Growth and Growth Factors

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This collection of articles aims to provide a wide spectrum of studies on the subject of growth and growth factors, particularly focusing on those with direct or potential clinical implications. Of course, given the limited number of papers to be selected, the choice of articles has been fully arbitrary and, inevitably, other equally valuable studies have been neglected. Among experimental studies, I favored those with major clinical implications. In the last 12 months, the research in the field of growth factors has provided important achievements. Molecular biology, applied to single clinical cases or large cohorts of patients, has elucidated the mechanisms underlying some conditions characterized by severe short stature. New therapeutic tools for treating growth impairment have been tested in phase 1 and 2 clinical trials. New relationships between the IGF system and predisposition to cardiovascular disease have been described. Finally, pharmacological modulation of IGF system has been attempted to inhibit cancer growth. Although most of these papers have not yet yielded conclusive results, they nonetheless provide the basis for further clinical and experimental research.

Wishing you a good reading, I would like to wrap up this short introduction quoting a sentence written on the office door of James Muorilyan Tanner, the founder of modern auxology: ‘the appetite comes reading’.

Important for clinical practice

Efficacy and safety of long-term continuous growth hormone treatment in children with Prader-Willi syndrome

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Background: Prader-Willi syndrome (PWS) is a rare, complex disorder characterized by failure to thrive, early obesity starting during the second year of life, hypotonia, short stature, other endocrine dysfunctions, learning disabilities, abnormal behavior, and psychiatric problems. Hypothalamic dysfunction may account for many features of the syndrome. GH insufficiency, reduced IGF-I levels and good response to GH therapy have been described in children with PWS. The long-term efficacy of GH treatment on adult height and body composition of patients with PWS has not yet been established. The purpose of this multicenter prospective study was to test efficacy and safety of GH therapy during a 4-year follow-up.

Methods: 55 prepubertal children (with a mean ± SD age of 5.9 ± 3.2 years) with PWS were recruited in the trial with GH therapy and followed for 4 consecutive years. All children had a genetically confirmed diagnosis of PWS by positive methylation test and were naive to GH treatment at the time of inclusion. Children were treated with rhGH 1 mg/m² daily. Body composition was evaluated by dual-energy x-ray absorptiometry.

Results: Fat body mass decreased only during the first year of therapy, and remained higher than +2 SDS after 4 years of therapy. Lean body mass transiently increased during therapy, remaining lower than –2 SDS after 4 years. Height and head circumference significantly increased during the first 3 years of therapy achieving a value not significantly different from 0 SDS. Body proportions expressed by the
Sitting height to height ratio improved during treatment and did not significantly differ from 0 SDS after 4 years. Hand and foot length as well as arm span did not normalize during treatment. GH therapy had no significant effect on bone maturation. GH significantly increased IGF-I up to more than +2 SDS with peak after the first year of therapy. IGFBP-3 also increased but to a less extent ultimately leading to a marked increase of the IGF-I/IGFBP-3 molar ratio, which might indicate that more free IGF-I was present in the systemic circulation. Glucose and insulin levels remained unchanged during GH therapy whereas LDL cholesterol decreased significantly.

Conclusions: GH long-term therapy proved to be safe and effective in improving height, body composition, head circumference and lipid profile in children with PWS.

This study substantially confirms previous reports showing the effects of GH therapy on a series of features typically associated with PWS, such as short stature, alterations of body composition and microcephaly. In addition, no major side effect was observed during the 4-year follow-up in this large cohort of patients. However, since 2002 [1], a significant number of deaths in children with PWS treated with GH have been reported [2], especially during the first 6–12 months of therapy. The major cause of death in PWS children is respiratory failure [3]. Although there is no direct evidence for a causative role of GH therapy, the safety of GH treatment in this high-risk population has been questioned and warnings have been added for the use of rhGH. Although there is no clear evidence of a connection between GH treatment and risk of death, and the results of this study look reassuring, the finding of the sharp increase of IGF-I even above the upper normal range raises concern. IGF-I is a potent stimulator of lymphoid tissue growth and the excessive and rapid increase in IGF-I at the start of GH treatment could induce tonsillar and adenoid hypertrophy which may concur in increasing the risk of sleep-obstructive apnea. Therefore, further research on longer-term effects of high IGF-I levels is warranted.

Serum insulin-like growth factor-binding protein-2 levels and metabolic and cardiovascular risk factors in young adults and children born small for gestational age

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Background: Subjects born small for gestational age (SGA) are at risk of developing insulin resistance, type 2 diabetes, metabolic syndrome and cardiovascular disease in adulthood. IGF-binding protein 2 (IGFBP-2) belongs to the family of 6 IGFBPs which bind IGF-I and IGF-II. IGFBP-2 has been reported to modulate intracellular insulin signaling, and IGFBP-2 levels are inversely related to insulin concentrations and insulin resistance. The aim of this study was to assess IGFBP-2 levels in a cohort of young adults and children born SGA.

Methods: 151 young adults and 147 children born SGA were studied. Subjects underwent anthropometry, blood lipid profile assessment and blood pressure evaluation. Body composition was assessed by dual-energy x-ray absorptiometry. A modified, frequently sampled intravenous glucose tolerance test with tolbutamide was performed in a subgroup of study subjects.

Results: SGA young adults showed reduced concentrations of IGFBP-2 independently of catch-up growth in height. Whereas IGFBP-2 did not correlate with birth size, it was inversely related to fat mass. Furthermore, in this group, IGFBP-2 correlated negatively with BMI, fat mass, blood pressure, fasting insulin, HOMA-IR, insulin secretion, cholesterol and triglycerides. On the other hand, IGFBP-2 correlated positively with insulin sensitivity. After adjustment for fat mass, the relationships with metabolic markers disappeared, thus suggesting that IGFBP-2 levels associate with FM and that a lower FM is associated with lower insulin levels and less insulin resistance. No correlation between IGFBP-2 and cardiovascular risk markers was seen in SGA children.

Conclusion: In SGA young adults, IGFBP-2 may represent an indicator of the cardiovascular risk.

Concentrations of IGFBP-2, binding both IGF-I and IGF-II, increase after a prolonged period of fasting, indicating that IGFBP-2 concentrations are metabolically regulated. Evidence suggests that IGFBP-2 may alter the activity of intracellular kinases that regulate insulin signaling by both IGF-
dependent and IGF-independent mechanisms, thereby modulating insulin sensitivity. Furthermore, studies in adult patients have shown a relationship between IGFBP-2 and cardiovascular risk factors. This study represents the first report on the association between IGFBP-2 levels and markers of cardiovascular risk in normal height and short SGA adults, and only in short SGA children. Although the results are in adults, and based on correlation analyses, the findings are suggestive for a potential use of IGFBP-2 in quantifying the metabolic risk in this population. However, it has to be pointed out that the whole set of independent variables (including IGFBP-2) only explained 20% of variance. It is noteworthy that such relationships were not present in SGA children. The search for a reliable marker of metabolic risk in children and adolescents with low birth weight looks worthwhile, but, unfortunately, no indicator has so far been identified. On the other hand, it may be argued that insulin sensitivity is mildly impaired in children born small for gestational age [4]. Efforts to evaluate metabolic risk are more meaningful in young adulthood but early predictors would be welcome.

**Impact of growth hormone therapy on adult height of children born small for gestational age**

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**Background:** The indication for growth hormone (GH) therapy in children born small for gestational age (SGA) has been approved worldwide. Although the primary outcome measure for evaluating the efficacy of such therapy is adult height, most trials have reported short-term results only. In addition, the quality of most studies is affected by the recruitment of small study cohorts, and, often, the use of historical controls. The aim of this systematic review was to examine the evidence that long-term randomized controlled trials (RCTs) of GH treatment in children born SGA may improve adult height.

**Methods:** A meta-analysis of all RCTs conducted up to the achievement of adult height and published until November 2008 was performed. Children with birth weight and/or length below –2 SD score (SDS) and pre-therapy height less than –2 SDS treated with two dose regimens (33–67 µg/kg per day) met the inclusion criteria. Adult height and total height gain expressed in SDS were considered as the primary outcome measures. The quality of trials and strength of recommendation were assessed using the Endocrine Society grade recommendations [5].

**Results:** Four RCTs were identified, including a total of 270 treated and 155 untreated children (controls). The mean differences in final height and overall height again between treated and untreated subjects were 0.85 SDS (approx. 6 cm) and 1.25 SDS (approx. 8 cm) respectively. No significant difference in adult height between the two dose regimens was observed. The positive predictive factors for GH efficacy were younger age and number of prepubertal years of therapy. Moderate-quality evidence was the score assigned to all four RCTs, whereas strong recommendation was assigned to 2/4 trials.

**Conclusions:** Despite the approval of GH therapy for improving adult height of short children born SGA, no single, long-term, randomized, controlled (in parallel), well-powered study conducted up to the achievement of adult height has been published so far. On average, GH therapy has been shown to be effective in reducing the adult height deficit, although the meta-analysis shows a wide individual variability in the response. Finally, there is no evidence for supporting the use of doses higher than 33 µg/kg per day.

This systematic review has important clinical practice implications representing a comprehensive meta-analysis appraising the effect of long-term GH therapy on adult height of short children born SGA. Although international and national drug agencies have approved the use of GH in this condition, this report re-opens the debate on the cost/benefit ratio and, more importantly, provides the evidence-based achievements that can be reasonably expected by children, parents and physicians. SGA children can expect to gain between 6 and 8 cm from years’ long daily injections of GH. Although GH seems effective in improving adult height, the magnitude of the growth-promoting effect is relatively low. In addition, the individual variability in the response to GH therapy should prompt further investigations aimed at identifying those who can substantially benefit from a long-term treatment.
Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype

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Background: Children born small for gestational age (SGA) represent a widely heterogeneous population. Genetic factors certainly play a role in determining birth size and postnatal growth, to date, however, only a few genetic alterations have been associated with intrauterine growth retardation. This study investigated copy number variations in 18 growth-related genes in short children born SGA.

Methods: Multiplex ligation-dependent probe amplification (MLPA) was used to test whether copy number variations in growth-related genes (SHOX, GH1, GHR, IGF1, IGF1R, IGF2, IGFBP1–6, NSD1, GRB10, STAT5B, ALS, SOCS2, and SOCS3) were present in a cohort of 100 children born SGA with persistent short stature. In 2 subjects a deletion of the IGF1R gene was identified. The extent of the two deletions was determined with single-nucleotide polymorphism (SNP) array analysis. Finally, functional studies on dermal fibroblasts were performed to investigate the IGF1R signal transduction pathway.

Results: Two patients with heterozygous de novo deletions of the insulin-like growth factor 1 receptor (IGF1R) gene were identified. Both subjects showed reduced birth length, dysmorphic features including proximal implanted thumbs, hearing problems and good response to growth hormone (GH) therapy. Patient A also showed delayed psychomotor development, whereas patient B had attention-deficit hyperactivity disorder. Unexpectedly, IGF-I circulating levels were low in patient A, probably due to partial GH deficiency.

Conclusion: IGF1R haploinsufficiency may be suspected in children born SGA with short stature, dysmorphic features and developmental delay, independently of GH responses to provocative tests and IGF-I levels. Interestingly, these patients seem to respond to GH therapy.

Familial short stature caused by haploinsufficiency of the insulin-like growth factor I receptor due to nonsense-mediated messenger ribonucleic acid decay

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Background: To date, only few cases of IGF-I receptor heterozygous mutations have been described in humans. These patients are characterized by severe pre- and postnatal growth retardation, microcephaly and mental retardation, a phenotype closely resembling that of subjects with IGF-I gene defects [6–9]. To ensure the accuracy of gene expression, eukaryotes have evolved several surveillance mechanisms. One of the best studied quality control mechanisms is nonsense-mediated mRNA decay (NMD), which recognizes and degrades transcripts harboring a premature translation-termination codon (PTC), thereby preventing the production of faulty proteins. NMD regulates approximately 10% of human mRNAs [10]. In this study, IGF-I receptor gene (IGF1R) was investigated in a family with severe short stature.

Case Description and Methods: The proband was a boy born small for gestational age (SGA), referred for short stature (height: –3.6 SDS) and microcephaly. No mental retardation was reported. He was on treatment with methylphenidate for a diagnosis of attention-deficit hyperactivity disorder. Family history revealed severe short stature on the maternal side of the family. GH responses to stimulation tests, IGF-I, IGFBP-3 and GHBP levels were normal. GH therapy did not induce catch-up growth. Primary fibroblast cultures
were established from skin biopsies taken from the patient and his siblings and parents. IGF1R was sequenced and both mRNA and protein expression was investigated.

Results: IGF1R sequencing showed a heterozygous duplication in exon 18 in the proband and other family members with growth failure. This duplication comprised nucleotides encoding part of the tyrosine kinase domain located within the β-subunit. This 19Dup mutation in the mutant IGF1R allele led to degradation of the mutant mRNA through the NMD pathway, resulting in haploinsufficiency of the wild-type IGF1R protein.

Conclusions: This study describes a novel heterozygous mutation in the IGF1R and indicates, for the first time, that the NMD pathway may play a key role in determining IGF1R haploinsufficiency eventually leading to the development of IGF-I resistance and human growth failure.

The study of Ester et al. found IGF1R haploinsufficiency in 2 of 100 short SGA children and confirms that alterations in IGF1R expression may cause, although in a proportion of children born SGA, intrauterine growth retardation, postnatal growth impairment, peculiar phenotype and developmental delay. These findings are consistent with the description by Abuzzahab et al. [6] of 2 children with fetal and postnatal growth failure caused by defects in the IGF-IR gene. Moreover, the 2 patients with IGF1R haploinsufficiency described in this paper showed a good response to GH therapy similarly to another child previously reported by the same authors [11]. This unexpected GH effectiveness in promoting growth despite the partial absence of IGF1R was explained by both a direct effect of GH on the epiphyseal chondrocytes independent of the biological actions of IGFS, and the increased serum IGF-I levels, which may overcome the reduced peripheral sensitivity. However, it has to be pointed out that the study children did not achieve adult height yet, making the final outcome of GH therapy uncertain. Another and probably stronger merit of Ester et al.’s paper is to offer an overview of the phenotypes associated with mutations and deletions of IGF1R gene, thus providing clinical indicators to drive the investigator toward the assessment of this gene. The results open many interesting questions. The expression of the IGF1R was minimally lower in the patient’s cells, and the authors speculate that haploinsufficiency may be cell type-dependent, with possibly a relatively strong effect in growth plate chondrocytes. Low serum IGF1 and short-term response to GH therapy remain to be explained. The second paper by Fang et al. describes a novel heterozygous mutation in the tyrosine kinase domain of the IGF1R in multiple subjects of the same family apparently characterized by ‘familial short stature’. However, the severity of short stature together with intrauterine growth retardation in the proband suggested a condition different from a simple normal variant of growth. In addition, this study has the merit to provide evidence, for the first time, that a mechanism involving nonsense-mediated mRNA decay may cause IGFIIR haploinsufficiency and, eventually, pre- and postnatal growth retardation.

New paradigms
Is there a relationship between IGF-I and cardiovascular risk?

A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability

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Background: The age-related decline of serum IGF-I has been associated with the risk of developing diabetes and cardiovascular disease. Although infectious diseases represent the major cause of death in black South Africans, the prevalence of cardiovascular disease is nevertheless high in urban areas. The aims of the study were to measure IGF-I concentrations in African and Caucasian subjects, and to correlate the IGF-I levels with risk parameters for cardiovascular disease.
Methods: This was a cross-sectional study involving 211 African and 316 Caucasian subjects (aged 20–70 years). IGF-I, parameters of glucose homeostasis, blood pressure, and pulse wave velocity were assessed.

Results: In African participants, blood pressure was significantly higher and serum IGF-I concentration significantly lower than in Caucasian counterparts. The decline of IGF-I with age was significantly faster in African subjects than in Caucasians. Finally, a significant negative correlation of IGF-I with blood pressure, pulse wave velocity, and high-density lipoprotein cholesterol was shown in young Africans.

Conclusions: Africans show an accelerated decline in IGF-I levels around the age of 40 years. This finding, together with the observed relationship between IGF-I levels and cardiovascular risk factors, suggests that earlier and greater reduction of IGF-I could be associated with cardiometabolic vulnerability. The age-related decline in IGF-I is associated with the increased incidence of cardiovascular diseases. Diabetes and cardiovascular disease are common in African urban areas. In this paper, Schutte et al. show, for the first time, an accelerated age-related decline in IGF-I circulating levels in African people. There is increasing evidence that IGF-I plays a key protective role in endothelial function, regulating nitric oxide production, improving insulin sensitivity, and exerting anti-inflammatory actions [12–14]. This study provides further indirect evidence on such a protective role of IGF-I against the development of cardiovascular disease. Although this study presents limitations such as the cross-sectional design and the lack of information on other important factors such as GH and IGF-binding proteins, and though correlation does not necessarily mean causation, the findings in African subjects are strongly consistent with the previous observations. This potential role of IGF-I warrants further research even envisaging a therapeutic implication involving perhaps low-dose IGF-I or GH treatment to prevent the early development of cardiometabolic diseases. It remains to be established whether the faster decline in IGF-I levels in Africans is due to genetic predisposition or environmental factors (such as alcohol abuse or poor nutrition) or both. Finally, it has to be pointed out that elevated levels of IGF-I have been implicated in the development and maintenance of many different cancers.

IGF-I bioactivity in an elderly population: relation to insulin sensitivity, insulin levels, and the metabolic syndrome

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Background: The GH-IGF-I axis has been implicated in the development of metabolic syndrome. The aim of this study to relate the IGF-I bioactivity, measured the IGF-I kinase receptor activation assay (KIRA), with insulin sensitivity and metabolic syndrome in an elderly population-based cohort.

Methods: The subjects were selected within the Rotterdam Study population, a prospective large-scale cohort study aimed at investigating incidence and risk factors of cardiovascular diseases in elderly people. 1,036 elderly subjects were recruited. The American Diabetes Association 2003 criteria were used to classify glucose tolerance. IGF-I bioactivity was determined by the IGF-I KIRA. This bioassay determines IGF-I bioactivity by measuring intracellular receptor autophosphorylation upon IGF-I binding [15].

Results: 697 subjects (69.7%) had normal fasting glucose (NFG), 165 subjects (16.3%) had impaired fasting glucose (IFG), and 153 subjects (15.1%) had diabetes. IGF-I bioactivity resulted positively related to insulin resistance markers. In non-diabetic subjects, after stratification according to deciles of HOMA-IR value, IGF-I bioactivity progressively increased up to and including the 9th decile. Thereafter, IGF-I significantly dropped in the 10th decile. IGF-I bioactivity was also directly related to the condition of metabolic syndrome, peaking when three components were present. However, a decline of IGF-I bioactivity was observed when five criteria of the metabolic syndrome were present.

Conclusions: IGF-I bioactivity is closely related with insulin resistance up to a maximum threshold being significantly lower in subjects with diabetes than in subjects with NFG and IFG.

There is a complex relationship between the GH-IGF-I axis and glucose metabolism. IGF-I influences blood glucose concentrations directly by stimulating glucose uptake in target cells and indirectly by increasing the sensitivity of tissues to insulin. Unlike insulin, circulating concentrations of IGF-I do not
fluctuate substantially with time. Instead, dynamic changes in IGF-I bioactivity are attributed to interactions with IGF-binding proteins (IGFBPs). Among the six IGFBPs, IGFBP-1 production is inhibited by insulin. Insulin is also essential for GH stimulation of hepatic IGF-I production either by regulating GH receptor expression or by modulating GH signaling. The main finding of this study is that circulating IGF-I bioactivity progressively increases with increasing severity of insulin resistance and hyperinsulinemia, reaching a plateau. Furthermore, this study shows that IGF-I bioactivity declines during progression of the metabolic syndrome. As subjects with the metabolic syndrome are chronically exposed to high insulin levels, the observed increase in IGF-I bioactivity may be secondary to an insulin-mediated suppression of IGFBP-1 levels. In subjects with more than three components of the metabolic syndrome, IGF-I bioactivity significantly declined, suggesting the development of hepatic insulin resistance (manifested by a relative increase of IGFBP-1) and hyperinsulinemia-induced GH resistance. This study suggests a close relationship between IGF-I, insulin sensitivity and, ultimately, cardiovascular risk and stimulates further studies aimed at elucidating whether IGF-I may prevent or, in specific conditions, predispose to metabolic and cardiovascular disease.

**Concepts revised**

**Partial primary deficiency of insulin-like growth factor (IGF)-I activity associated with IGF1 mutation demonstrates its critical role in growth and brain development**


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**Background:** Insulin-like growth factors-I and -II (IGF-I and IGF-II) play a key role in fetal growth and development. Only few specific IGF-I gene (*IGF1*) defects have been described so far, all were characterized by severe pre- and postnatal growth retardation, sensorineural deafness and severe mental retardation associated with microcephaly. This study describes a child with *IGF1* mutation associated with severe intrauterine growth retardation, postnatal growth impairment and brain alterations.

**Case Description:** The patient was born at 40 weeks, to consanguineous parents, with birth weight 2,350 g (–2.4 SDS), birth length 44 cm (–3.7 SDS) and head circumference 32 cm (–2.5 SDS). At referral, the child showed severe short stature (–3.7 SDS), low weight (–5 SDS) and microcephaly (–2.5 SDS). No dysmorphic features were present. The child had anorexia and scarce adipose tissue. Hearing test was normal. Mild developmental delay was present. Conventional work-up for growth retardation was normal. GH stimulation test and spontaneous GH secretion assessment revealed normal GH concentrations. IGF-I levels were almost undetectable if measured with a highly specific monoclonal assay but elevated in a polyclonal assay. In contrast, IGFBP-3 and ALS levels were in the upper normal range or above. GHB levels were within the normal range. The response to IGF-I generation test was subnormal. He was given GH therapy at standard dose for children born small for gestational age (SGA) with no significant improvement of his growth curve. However, when a higher dose was used, partial catch-up growth was observed. Brain MRI scan was normal.

**Results:** Due to the discrepancy between the extremely low IGF-I concentrations and normal/elevated IGFBP-3 and ALS levels, a potential *IGF1* defect was investigated. A previously unidentified homozygous missense mutation in exon 4 leading to replacement of an arginine in position 36 of the C domain by a glutamine was identified. This substitution was shown to affect protein function leading to a partial loss of protein affinity for the receptor and significant reduction of mitogenic activity.

**Conclusions:** Partial loss of IGF-I activity may cause a milder phenotype than complete *IGF1* deficiency and allow a partial response to high-dose GH therapy. It is plausible that such *IGF1* mutations may be not uncommon in children born SGA with microcephaly and poor response to conventional GH therapy.
To date, 3 patients have been described with an IGF1 molecular defect leading to complete or very severe IGF-I deficiency. This paper describes the fourth case with alterations in the IGF-I gene [16–18]. The hallmark of the diagnosis is the discrepancy between the reduced IGF-I levels and IGFBP-3 and ALS serum levels in the upper normal range. Unlike the previous 3 children, this case showed a milder phenotype and only a partial loss of IGF-I binding and activity. Once again this study shows the heterogeneity of the phenotype and raises the question whether this kind of missense mutations may be commoner than expected in children born SGA. Despite the mild IGF-I deficiency, this child showed severe pre- and postnatal growth failure and mental retardation, thus confirming the pivotal role of IGF-I in growth and neurodevelopment. Therefore, this report demonstrates that even partial IGF-I deficiency has marked effects on brain development and cognitive functions. The clinical implication is that these patients are able respond to doses of GH higher than those conventionally used. Moreover, these children may also benefit from IGF-I treatment. Finally, the article reports that low IGF-I serum concentrations using a monoclonal antibody assay were found to be normal in a polyclonal antibody assay – an issue worth remembering.

**Growth hormone and insulin-like growth factor I insensitivity of fibroblasts isolated from a patient with an IκBα mutation**

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**Background:** Nuclear factor (NF)-κB represents a family of transcription factors including five members which in resting cells are bound to specific inhibitory proteins, the IκBs, that also include IκBα. When the cell is stimulated, NF-κB is released from the complexes and translocates to the nucleus where it modulates the expression of target genes. In rodents, NF-κB regulates bone growth and chondrogenesis. In a patient with heterozygous mutation of IκBα, partial GH insensitivity was noted [19] and the effects of GH and IGF-I on NF-κB DNA-binding activity, cell proliferation, and target gene expression were investigated.

**Methods:** Skin fibroblasts from the patient, his father and controls were cultured. Fibroblasts were tested for NF-κB DNA-binding activity, cell proliferation assessed by 3H-thymidine incorporation, phosphatidylinositol 3-kinase (PI3K) assay, STAT5 phosphorylation and expression of specific genes such as GH receptor (GHR), IGF-I receptor (IGF-IR) and TDAG51, a target gene of IGF-I.

**Results:** GH and IGF-I dose-dependent effect on NF-κB DNA-binding activity and fibroblast incorporation of 3H-thymidine was observed in the controls and patient’s father, whereas no effect was elicited in the patient’s fibroblasts. GHR and IGF-IR expression was normal in the patient’s cells. Whilst GH addition to culture medium induced STAT5 phosphorylation and IGF-I mRNA expression in controls’ and father’s fibroblasts, no effect was elicited in the patient’s cells. Finally, IGF-I failed to stimulate PI3K activity and TDAG51 expression.

**Conclusions:** GH and IGF-I do not stimulate cell proliferation and gene expression in fibroblasts isolated from this patient harboring a mutation of IκBα, thus suggesting that NF-κB activity is necessary for the growth-promoting actions of both hormones.

This study shows for the first time the key role of NF-κB activity in signal transduction, reporting a child with severe postnatal growth retardation associated with GH insensitivity, immunological defects, ectodermal dysplasia and delay of psychomotor development. GH and IGF-I intracellular signaling takes place through a complex network of factors whose function is finely tuned to allow the proper cell response. Alterations of this intracellular cascade induce the blockade of signal transmission ultimately leading to growth arrest. Mutations/deletions of GH and IGF receptors, and STAT5b have been described. It was previously demonstrated in rats that NF-κB signaling facilitates longitudinal bone growth and growth plate chondrogenesis and that NF-κB p65 in rats mediates the growth-promoting effects of IGF-I. The results of this study indicate that both GH and IGF-I independently stimulate NF-κB DNA-binding activity and cell proliferation in human fibroblasts. The phenotype of this patient with impairment of NF-κB activity secondary to a mutation of IκBα was complex, growth retardation representing only a tiny portion of a constellation of severe symptoms and signs. The patient unfortunately died shortly after stem cell transplantation performed for the immune disorder. This report demonstrates that any alteration in the steps of the post-receptor signals may affect...
Involvement of pregnancy-associated plasma protein-A2 in insulin-like growth factor (IGF)-binding protein-5 proteolysis during pregnancy: a potential mechanism for increasing IGF bioavailability

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Background: The majority of IGFs circulate in blood in high molecular mass ternary complexes containing either IGFBP-3 or IGFBP-5 and the acid-labile subunit. IGFBP proteases are proteolytic enzymes that fragment IGFBPs and reduce their affinity for IGFs, ultimately leading to increased free IGF concentrations. Such proteolytic activity has been reported to be augmented in pregnancy, GH deficiency and catabolic conditions, coincident with an increased demand for bioavailable IGFs. During pregnancy, IGFBP-5 undergoes substantial size redistribution, the majority of the protein being in either free or IGF-IGFBP-5 binary complexed forms. The formation of binary complexes may facilitate the transfer of IGFs from circulation to tissues, eventually increasing local IGF bioactivity. The aim of the study was to characterize the IGFBP-5 proteolytic activity and determine its physiological function.

Methods: Plasma from healthy non-pregnant and pregnant women was fractioned by gel-filtration chromatography. IGFBP-5 circulating forms were identified by immunoblotting. Plasma proteolytic activity against IGFBP-5 was determined. IGFBP-5 fragments were purified from pregnancy samples and analyzed by mass spectrometry.

Results: Whilst the intact form of IGFBP-5 was present in non-pregnancy samples, it was absent in specimens at all stages of pregnancy which showed the presence of proteolyzed fragments in the 18- to 25-kDa range, particularly in the third trimester. The level of IGFBP-5 proteolytic activity increased progressively during pregnancy. Size exclusion chromatography revealed two major sources of proteolytic activity, one in the >150-kDa fraction and the second one eluting in the approximately 40-kDa fraction. The former was present only in pregnancy plasma whereas the latter was present in both pregnant and non-pregnant samples. The protease inhibitor profile and mass spectrometry analyses showed that the >150-kDa fraction contained PAPP-A2 (pregnancy-associated plasma protein-A) or a PAPP-A2-like protease responsible for the proteolysis of IGFBP-5 during pregnancy. Finally, pregnancy plasma was able to induce proteolysis of IGF-I-IGFBP-5 complexes, and to increase IGF-I receptor phosphorylation, thus suggesting that IGFBP-5 proteolysis in pregnancy leads to increased IGF-I bioactivity.

Conclusions: Circulating IGFBP-5 is fully proteolyzed by PAPP-A2 during pregnancy. This proteolysis leads to increased IGF bioavailability, which may play a key role in growth and development of fetus as well as maternal well-being.
suggests that PAPP-A2 up-regulation reflects a compensatory mechanism finalized to preserve fetal growth and development under unfavorable conditions such as eclampsia.

Inhaled growth hormone (GH) compared with subcutaneous GH in children with GH deficiency: pharmacokinetics, pharmacodynamics, and safety

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Background: Since the description of the first patient treated with growth hormone (GH) in 1958, GH has been administered initially by intramuscular and subsequently by subcutaneous injections. This annoying method of administration affects long-term compliance leading to dose missing and therapy discontinuation, sometimes even to the refusal of treatment. Therefore, a less invasive method looks desirable to guarantee a better adherence to treatment and better final results. Aerosol technology has recently allowed the development of an innovative formulation of GH, termed somatropin inhalation powder (SIP). Preliminary pharmacokinetic (PK) and pharmacodynamic (PD) data in primates were promising. This study aimed at determining bioavailability and biopotency of SIP in comparison with GH given subcutaneously. In addition, PK, PD and safety of SIP treatment in children were tested.

Methods: The design was a multicenter, randomized, double-blind, placebo-controlled, crossover trial. 22 GH-deficient children were recruited. Patients underwent two 7-day treatment phases with either inhaled GH or subcutaneous GH + placebo separated by 6–13 days of washout.

Results: Although the absorption of SIP was faster, the overall PK profile was similar to that of subcutaneous GH. The mean relative bioavailability for SIP administration compared with subcutaneous GH was 3.5%. The mean relative biopotency, based on IGF-I response, was 5.5%. The two routes induced similar increases in mean serum GH area under the curve and in IGF-I levels in a dose-dependent fashion. The short-term administration of SIP resulted in being safe, without any major side effects, though vomiting, headache and cough were observed in some patients. No change in pulmonary function was recorded. The questionnaires provided to patients and parents showed a clear preference for the inhalation route.

Conclusions: This is the first study in children showing that inhaled GH administered to GHD children for 7 days was effective in inducing a dose-dependent raise in GH and IGF-I levels and was well tolerated. However, the bioavailability of GH administered by this route is low relatively to GH administered subcutaneously.

The search for an alternative way for administering GH in children is certainly worthwhile. Despite the use of ad-hoc devices to alleviate the burden of daily injections, long-term GH therapy remains poorly acceptable for parents and children. Recent advances in aerosol technology, by increasing particle size and lowering their density and tendency to agglomerate, have increased efficiency of deep lung delivery and improved systemic absorption. This preliminary short-term investigation on inhaled GH demonstrates proof of principle and looks promising in terms of tolerability and efficacy in increasing both GH and IGF-I. Although this study demonstrates the potential feasibility of this alternative route of administration in children, the relatively low bioavailability and biopotency of inhaled GH require further extensive studies to refine this aerosol technology.
A pharmacokinetic and dosing study of intravenous insulin-like growth factor-I and IGF-binding protein-3 complex to preterm infants
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Background: Preterm babies show almost undetectable circulating levels of insulin-like growth factor-I (IGF-I) and previous reports from the same authors demonstrated an association between low IGF-I concentrations and risk of developing the retinopathy of prematurity. Moreover, IGF-I plays a key role in proliferation, maturation, differentiation and migration of neural cells during embryo-fetal brain development, and the low IGF-I levels of premature neonates have been associated with brain vulnerability. The aim of this study was to test tolerability and pharmacokinetics of IGF-I and IGFBP-3 complex administered intravenously to a small group of premature newborns.

Methods: Five infants born at gestational age 26–29 weeks with IGF-I levels <25 µg/l at postnatal day 2 were studied. The study substance was an equimolar preparation of recombinant protein complex of rhIGF-I and rhIGFBP-3 diluted with 10% glucose solution. The individual dose of rhIGF-I ranged from 1 to 12 µg/kg and was infused over 3 h.

Results: The infusion of rhIGF-I and rhIGFBP-3 complex determined a marked increase of both IGF-I and IGFBP-3 concentrations. The estimated half-lives of IGF-I and IGFBP-3 were 0.86 and 0.90 h respectively for a child of 1,000 g. All the safety measures were reassuring.

Conclusions: Infusion of rhIGF-I and rhIGFBP-3 complex is able to increase both circulating peptides in extremely preterm infants and is well tolerated. This study may thus represent a basis for further investigations on efficacy and safety of IGF-I in severe prematurity to stimulate growth and prevent retinopathy and brain damage.

IGF-I is a fetal growth factor essential for the development of the central nervous system. In preterm infants, low serum levels of IGF-I have been associated with slow weight gain and slow head (brain) growth as well as with the later development of retinopathy of prematurity. IGF-I promotes proliferation, maturation, and differentiation of neural stem cells. Moreover, IGF-I has been demonstrated to have neuroprotective properties both in vivo and in vitro. Finally, IGF-I plays an important role in retinal vascular development in both experimental and clinical studies. This Swedish group previously reported that serum IGF-I levels can predict which preterm babies will develop the retinopathy of prematurity [21, 22]. This paper represents the logical step ahead towards the therapeutic use of IGF-I in combination with IGFBP-3 to prevent the development of retinopathy in preterm infants. The major weakness of the study is the small number of study subjects (only 5), nevertheless it sheds, for the first time, some light on the pharmacokinetics and safety of IGF-I administration in extreme premature infants.

New paradigms
Cooperation between oncogenic viruses and IGF-I receptors

Physical and functional interaction between polyoma virus middle T antigen and insulin and IGF-I receptors is required for oncogene activation and tumor initiation
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Background: An oncogenic role of polyoma viruses in human cancers has been repeatedly proposed. Tumorigenesis seems to be mediated by polyoma virus middle T antigen (PyVmT) oncogene. PyVmT-
transforming activity is linked with the downstream signaling after incorporation to the cell membrane. PyVnT undergoes tyrosine phosphorylation and activates MAPK and PI3K/Akt pathways. However, the mechanisms of PyVmT activation are poorly understood. The aim of this study was to test the role played by the interactions between insulin and IGF-I receptors with PyVnT in oncogene activation and tumor initiation.

Methods: Met-1 and DB-7 cells derived from two different lines of mouse mammary tumor virus (MMTV)-PyVmT transgenic mice and human mammary carcinoma MCF-7 and MDA-MB-231 cells were studied. In Met-1 cells, insulin and IGF-I receptor gene silencing was induced by transfection. Thereafter, Met-1 cells were injected into inguinal mammary fat pads of wild-type FVB/N mice. After 4 weeks the size of Met-1 orthograft tumors was evaluated.

Results: Insulin and IGF-I receptor (IR and IGFIR) are markedly expressed and phosphorylated in Met-1 cells overexpressing PyVmT. Treatment of these cells with insulin and IGF-I activates both PI3K and MAPK pathways inducing mitogenesis, inhibiting apoptosis, and stimulating migration and invasion. IR and IGF-IR interact with PyVmT and both insulin and IGF-I enhance this interaction and induce PyVmT tyrosine phosphorylation. IR and IGF-IR knockdown abrogates the ability of Met-1 cells to initiate tumor formation in vivo when implanted into the inguinal mammary fat pads of recipient mice.

Conclusion: Although PyVmT mimics the action of tyrosine kinase receptors, it lacks intrinsic tyrosine kinase activity and requires cellular kinases for its activation. These results indicate that the interaction between PyVmT and cellular receptor tyrosine kinases such as IR and IGF-IR, plays a key role in PyVmT activation and, consequently, cancer initiation. This mechanism may represent a novel biological paradigm for oncogene activation in mammalian cells.

A number of human cancers have been shown to be caused by viral infections, including Burkitt’s lymphoma, nasopharyngeal carcinoma, hepatocellular carcinoma, cervical cancer, T-cell leukemia and Kaposi’s sarcoma. The IGF system activation is also involved in the development and maintenance of tumors. High circulating IGF-I and insulin levels are associated with increased risk of many cancers, whereas low levels of these hormones are accompanied by delayed tumor development. This study demonstrates cooperation between IGF system and a viral oncogen such as polyoma virus, in promoting cell transformation via activation of common intracellular pathways including PI3K/Akt and MAPK. In particular, the tumorigenic action of polyoma virus is mainly determined by PyVmT, one of the most powerful viral oncogenes. IGFR and IR provide PyVmT, the tyrosine kinase activity necessary to initiate intracellular signaling ultimately leading to cell transformation. The potential clinical implication is that oncogenesis might be targeted by a double strategy directed to both viral infection and IGF system elements.

**New paradigms**

**Blocking IGF-I, the elixir of life?**

**Systemic signals regulate ageing and rejuvenation of blood stem cell niches**

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Background: Aging is associated with progressive decline in cell replacement and repair processes, and alterations of hematopoietic stem and progenitor cells (HSPCs) ultimately leading to defects of immune system and increased risk of malignancy. Stem cell supportive microenvironment, or niche, plays a key role in determining the fate of HSPCs in bone marrow and the authors asked whether systemic and/or local factors may affect HSPC number and function.

Methods: Heterochronic parabiotic pairs were generated by surgically joining young (2 months) with older (>21 months) mice. These animals were compared with isochronic pairs (young-young or aged-aged) joined at identical ages. This parabiotic procedure provides a biological model in which the ani-
mals share a common blood circulation, thus enabling to test whether physiological levels of circulating cells or factors can significantly alter tissue function. The possible role of niche and systemic factors in HSPC ageing was investigated using direct isolation of hematopoietic stem cell (HSC)-regulatory niche cells. Finally, osteoblastic niche cells were isolated using fluorescence-activated cell sorting (FACS) from collagenase-treated bones.

**Results:** The exposure to young circulation of aged-heterochronic partners showed significant recovery of HSC number and function which approached normal ‘youthful’ levels. In particular, bone marrow cells from aged mice exposed to young systemic factors showed recovery of engraftment potential, manifested by increased reconstitution of peripheral blood leukocytes, as well as restoration of youthful ratios of B lymphoid to myeloid. The interaction of HSC with aged osteoblastic niche cells was sufficient to induce HSPC accumulation similarly to that observed in aged marrow. The age-dependent alterations in osteoblastic niche cell number and function were restored to youthful levels when aged animals were exposed to young circulation. Locally, the inhibition of IGF-1 signaling in aged osteoblastic niche cells promoted youthful HSC-regulatory function, indicating that IGF-1 impairs the osteoblastic niche cells appropriate regulation of HSCs, thus contributing to the age-associated hematopoietic dysfunction. Tissue, but not systemic, IGF-1 seems to induce ageing of HSC-regulatory niche cells, and neutralization of IGF-1 signaling in the bone marrow microenvironment reverts age-related changes in osteoblastic niche cells.

**Conclusions:** The results suggest that the age-associated changes in bone marrow niche cells are both systemically and locally regulated. They can be reversed by exposure to a young circulation or by inhibition of IGF-1 in the marrow microenvironment. The finding that IGF-1 neutralization restores youthful function to aged osteoblastic niche cells highlights a new and important activity for this growth factor in controlling the fate of stem cells.

In the hematopoietic system, ageing is associated with deficient immune function and increased incidence of malignancy. Age-associated blood diseases are thought to arise in part owing to discrete changes in aged hematopoietic stem and progenitor cells. Local tissue environment regulates the number and function of cells in all tissues, particularly in bone marrow. The hematopoietic stem cells (HSCs) have the potential to self-renew to maintain the HSC pool. HSCs need to be localized in a particular location (termed the HSC niche) within the bone marrow to retain their multipotency and if the HSCs are located elsewhere, they would probably commit to differentiation rather than self-renew. Studies in genetically modified mice supported roles for cells of the osteoblast lineage in the retention and regulation of HSCs in the bone marrow. The study of Mayack et al. indicates for the first time that the regulatory role of osteoblast niche cells in hematopoietic stem cell is closely dependent on IGF-1 in mice. Switching off osteoblast IGF-1 signaling restores the cell youthful function, thus suggesting that IGF-1 impairs the osteoblast niche cell regulation of HSCs thereby contributing to age-associated hematopoietic dysfunction. Such observation has potential major clinical implications envisaging the development of new therapeutic strategies aimed at reversing the age-related immune dysfunction and cancer risk, specifically targeting IGF-1 in the marrow microenvironment.

**New anti-cancer treatments**

**Anti-insulin-like growth factor I receptor immunoliposomes: a single formulation combining two anticancer treatments with enhanced therapeutic efficiency**

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**Background:** Insulin-like growth factors (IGFs) I and II are overexpressed in the vast majority of tumors and play a pivotal role in transformation, growth and survival of cancer cells. A number of strategies have been proposed to silence the IGF system in various types of tumor cells, particularly targeting the IGF-I receptor (IGF1-R) which mediates the biological action of both IGF-I and IGF-II. The aim of this
study was to investigate the antitumor action of a monoclonal anti-IGFI-R antibody coupled to liposomes loaded with doxorubicin (DXR) in neuroendocrine (NETs) tumors of the gastroenteropancreatic (GEP) system.

Methods: Samples of gastrointestinal NETs from 59 patients and samples from normal tissues were analyzed. The anti-IGFI-R Ab was coupled to the liposomal surface via sterol-based postinsertion technique using a succinimide activated sterol-PEG1300-anchor. Tumor cell lines were used to test antibody association and internalization in vitro. For in vivo experiments commercially available sterically stabilized liposomes loaded with DXR were used. Female athymic NMRI v/v mice were inoculated with BON (GEP-NET) cells for inducing tumor development and when longest tumor diameters ranged between 0.5 and 0.7 cm, therapeutic treatments were given intravenously as single bolus.

Results: All tumor tissues showed overexpression of IGFI-R. Anti-IGFI-R immunoliposomes significantly associated to cells, were internalized and resulted effective in inhibiting proliferation and inducing apoptosis of cancer cells. Treatment of human neuroendocrine BON cell xenografts increased the survival time of animals. The anti-IGFI-R immunoliposomes were also tested in other human tumor cell lines different from GEP-NET such as neuroblastoma, breast and prostate cancer cells, showing a similar capacity of binding to the cell surface and being internalized.

Conclusions: In vitro and in vivo treatment with these novel anti-IGFI-R immunoliposomes resulted effective in reducing cell proliferation, inducing apoptosis, and increasing lifespan in animals bearing GEP-NET tumors. Preliminary in vitro experiments indicated that this agent could also represent a promising therapeutic tool for different cancer types.

This report describes the development of DXR-encapsulated immunoliposomes coupled with an IGFI-R blocking Ab to both target IGFI-R-overexpressing tumor tissues and inhibit IGF-dependent pathways. The IGFI-R is important for cancer development and progression. This role was elegantly discovered by Sell et al. who described the resistance of fibroblasts harboring a null mutation of IGFI-R to transformation induced by various viral and cellular oncogenes [23]. As IGFI-R is overexpressed in the majority of tumor cells, a number of inhibition strategies for IGFI-R signaling have been developed [24]. These include: (1) the use of antisense molecules to reduce IGF-I-R translation; (2) the use of blocking antibodies directed to the extracellular part of the receptor; (3) the use of peptides mimicking IGFI-I to block the ligand/receptor interaction; (4) the use of specific inhibitors of the receptor catalytic activity; (5) the use of dominant negative gene variants, and (6) the use of peptide aptamers, a class of molecules genetically selected for specific binding to the receptor. Unfortunately the in vitro promising results obtained with all these methods were not replicable in vivo. The novelty of this study was the combined approach with a cytostatic molecule such as doxorubicin coupled to anti-IGFI-R antibodies in the same carrier. The preliminary in vitro and in vivo results are encouraging but the therapeutic efficacy and safety of such an approach in humans remain to be established.

New mechanisms

Distinct alterations in chromatin organization of the two IGF-I promoters precede growth hormone-induced activation of IGF-I gene transcription

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Background: Most growth hormone (GH) biological actions are mediated by IGF-I whose expression and secretion is directly stimulated by GH in liver and other tissues. IGF-I gene contains two promoters differently active in the different tissues with the only exception of liver where both promoters are functionally active. The mechanisms by which IGF-I promoters are regulated by GH are still largely unknown. The aim of this study was to investigate the effect of GH on IGF-I promoter function.

Methods: Liver from hypophysectomized male Sprague-Dawley rats was studied. IGF-I mRNA expression was assessed by RT-PCR. Quantitative Stat5 chromatin immunoprecipitation assay (ChIP) was
performed to determine whether GH causes recruitment of Stat5b to the IGF-I promoters. Histone acetylation and methylation were determined. A series of quantitative ChIP experiments were performed.

Results: Whereas in absence of GH, IGF-I gene transcription was negligible, a single systemic GH administration induced a significant increase in transcription from both liver IGF-I promoters. Stat5 ChIP experiments failed to detect a substantial association of Stat5b with either IGF-I promoter. The activation of IGF-I promoters associated with a rise in acetylation of histones H3 and H4 in promoter-associated chromatin. GH acutely modified histone lysine methylation at the IGF-I promoters. Finally, GH treatment was able to induce recruitment of polymerase II (Pol II) to promoter 2.

Conclusion: The authors conclude that GH induces rapid and dramatic changes in hepatic chromatin at the IGF-I locus and activates IGF-I gene transcription in the liver by distinct promoter-specific mechanisms. Whereas GH treatment does not influence recruitment of Pol II to promoter 1 which in absence of GH shows Pol II already present in a preinitiation complex, at promoter 2, GH facilitates recruitment and then activation of RNA Pol II to initiate transcription.

This exquisite molecular study sheds light on the mechanisms involved in GH-dependent activation of IGF-I gene promoters showing how GH-mediated signaling causes acute alterations in hepatic chromatin architecture at the IGF-I locus, and that GH activates IGF-I gene transcription in the liver via distinct promoter-specific mechanisms. A single GH systemic administration induces instantaneous acetylation and methylation of core histones, as well as recruitment of polymerase II. Recruitment and modification of transcriptional coregulators may represent fundamental and physiologically relevant dynamic genomic effects of GH. As Stat5b represents the connecting link between the activation of the cell membrane GH receptor and the final biological action of GH on chromatin reorganization, which leads to IGF-I gene transcription, next investigations will be targeted to elucidate the pathways downstream Stat5b activation which eventually orchestrate chromatin structure and function. The knowledge of these mechanisms could unravel processes involved in peripheral altered responses to GH and devise interventions based on the fine tuning of these pathways.

References


