised their infants in the open, while we hide them in semi-dark rooms. Alterations in the exposure of human babies to light stimuli may affect granule cell migration and may have long-lasting consequences on brain development and function later in life, because the cell density and dendrite length of granule cells are significantly reduced at an adult age in the cerebellum of mice exposed to altered light stimuli during early postnatal development. (2) IGF-I, a well-known neurotrophic factor, plays a key role in mediating the light-induced brain maturation and may represent a molecule effective in restoring normal development of cerebellum in babies at high risk of cerebellar damage.

Noonan syndrome-causing SHP2 mutants inhibit insulin-like growth factor 1 release via growth hormone-induced ERK hyperactivation, which contributes to short stature

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Background: Noonan syndrome (NS), a genetic disease clinically characterized by heart defects, facial dysmorphism and short stature. Half of the patients with NS carry activating mutations of the tyrosine

phosphatase SHP2 (PTPN11) but the mechanism through which SHP2 induces growth retardation is still undefined. The aim of this study was to explore how SHP2 mutations may alter GH response.

Methods: A mouse model of NS expressing the D61G mutant of SHP2 and different cellular approaches were used.

Results: Early postnatal growth retardation in mutant mice was associated with reduced IGF-I and IGFBP-3 circulating levels. The experiments in cell lines revealed a negative effect of SHP2 on GH-induced IGF-I production whereas a positive effect of SHP2 on GH-evoked ERK1/2 phosphorylation was observed. Furthermore, SHP2 cooperated in GH-induced RAS activation by dephosphorylation of the adaptor Grb2-associated binder-1 (GAB1) on its RAS GTPase-activating protein (RASGAP) binding sites. NS mutant mice showed increased ERK1/2 phosphorylation upon GH treatment. Finally, treatment with a specific inhibitor of ERK1/2 restored a normal GH-induced IGF-I production and partially rescued growth retardation.

Conclusions: SHP2 mutants show inhibition of GH-induced IGF-1 release through RAS/ERK1/2 hyperactivation, a mechanism that could ultimately lead to growth failure. These findings open avenue for potential treatments based on the modulation of RAS/ERK1/2 in NS caused by PTPN11 mutations.

Clinical data show that children with NS carrying PTPN11 (SHP2) mutations display reduced IGF-I levels [25]. The SHP2 mutant mice used in this study showed similar growth phenotype, characterized by early onset of growth retardation and reduction of both IGF-I and IGFBP-3 levels. Interestingly, whereas liver-selective *IGF1* gene disruption, determining a 75% reduction of circulating IGF-I, was not associated with growth failure [26], the 40% reduction of IGF-I concentrations in NS mice was accompanied by severe growth retardation, suggesting a concomitant impairment of local IGF-I autocrine/paracrine actions, particularly in bone and cartilage. The merit of this study was to elucidate the mechanism underlying the association of SHP2 mutation and growth failure. The authors identified in the dysregulation of RAS/ERK1/2 activation the critical checkpoint affecting IGF-I secretion, eventually leading to growth failure. The finding of the negative influence of mutated SHP2 on GH-induced IGF-I production may have potential clinical implications. GH treatment has been shown to stimulate growth in children with NS [27] but long-term efficacy results are modest. The results of this study clearly show that subjects with SHP2 mutations have partial GH insensitivity which may account for the moderate response to therapy. In the light of these data, short children with NS might need a higher GH dose, IGF-I therapy or a MEK inhibitor which has already been shown to improve growth and alleviate cardiac and craniofacial defects in animal models of NS [28].

Skeletal muscle mitochondrial function is associated with longitudinal growth velocity in children and adolescents

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Background: Mitochondrial diseases are associated with the impairment of growth suggesting that mitochondrial function, providing energy and substrates, may play a key role in longitudinal growth. The aim of this study was to determine the relationship between mitochondrial function and linear growth in healthy children and adolescents.

Methods: 29 children aged 8–15 years were enrolled in this prospective longitudinal study. Fasting laboratory studies and an estimate of mitochondrial function (as assessed by the time to recovery of phosphocreatine (PCr) concentration after submaximal quadriceps extension/flexion exercise using ³¹P magnetic resonance spectroscopy) were obtained at baseline and annually for 2 years.

Results: Data were complete at the 1 year time point for 23 subjects. More rapid skeletal muscle oxidative phosphorylation, as suggested by a shorter time to PCr recovery after submaximal exercise, was significantly associated with growth velocity in the subsequent year. This result was significant in the first year. In multivariate modeling, baseline mitochondrial function remained significantly and independently associated with growth, controlling for age, gender, pubertal stage, body mass index Z-score, height Z-score, sex steroid levels and IGF-I concentrations.

Conclusions: A novel association between mitochondrial function, expressed by time to recovery of PCr concentration after submaximal exercise, and linear growth in childhood and adolescence is shown.

The growth process requires both energy and substrate to fulfill physiological demands. Consistent with the pivotal role played by mitochondria in energy metabolism during growth is the observation that mitochondrial diseases are often associated with growth impairment. This study for the first time shows an association between linear growth and mitochondrial function as investigated by an indirect and noninvasive method based on ^{31}P magnetic resonance spectroscopy. This association might be explained by a GH-dependent regulation of mitochondrial function [29]. However, there are many potential biases affecting the interpretation of these results. The small size and the heterogeneity of the study cohort, including both prepubertal and pubertal children of whom two thirds were overweight, calls for caution in drawing conclusions. Moreover, association does not necessarily mean causation. Nevertheless, the originality of the results and their potential physiological and clinical fallout warrant further large-scale studies to elucidate the extent of the interplay between muscle energy production and growth plate growth and maturation during childhood and adolescence.

Important for clinical practice

Incidence of diabetes mellitus and evolution of glucose parameters in growth hormone-deficient subjects during growth hormone replacement therapy: a long-term observational study

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Background: Growth hormone (GH) exerts anti-insulin effects in liver and muscle and GH therapy has been associated with the risk of developing insulin resistance and diabetes. The aim of the current study was to determine the incidence of diabetes during GH replacement therapy (GHRT) in adult patients. In addition, results of a longitudinal study analyzing yearly fasting plasma glucose and HbA_{1c} concentrations in a subgroup of patients over an observation period of 6 years are reported.

Methods: KIMS database was the data source. Only patients with severe adult-onset GH deficiency and naive to GH treatment were selected. A total of 5,143 GH-deficient patients (mean age $\pm$ SD, 49 ± 13 years) were analyzed. Mean observation time was 3.9 years. Total number of patient-years was 20,106. Observed number of cases-to-expected number of cases (O/E) ratios of patients diagnosed with diabetes were calculated, and stratified for age and sex. The expected number of cases was calculated using normal population-based age- and sex-specific reference incidence rates from Sweden and three additional European regions, plus one US region.

Results: 523 patients (10.2%) developed diabetes after a median period of 1.7 years. Patients who developed diabetes were significantly older, had higher BMI, waist circumference, waist-to-hip ratio, and triglyceride concentrations, and had significantly higher systolic and diastolic blood pressure and significantly lower HDL cholesterol concentrations than those who did not develop diabetes. Overall diabetes incidence was 2.6 per 100 patient-years, significantly higher than the expected according to the Swedish reference, the overall O/E ratio was 6.0 (95% CI 5.5–6.6), as well as to the four other populations references, the O/E ratios ranging from 2.11 (1.86–2.39) to 5.22 (4.76–5.72). In the subgroup of patients with available yearly fasting glucose and HbA_{1c} data over a period of 6 years, plasma glucose concentrations increased from 84.4 ± 0.9 to 89.5 ± 0.8 mg/dl (0.70 mg/dl/year) and HbA_{1c} increased from 4.74 ± 0.04 to $5.09 \pm 0.13\%$ (0.036%/year).

Conclusions: Diabetes incidence appears to be increased in GH-deficient patients receiving GHRT, thus strongly suggesting that patients on GHRT, particularly those with an adverse baseline risk profile, should be carefully followed regarding parameters of glucose metabolism.

Although this study reports data from a large database of adult patients (KIMS), the results are relevant for clinical practice raising concern on the safety of GH replacement therapy, and should drive the attitude of pediatric endocrinologists as well. Indeed the finding of increased incidence

of diabetes in adult patients undergoing GH replacement therapy strengthen the results of previous studies reporting a similar increase in metabolic risk in both adults and children treated with GH [30–32]. These data should prompt the clinicians who treat subjects with GH to monitor glucose homeostasis especially in those patients with predisposing factors (i.e. familial history for type 2 diabetes, high BMI and waist circumference, etc.). As in the case of the potential cancer risk associated with long-term GH therapy, it may be argued that the metabolic risk may be intrinsically related to the disease (GH deficiency) rather than to treatment, and that, ideally, only a randomized controlled trial might clarify the real impact of GH therapy on the development of diabetes. However, realistically speaking, it is extremely unlikely that a study involving an ideal age-, sex- and risk factor-matched GH-deficient group not receiving GHRT in a prospective controlled trial will ever be performed. Regulatory authorities in several countries have recently questioned the beneficial effect of GH in adult GHD. This paper adds another point to the hesitant. Alternatively, we may be giving GH in a wrong fashion; after all, normal secretion of GH is pulsatile.

A novel missense mutation in the SH2 domain of the STAT5B gene results in a transcriptionally inactive STAT5B associated with severe IGF-I deficiency, immune dysfunction, and lack of pulmonary disease

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Background: Signal transducer and activator of transcription 5b (STAT5B) deficiency accounts for rare forms of GH insensitivity (GHI) characterized by IGF-I deficiency (IGFD), and severe immune dysregulation manifesting as progressive worsening of pulmonary function. The aim of this study was to report the phenotype of a patient with a novel mutation of STAT5B.

Patient: A girl from Argentina developed severe generalized seborrheic dermatitis at the age of 1 year. Autoimmune thyroiditis was diagnosed at 4.5 years, and she was started on L-T₄ therapy. In later years, she developed severe varicella with cutaneous infection and *Streptococcus pyogenes* septicemia. Impairment of pulmonary function was not observed throughout childhood or adolescence. She concomitantly exhibited severe growth failure, achieving an adult height of 124.7 cm. Endocrine evaluations (normal provocative GH tests, low serum IGF-I, –3.7 SDS, and IGF-binding protein-3, –4.5 SDS) were consistent with primary IGF-I deficiency.

Results: Sequencing of the *STAT5B* gene revealed a T→C transition in exon 16, corresponding to the second nucleotide of codon 646 (c.1937T3C), which changed phenylalanine (Phe, F) to serine (Ser, S) (p.Phe646Ser; p.F646S). In reconstitution studies, GH-induced phosphorylation of the STAT5B p.F646S variant was observed but was consistently less than that detected with wild-type STAT5B. Similar observations were made when treated with IFN-γ. These findings suggested that STAT5B p.F646S had retained the ability to associate with critical pY motifs on the GHR and IFN-γ receptors.

Conclusion: A novel homozygous *STAT5B* missense mutation, located within the critical SH2 domain, was identified in a patient with profound postnatal growth failure due to IGF deficiency but who lacked the severe chronic pulmonary disease and immunodeficiency observed in the patient carrying the first described *STAT5B* missense mutation in SH2 domain.

This case report describes the clinical features of a novel homozygous missense mutation in the *STAT5B* gene. This is the ninth case of *STAT5B* deficiency. Functional studies and clinical presentation of this novel mutation were compared with p.A630P missense mutation previously identified in the same critical SH2 domain. Unlike the previously described missense mutation however, this new mutation was associated with a residual capacity of *STAT5B* to be phosphorylated in response to GH and IFN-γ, although could not drive transcription. Another striking difference between the 2 patients was the absence of pulmonary symptoms in the new case, despite a diagnosed T-cell lymphopenia and other immune irregularities. The mechanism underlying the relative immune competency in this and all previous patients relates to the overlap in STAT signaling between GH and several cytokines

and interferon. This case confirms the critical importance of the SH2 domain for mediating the GH-induced regulation of *IGF1* expression. Moreover, this patient shows a new genotype-phenotype association among the STAT5B mutations, mainly characterized by the severe growth failure, which is typical of patients carrying other types of STAT5B mutations, but spared pulmonary function.

Food for thought

Targeted loss of GHR signaling in mouse skeletal muscle protects against high-fat diet-induced metabolic deterioration

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Background: It is difficult to distinguish the metabolic actions directly exerted by growth hormone (GH) in tissues from those mediated by IGF-I and/or insulin. The aim of this study was to identify the direct metabolic effects of GH in the muscle using mice with selective postnatal inactivation of growth hormone receptor (GHR) gene in skeletal muscle.

Methods: Specific GHR inactivation in muscle was achieved using the Cre/loxP system (mGHRKO model). Body composition analysis was performed using the EchoMRI 3-in-1 NMR system. Total triglycerides (TG) were extracted from the liver and gastrocnemius using the chloroform-methanol method. Metabolic cage studies were performed in unrestrained mice fed a high-fat (60% fat) diet (HFD) for 12–14 weeks. The metabolic state of the mGHRKO mice was characterized under lean and obese states.

Results: High-fat diet feeding in the mGHRKO mice was associated with reduced adiposity, improved insulin sensitivity, lower systemic inflammation, decreased muscle and hepatic triglyceride content, and greater energy expenditure compared with control mice. The obese mGHRKO mice also had an increased respiratory exchange ratio, suggesting increased carbohydrate utilization. Muscles of obese mGHRKO mice had decreased *socs2* and muscles of both lean and obese mGHRKO mice showed a higher interleukin-15 and lower myostatin expression relative to controls.

Conclusions: These data suggest a role for muscle GHR signaling in mediating whole-body insulin resistance in obesity either by altering substrate utilization or by modifying energy expenditure.

As most GH actions are mediated by IGF-I via endocrine, paracrine and autocrine actions, it is difficult to dissect pure GH actions from those actually mediated by IGF-I. The selective postnatal knockout of muscle GHR has enabled the authors to elucidate the role of skeletal muscle GHR signaling in regulating metabolism. The inactivation of muscle GHR has revealed a novel role of muscle GHR signaling in facilitating cross-talk between muscle and other metabolic tissues such as liver and adipose tissue, probably via the regulation of secreted myokines, such as myostatin. However, these results are in contrast with that of Mavalli et al. [33] showing in a similar model of selective muscle inactivation of GHR increased body weight, body adiposity, and circulating TG levels and worsened insulin sensitivity. The discrepancies between the two studies may arise from the different genetic background of the mice used or from the different timing of GHR inactivation. Therefore, different animal models of muscle GHR inactivation yield different opposite results, suggesting that the genetic, endocrine and metabolic mechanisms involved in the interplay between muscle GHR signaling and metabolism are complex and still largely unknown. We knew that GHD and GHIS are associated with muscle weakness and adiposity; here may lay the mechanism.

Growth hormone receptor regulates β -cell hyperplasia and glucose-stimulated insulin secretion in obese mice

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Background: β cells express the GH receptor and GH stimulates insulin gene expression, biosynthesis, and release in β cells of rodents and humans. However, it is unclear whether these effects are directly exerted by GH or are mediated by IGF-I or by changes in body composition. The aim of this study was to determine the role of GHR in determining β -cell mass and function during normal and pathophysiological conditions.

Methods: Specific *Ghr* inactivation in β cells was achieved using the Cre/loxP system to generate β -cell-specific GHRKO mice.

Results: β GHRKO mice fed a standard chow diet showed impaired glucose-stimulated insulin secretion but had no changes in islet size or insulin content. When challenged with a high-fat diet (HFD), β GHRKO mice exhibited impaired β -cell hyperplasia and a β -cell secretory defect, with deterioration of glucose homeostasis. Obese β GHRKO mice exhibited decreased β -cell proliferation.

Conclusions: Deletion of GHR specifically in pancreatic islet β cells was associated with a lack of compensatory hyperplasia in response to HFD-induced obesity, thus suggesting that GH receptor plays critical roles in glucose-stimulated insulin secretion and β -cell compensation in response to a high-fat diet.

This is another paper reporting the structural and functional consequences of targeted loss of GHR, in this case specifically in the pancreatic β cell. The objective was to clarify the role, if any, of GH and its receptor in regulating β -cell proliferation and function in mice either fed a standard chow diet or a high-fat diet. Previous studies yielded conflicting results showing that loss of GHR in β cells was followed by reduced β -cell mass, impaired glucose tolerance, and increased insulin sensitivity. However, these animals displayed compromised growth and significant changes in body adiposity, thus questioning a direct causal effect of GHR ablation [34]. Furthermore, the ablation of STAT5A/B had negligible effects on β -cell mass or function under normal conditions, but in obese mice was associated with hyperglycemia and glucose intolerance [35]. These results are consistent with a metabolic role played by GHR during obesogenic conditions, GHR ablation negatively influencing glucose-stimulated insulin secretion and β -cell compensation in response to a high-fat diet. These targeted knockout studies are valuable in identifying the direct actions exerted by specific molecules, in this case GHR. However, physiology is far more complex involving a network of interactions among multiple players influencing each other. Therefore, it would be simplistic and potentially misleading trying to speculate that GHR may mediate potential protective effects on β cells in conditions of overfeeding.

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