Growth and Growth Factors

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The selection of papers reporting important advancements in the field of growth and growth factors is not an easy task. My choice has focused on the studies apparently more promising for future translations into clinical practice. Clinically important papers relate to the effects of GH therapy on adult height, metabolism, body composition and cognitive function of patients with Prader-Willi syndrome, as well as to long-term drawbacks of the conventional dose of recombinant human IGF-I used in children with growth hormone insensitivity syndrome. The role of the IGF system in the development of fetal tissues continues to be extensively investigated. I have selected some papers showing the key role played by IGFs and IGF-BPs in the development of lung, retina and brain. New evidence supports the role of IGFs in glucose and bone metabolism, which I have included for their potential clinical implications. Finally, in the section 'Food for thought', the reader will find an intriguing experimental study showing the programming effects of infancy duration on the tempo of physiological development and maturation. This selection of papers obviously represents my own bias, but I hope you may find them interesting to read and helpful for your activity in clinical and experimental work.

Important for clinical practice

Breast-feeding vs. formula-feeding for infants born small-for-gestational-age: divergent effects on fat mass and on circulating IGF-I and high-molecular-weight adiponectin in late infancy

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Background: Subjects who are born small for gestational age (SGA) are at high risk of developing insulin resistance, type 2 diabetes and metabolic syndrome in adulthood. A protective role of breastfeeding on metabolic and cardiovascular risk has been demonstrated. The aim of this study was to assess the effects of nutrition in early infancy (breastfeeding vs. formula-feeding; BRF vs. FOF) on body composition and endocrine/metabolic markers in late infancy.

Methods: 130 infants were enrolled in a longitudinal controlled study. Body composition, fasting glycaemia, insulin, IGF-I, and high-molecular-weight (HMW) adiponectin were assessed at 4 and 12 months in BRF controls born appropriate-for-GA ($n = 31$) and in SGA infants receiving either BRF ($n = 48$) or FOF ($n = 51$), the latter being randomized to receive a standard formula (FOF1) or a protein-rich formula (FOF2).

Results: SGA-BRF infants maintained low adiposity and high insulin sensitivity with normal IGF-I and HMW adiponectin levels. In contrast, SGA-FOF2 infants normalized body composition gaining more fat mass, and showed high IGF-I and low HMW-adiponectin levels. Results of SGA-FOF1 infants were intermediate.

Conclusions: Breastfeeding is associated with a better body composition and metabolic status in infants born SGA. Nutritional strategies in SGA infants should aim to achieve high insulin sensitivity and normal IGF-I and HMW adiponectin levels rather than normal body composition.

It is well established that breastfeeding is associated with a reduced cardiometabolic risk in adult life [1, 2]. Epidemiological studies conducted in many countries and different ethnicities have shown that subjects with low birth weight have a higher metabolic and cardiovascular risk in adulthood [3].

Subjects born SGA have been extensively investigated for markers of metabolic risk and it is recognized that whereas their metabolic status is substantially normal during childhood and adolescence, in a minority of them it starts deteriorating in young adulthood [4]. This longitudinal study addresses the issue of early change in body composition and metabolic parameters in relation to the type of feeding in infancy. The results confirm the cardiometabolic protective role of breastfeeding whose beneficial effects on insulin sensitivity are already visible in the first year of life. Formula feeding for SGA infants should aim at maintaining normal HMW-adiponectin levels rather than at achieving a quick normalization of body composition associated with a rapid catch-up growth in fat mass. With regard to the more general issue of the use of enriched or non-enriched formulas in low birth weight infants, an appropriate nutritional strategy should be based on the balance between the need to induce a normal development of brain and, at the same time, to reduce the long-term cardiometabolic risk.

Growth hormone therapy for children and adolescents with Prader-Willi syndrome is associated with improved body composition and metabolic status in adulthood

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Background: GH therapy is a worldwide-approved indication for treatment of children with Prader-Willi syndrome (PWS). GH therapy accelerates growth rate and improves body composition by decreasing fat mass (FM) and increasing lean body mass (LBM). However, the persistence of such GH-induced effects on body composition after discontinuation of therapy is still object of debate. The aim of this study was to assess body composition and metabolic status in adults with PWS treated with GH treatment during childhood and adolescence.

Methods: 64 adults (mean age 25.4 years) with genetically confirmed PWS were included. Anthropometry, body composition by DXA, resting metabolic rate (RMR) and metabolic biochemical parameters were assessed. Comparison was performed between GH-treated ($n = 20$) and untreated groups ($n = 40$). The mean duration of GH treatment in the treated group was 4.4 ± 2.7 years, and the mean time between the end of treatment and the current evaluation was 7.0 ± 4.4 years.

Results: GH treatment was associated with lower BMI and FM and higher LBM. Insulin sensitivity was higher in treated patients, whereas no difference in lipid profile was observed.

Conclusions: The beneficial effects of GH treatment on body composition and metabolic status of patients with PWS are still present even several years after discontinuation of treatment.

Prader-Willi syndrome (PWS) is a genetic disorder, caused by either a microdeletion on the paternally derived chromosome 15q11-13 or a uniparental maternal disomy affecting the same region [5]. In rare cases, PWS is due to an imprinting center mutation which results in silencing of genes that are normally active in the paternally inherited chromosome 15q11-13 [6]. PWS is characterized by muscular hypotonia, psychomotor delay, short stature and feeding difficulties in infancy. Thereafter, excessive appetite may result in rapid weight gain and obesity. Obesity in PWS is associated with increased FM and reduced LBM with reduction of energy expenditure and resting metabolic rate [7], ultimately increasing the metabolic risk of patients with PWS who are prone to develop type 2 diabetes mellitus, hypertension and dyslipidemia [8]. Whilst the efficacy of GH therapy in improving adult height and body composition in childhood and adolescence is well known, there was previously no evidence that the metabolic benefits persist after the end of GH treatment. This study has the merit to demonstrate, for the first time, that the GH beneficial effects on BMI, body composition and insulin sensitivity of subjects with PWS persist up to 7 years after discontinuation of therapy. The main limitations of the study are the relatively small size of the sample, the genetic heterogeneity of population, the lack of clinical and metabolic data at diagnosis and the unavailability of data on both physical activity and diet.

Adult height in short children born SGA treated with growth hormone and gonadotropin-releasing hormone analog: results of a randomized, dose-response GH trial

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Background: GH therapy for short children who were born small for gestational age (SGA) is a worldwide approved indication and is effective in partially reducing the adult height (AH) deficit. An earlier start of GH treatment is associated with a better growth outcome. However, many short children born SGA present to medical attention around the onset of puberty thus reducing the probability of therapeutic success. The aim of this trial was to test the efficacy of the combination of high dose GH with GnRH analog (GnRHa) therapy in improving AH of short SGA children.

Methods: The authors performed a randomized, dose-response GH trial in short SGA children ($n = 121$) of at least 8 years of age, evaluating GH doses 1 vs. 2 mg/m² daily from early puberty until adult height. 84 children reached AH. A subgroup ($n = 40$) of pubertal children with heights <140 cm at start was treated with GH+GnRHa for 2 years.

Results: Height improved from -2.9 SDS at start of treatment to -1.7 SDS at AH ($p < 0.001$). 62% (52 of 84) reached an AH >-2 SDS. AH was 0.6 SDS higher in children randomized to the higher GH dose. The growth outcome of children who started puberty at <140 cm and were treated with GH+GnRHa was similar to that of children who started puberty taller than 140 cm and received GH only.

Conclusions: (1) GH therapy increases AH even when started around puberty. (2) The high dose regimen (2 mg/m² daily) is more effective in improving AH. (3) The combined GH/GnRHa treatment could represent a strategy to improve AH in SGA children with more severe growth retardation at puberty.

Long-term GH treatment increases AH in children born SGA [9]. The main predictors of response to GH therapy are GH dose, age at the start of therapy, weight at the start of therapy, and mid-parental height [10]. In particular, the best growth outcome is observed in children who start GH therapy more than 2 years before the onset of puberty [11]. Lem et al. now show the possibility of a good response to GH even when therapy is started at puberty provided that high doses are used. In addition, it suggests that the combination of GH+GnRHa could represent a successful therapeutic approach in shorter SGA children. However, these results should be taken with caution. Firstly, the high dose of GH (2 mg/m² daily) was above and beyond the threshold of 50 µg/kg/day recently associated with increased risk of mortality [12] and resulted in significantly higher IGF-I levels, thus raising the issue of safety. Secondly, the combined GH/GnRHa therapy was not randomized, but was allocated on the basis of an arbitrary cut-off value of height (140 cm) at the onset of puberty. Although the authors found no adverse effects on bone mineralization and body composition [13, 14], the use of GnRHa in combination with GH has still to be considered experimental and even potentially detrimental for the growth outcome [15].

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: GH therapy improves height and body composition of children with PWS. The effects of such therapy on cognitive function are still uncertain. The aim of this study was to investigate the effect of long-term GH treatment on cognitive function in children with PWS.

Methods: 50 prepubertal children with genetically confirmed diagnosis of PWS were studied. Cognitive function was measured biennially. The study population was part of a randomized control trial (RCT) investigating the effects of GH treatment vs. no GH treatment. After 2 years, all children entered the treatment group and were continuously treated for 4 years. To assess intelligence, a short form of four subtests (Vocabulary, Similarities (verbal IQ subtests), Block design, and Picture arrangement (performance IQ subtests)) of the Wechsler Intelligence Scale for Children-Revised, Dutch version (WISC-R), was used in children over 7 years of age. A short form of four subtests (Vocabulary, Similarities (verbal IQ subtests), Block design, and Picture completion (performance IQ subtests)) of the Wechsler Preschool and Primary Scale of Intelligence-Revised, Dutch version (WPPSI-R) was used for children younger than 7 years of age. The total IQ (TIQ) score was estimated.

Results: During the first 2 years of RCT, mean SD scores of all subtests and mean TIQ score remained similar compared to baseline in GH-treated children with PWS, whereas in untreated patients a significant decrease for the Similarities and Vocabulary subtests in comparison with healthy controls was observed. No significant differences between treated and untreated groups were seen after 2 years. After 4 years of GH treatment, mean SD scores on the Similarities and Block design subtests were significantly higher than at baseline thus indicating that long-term GH treatment had significantly improved abstract verbal reasoning (Similarities subtest) and visuospatial skills (Block design subtest). Scores on Vocabulary and TIQ remained similar compared to baseline. At baseline, children with maternal uniparental disomy had a significantly lower score on the Block design subtest but a larger increment on this subtest during 4 years of GH treatment than children with a deletion.

Conclusions: GH therapy in children with PWS seems to be an effective treatment for improving cognitive functioning, especially their abstract verbal reasoning and visuospatial skills.

This study points out a positive effect of GH therapy on abstract verbal reasoning and visuospatial skills of children with PWS. GH regulates development and survival of neurons, astrocytes, and oligodendrocytes [16] either directly or indirectly through the local production of IGF-I. The lack of correlation between cognitive function and circulating IGF-I levels suggests that this potential effect is mainly mediated through autocrine/paracrine actions. Although the authors suggest that GH exerts its beneficial central actions by exploiting the plasticity of nervous system, it has to be considered that the mean age of study population was about 7 years, far from the age of maximum human brain plasticity (first 3 years of life). Two limitations of this study are the relatively small study cohort and the lack of a placebo-control group. Despite these weaknesses, this study could open avenues for further investigations on the effects of GH therapy on cognitive skills even in different conditions such as Turner syndrome or in children born small for gestational age.

Recommended IGF-I dosage causes greater fat accumulation and osseous maturation than lower dosage and may compromise long-term growth effects

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Background: Children with GH insensitivity syndrome (GHIS) are treated with exogenous IGF-I with doses ranging from 80 (low dose, LD) to 120 (high dose, HD) $\mu\text{g/kg}$ b.i.d., the latter representing the worldwide standard dose for treatment of GHIS. The aim of this study was to compare the promoting growth efficacy of low and high IGF-I dosage in children with GHIS.

Methods: 21 Ecuadorian children and adolescents with GHIS due to a splice site mutation on codon 180 of exon 6 of the GH receptor gene were studied. 7 patients were treated with LD and 14 with HD for 3 years. Children underwent anthropometry, bone age evaluation, DXA for body composition analysis, abdominal ultrasound scan and assessment of side effects.

Results: Growth velocity and height gain over the 3-year period were similar between the LD and HD groups, whereas the rate of bone maturation in HD patients was nearly twice as rapid as in LD children. Osseous maturation correlated with body fat and adrenal size increase. Percentage body fat increased significantly more in the HD group over the study period. Adrenal size increase over the first year of therapy was significantly higher in HD group. Kidney, liver, and spleen size increased similarly in the two groups.

Conclusions: LD IGF-I in children and adolescents with GHIS had a more favorable effect on the accretion of fat mass and advancement of osseous maturation, thus likely improving adult height. In addition, decreasing the IGF-I dose by one-third substantially reduced the cost/benefit ratio.

IGF-I is effective in promoting growth in children with severe primary IGF-I deficiency due to GHIS although the growth response is less than that observed when GH-deficient children are given GH replacement [17]. The first-year growth response is robust, with a tripling of baseline growth velocity; thereafter IGF-I-treated subjects grow at approximately normal rates and do not reach adult heights within the normal range. This unsatisfactory long-term response reflects the inability to replicate physiological IGF-I distribution and actions which involve both endocrine and paracrine/autocrine mechanisms [18]. As the exogenous administration of IGF-I does not correct the reduced concentrations of IGFBP-3 and acid-labile subunit [19], the ternary complex is not formed and the clearance of the administered IGF-I is accelerated. Finally, the lack of the direct actions of GH on growth cartilage may contribute to the poor growth response. The current study shows that the standard dose of IGF-I used in children with GHIS (120 $\mu\text{g/kg}$ b.i.d.) may be too high and have detrimental effects in the long run, accelerating bone maturation and increasing percentage body fat. The authors speculate that both fat mass accrual, with consequent enhanced androgen aromatization, and adrenal enlargement, leading to increased androgen secretion, may induce the acceleration of skeletal maturation. The use of a lower dose of IGF-I (80 $\mu\text{g/kg}$ b.i.d.) could be more effective in improving adult height as it stimulates linear growth to the same extent, but with reduced effects on osseous maturation and body fat accrual. Unfortunately, adrenal androgens were not measured and patients were not followed up to the achievement of adult height.

Significance of IGFBP-4 in the development of fetal growth restriction

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Background: Fetal growth restriction (FGR) is associated with high risk of perinatal morbidity and mortality as well as postnatal metabolic alterations. Experimental evidence indicates that IGF-II and IGFBP-4, an inhibitor of IGF activity, play key roles in the regulation of placenta growth and development. The aim of this study was to test whether IGFBP-4 was associated with the risk of FGR in humans.

Methods: Placental villi and tissues at maternal-fetal junctional zones were collected from 3 healthy donors during early pregnancy and immunohistochemistry for IGFBP-4 and pregnancy-associated plasma protein-A (PAPP-A) was performed. Serum samples from healthy non-pregnant and pregnant women during early, mid-, and late gestation were tested for IGF-I, IGF-II and IGFBP-4 by Western blotting (WB). A nested case-control study was conducted to measure circulating levels of IGF-I, IGF-II, IGFBP-4, and PAPP-A in early gestation in 36 women who delivered a fetus with FGR and 36 controls who had normal-weight babies.

Results: IGFBP-4 and PAPP-A were highly expressed by decidual cells and extravillous trophoblasts at the maternal-fetal interface. Circulating IGFBP-4 protein concentrations were highest in early pregnancy, whereas PAPP-A content increased in the maternal circulation as gestation progressed. Elevated maternal IGFBP-4 concentrations in early gestation were associated with the development of FGR.

Conclusions: IGFBP-4 concentrations in the maternal circulation in early pregnancy are closely related to the risk of FGR. This finding raises the possibility of clinical use of IGFBP-4 as an early biomarker for this condition.

Intrauterine growth retardation (IUGR) is associated with increased perinatal morbidity and mortality and is linked to a higher incidence of adult-onset diseases such as hypertension, diabetes, hyperlipidemia, and cardiovascular diseases [20]. Unfortunately, it is currently impossible to accurately predict, prevent, or treat IUGR. IGF-II is expressed in early pregnancy by maternal, fetal, and placental tissues and regulates placenta growth and development, thereby controlling nutrient transfer to the developing fetus [21, 22]. IGF bioavailability is regulated by at least six different binding proteins (IGFBP-1 to IGFBP-6). IGFBP-4, a potent inhibitor of IGF actions, is the second most abundant IGFBP in the placental bed, being expressed exclusively by the maternal decidua. IGFBP-4 activity, in turn, is directly regulated through the proteolytic activity of pregnancy-associated protein A (PAPP-A) [23]. Proteolysis of IGFBP-4 results in decreased affinity for IGF-II peptide, thereby enhancing local IGF-II actions [24]. This study shows that elevations in circulating levels of IGFBP-4 in the maternal circulation during the first trimester of pregnancy are strongly associated with the development of IUGR. A likely explanation for this elevated IGFBP-4 is deficiency in IGFBP-4 proteolysis by PAPP-A [24]. The high concentrations of IGFBP-4 would inhibit the IGF-II growth-promoting action in placenta, eventually leading to IUGR. Consistent with this, PAPP-A knockout in mice results in increased circulating maternal IGFBP-4 levels and a 34% growth deficiency compared with wild-type littermates [25]. This study also reveals that PAPP-A is present at the maternal-fetal interface, and is expressed by extravillous trophoblasts and maternal decidual cells. The colocalized expression of IGFBP-4 and PAPP-A at this interface highlights PAPP-A's proteolytic processing function of IGFBP-4 at the maternal-fetal interface. PAPP-A is exclusively expressed by syncytiotrophoblasts, directly in contact with the maternal circulation, thus representing the primary source of this protease within the maternal circulation. The clinical implication of these findings is that IGFBP-4 could be used as a specific biomarker for the risk of IUGR. The odds ratio for IUGR associated with high IGFBP-4 in early pregnancy reached 22 (95% CI 2.7–181), which is much higher than any other reported single biomarker. Therefore, IGFBP-4 may represent an early and reliable predictor of IUGR, thus permitting early identification of which pregnancies may warrant closer follow-up and potential therapeutic interventions. The major limitation of this study was the use of a semiquantitative method such as WB for measuring IGFBP-4 levels. The results need to be validated by a quantitative assay for the measurement of IGFBP-4 in maternal serum.

Maternal and fetal IGF-I and IGF-II levels, fetal growth, and gestational diabetes

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Background: The relationships between maternal IGF-I and IGF-II concentrations and fetal growth are still uncertain. Gestational diabetes is a common cause of fetal macrosomia. The aim of this study was to determine the associations between maternal and fetal levels of IGF-I and IGF-II with fetal growth and gestational diabetes.

Methods: Maternal and cord blood specimens from a singleton pregnancy cohort (n = 307) were used for measuring maternal and fetal IGF-I and IGF-II. Maternal venous blood specimens were collected at 24–28 and 32–35 weeks of gestation. Cord venous blood specimens were collected immediately after delivery. All pregnant women were studied by anthropometry and OGTT. Birth weight was the primary outcome measure.

Results: Both maternal IGF-I and IGF-II concentrations rose from 24–28 to 32–35 weeks of gestation (average increase 55.4 and 11.8% respectively). IGF-I levels in both maternal and fetal circulations were significantly higher in gestational diabetic vs. non-diabetic pregnancies, whereas IGF-II levels were similar. Maternal IGF-I, but not IGF-II, levels were correlated with birth weight and placental weight. IGF-I, but not IGF-II, concentrations were correlated with insulin and proinsulin concentrations in both maternal and fetal circulations. After adjusting for maternal characteristics, gestational age at blood sampling, infant sex, and gestational age, each SD increase in plasma concentration was associated with a birth weight increase of 75 g for maternal IGF-I at 24–28 weeks, 86 g for maternal IGF-I at 32–35 weeks, and 68 g for the change in IGF-I concentration between the two gestational age windows, respectively. The corresponding increases in placental weight were 20, 25, and 22 g, respectively.

Conclusions: Maternal IGF-I (but not IGF-II) levels may be predictive of fetal and placental growth. IGF-I, but not IGF-II, is associated with fetal hypertrophy in gestational diabetes.

The IGFs play important roles in regulating and controlling placental development and growth, but data on the role played by maternal IGFs in promoting fetal growth are conflicting [26]. This study conducted on a large number of singleton pregnancies reveals that maternal IGF-I increases at mid- and late gestation and is closely related to fetal growth in both diabetic and non-diabetic mothers. Although a concomitant rise in maternal IGF-II was observed, no association between IGF-II and fetal or placental growth was found. However, maternal IGF-I is unlikely to have a direct effect on fetal growth because IGF-I does not cross the placental barrier. The positive association between maternal IGF-I and placental weight suggests that maternal IGF-I level may represent a biomarker of placental function. The potential clinical implication of these findings is the use of maternal circulating IGF-I concentrations as an indicator of placental health and fetal growth.

Early origins of the metabolic syndrome: role of small size at birth, early postnatal weight gain, and adult IGF-I

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Background: Low birth weight is associated with high risk of developing metabolic disorders in adult life, but the mechanisms underlying this association have not yet been elucidated. The aim of this study was to investigate the effect of the growth pattern during the first year of life on long-term metabolic risk.

Methods: In 280 born at term young adults, the associations of low birth weight, weight gain in the first year of life and current IGF-I values, with the components of metabolic syndrome (MetS) were investigated.

Results: Gain in weight SDS in the first 3 months of life was associated with an increased number of MetS components at the age of 21 years, adjusted for age, gender, gestational age, socioeconomic status, and gain in length in the same period. Per 1 SDS increase in weight gain, the chance of having a higher number of MetS components increased by 34%. Early weight gain was also significantly associated with the prevalence of low high-density lipoprotein cholesterol, prevalence of MetS, increased C-reactive protein levels, and lower insulin sensitivity. Low birth weight and adult IGF-I levels were not associated with any of the MetS components or MetS prevalence at 21 years.

Conclusions: The reported association of low birth weight with MetS in adulthood is mainly due to an accelerated weight gain in the first 3 months after birth. This finding points to the need to identify the optimal target of early postnatal weight gain in all infants, regardless whether they are born SGA or appropriate for gestational age.

Low birth weight has been associated with cardiovascular disease and type 2 diabetes in adulthood [3]. The importance of the growth trajectory in early postnatal life as an additional determinant of the long-term metabolic risk has been progressively realized over the last two decades [1, 27, 28]. The findings of this study, showing a significant association between early weight gain and MetS components in young adulthood, are consistent with their previous report showing that a rapid growth in the first 3 months of life is associated with several determinants of adulthood cardiovascular disease and type 2 diabetes [29]. In addition, the authors extend the previous investigation by measuring IGF-I levels in adulthood. IGF-I could potentially contribute to the relationship between SGA birth and later risk for MetS. Decreased serum levels of IGF-I have been reported in adults born SGA [30] and low IGF-I levels have been associated with each of the components of MetS [31]. However, no significant relationship between IGF-I and MetS components was found. Altogether these observations suggest a detrimental effect of early growth acceleration on metabolic status in adulthood, and indicate that weight gain during the first months of postnatal life deserves careful monitoring to avoid overgrowth in both SGA and AGA children. The mechanisms underlying this association remain unknown. Early nutrition could induce permanent changes having adverse effects on cardiovascular risk factors later in life. Consistent with this, formula-fed infants grow at a faster rate than breast-fed infants and have a higher risk of being overweight later in life [32]. Genetic predisposition could represent the key factor in determining both early growth acceleration and metabolic risk independently of birth size.

Protection of blood retinal barrier and systemic vasculature by insulin-like growth factor-binding protein-3

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Background: IGFBP-3 has been shown to act as a vasoprotective substance, facilitating perfusion to ischemic areas, reducing endothelial apoptosis, recruiting precursor cells to sites of injury, and preventing microvascular leakage. The aim of this study was to test whether IGFBP-3 influences blood retinal barrier (BRB) integrity and function, in developing mouse retina and in vitro.

Methods: After injection of IGFBP-3 plasmid into the eye of C57BL/6 mice on postnatal day 1, on postnatal day 7, mice were placed in a 75% oxygen atmosphere for 5 days. The barrier properties of retinal vessels in the mouse oxygen-induced retinopathy (OIR) model were determined by intravascular injection of horseradish peroxidase on postnatal day 17. To examine the direct effect of IGFBP-3 on vasculature, an ex vivo whole vessel model based on rat cerebral arteries assessment was used. Posterior cerebral arteries (PCAs) were isolated and IGFBP-3 and the non-IGF-binding mutant were applied intraluminally. To evaluate constriction to different pressures, intraluminal pressure was progressively increased. To characterize the impact of IGFBP-3 on the BRB, immunohistochemistry of the adherence junction protein, VE-cadherin and of the tight junction protein, claudin-5, was performed. The nitric oxide (NO) generation in intact arteries and the expression of scavenger receptor-B1 (SRB1) in PCAs were determined.

Results: IGFBP-3 overexpression by the retinal endothelium restored BRB integrity following hyperoxia-induced injury, and corrected the retinal morphology of OIR mice towards normal. When applied intraluminally, IGFBP-3, independently of IGF-1, reduced the induced vasoconstriction through NO release via SRB1 activation.

Conclusions: This study suggests that IGFBP-3 directly affects vascular tone and may represent a therapeutic candidate for ocular complications, such as diabetic retinopathy or retinopathy of prematurity.

This complex study evaluated the protective effect of IGFBP-3 in the retina of mouse pups exposed to oxygen-induced retinopathy (OIR). The local expression of IGFBP-3 preserved the blood retinal barrier integrity and promoted vasodilation, independently of the IGFBP-3 IGF-binding property. Previous studies showed that IGFBP-3 expression increases in response to hypoxia, suggesting that it may contribute to the physiological response of a tissue to hypoxic injury [33]. The results of the present study elegantly demonstrate that IGFBP-3 exerts direct positive effects on BRB function, protects endothelium from VEGF-induced disruption, and induces vasodilation. These novel actions are linked to the ability of IGFBP-3 to stimulate physiological NO generation by the endothelium through a cascade of events involving SRB1. Therefore, this study, for the first time, sheds light on a novel physiological role of IGFBP-3 whose local release following injury may represent a generalized compensatory mechanism or a response to cellular or tissue stress.

Interaction between IGF-binding protein-3 and TGF- β in the regulation of adipocyte differentiation

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Background: The development and expansion of white adipose tissue involves both the hypertrophy of existing adipocytes and the proliferation and differentiation of precursor cells (preadipocytes). TGF- β is a potent inhibitor of adipogenic differentiation through the activation of Smad2/3 signaling. IGF-binding protein-3 (IGFBP-3) is also known to activate Smad2/3 signaling in some cell types. Exogenous IGFBP-3 or overexpressed IGFBP-3 is known to inhibit adipogenesis in 3T3-L1 cells. The aim of this study was to investigate the role of endogenous IGFBP-3 in preadipocyte differentiation.

Methods: Murine 3T3-L1 preadipocytes differentiation was induced by insulin, 3-isobutyl-1-methylxanthine (IBMX), and dexamethasone. The expression and/or secretion of IGFBP-3, TGF- β , adiponectin, and resistin were determined. Two small interfering RNA (siRNA) duplexes targeting the endogenous IGFBP-3 gene were used to induce IGFBP-3 knockdown.

Results: During adipocyte differentiation, 3T3-L1 cells expressed increasing levels of IGFBP-3 and TGF- β_1 . Smad2 phosphorylation in 3T3-L1 preadipocytes significantly increased after exposure to both TGF- β and IGFBP-3 but no additive effect was observed for the two agents. The knockdown of endogenous IGFBP-3 by siRNA significantly impaired Smad2 activation by TGF- β_1 . The expression of human IGFBP-3 significantly inhibited the induction of adipogenic markers and the appearance of lipid droplets. Downregulation of endogenous IGFBP-3 reversed the inhibitory effect of TGF- β_1 on both adiponectin and resistin induction.

Conclusions: IGFBP-3 activates inhibitory Smad signaling in 3T3-L1 cells and influences the extent of preadipocyte differentiation by affecting 3T3-L1 sensitivity to the inhibition by TGF- β .

In experimental conditions, exogenous IGFBP-3 inhibits adipocyte differentiation [34]. However, the role of endogenous IGFBP-3 in regulating adipogenic differentiation in vivo has been difficult to elucidate. Previous evidence showed that IGFBP-3 activates signaling through the TGF- β receptor pathway, inducing phosphorylation of the receptor-regulated Smads, Smad2 and Smad3 [35, 36]. Since both Smad2 and Smad3 inhibit adipogenesis, IGFBP-3 could negatively regulate adipogenic differentiation through modulation of TGF- β signaling. The present study provides evidence for a permissive role of endogenous IGFBP-3 on TGF- β action in the 3T3-L1 differentiation process. This represents a novel physiological action of IGFBP-3 which, in an IGF-independent fashion, could be implicated in the regulation of adipogenesis and, potentially, fat mass accrual. These results have been obtained in a murine cellular model (3T3-L1) and it is, therefore, arduous to extrapolate these

in vitro data to human physiology. However, this study opens avenue for further investigations aimed at determining the IGFBP-3 effects on development and differentiation of adipose cells in vivo and the potential involvement of IGFBP-3 in the development of obesity and its metabolic complications.

Knockout of insulin-like growth factor-1 receptor impairs distal lung morphogenesis

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Background: Mice lacking IGF-1 receptor (IGF-1R) reach only 45% of normal birth size, are unable to expand their lungs and die shortly after birth. Similarly, mice lacking IGF-I are markedly growth-retarded and show high postnatal mortality due to hypoplastic lungs. The aim of this study was to investigate the role of IGF signaling in lung development.

Methods: IGF receptor null (IGF-1R^{-/-}) and IGF-1R knockdown mice (IGF-1R^{neol/-}) expressing only 22% of wild-type IGF-1R levels in lung tissue were generated. Lung morphometry of adult mice and embryos was performed. The ventilatory capacity was tested in young adult males.

Results: IGF-1R^{neol/-} mice showed normal lung morphometry and normal breathing response to hypercapnia. IGF-1R^{-/-} mice showed cyanosis, agonal breathing, and death from hypoxia soon after birth. Analysis of lung morphology showed lung hypoplasia and markedly underdeveloped diaphragms. E17.5 knockout lungs contained significantly less epithelium and less alveolar space than controls.

Conclusions: Lung development progresses to completion in knockdown IGF-1R^{neol/-} mutants, demonstrating that partial IGF-1R inactivation is well tolerated. Complete IGF-1R inactivation, in contrast, produces severe delay in lung maturation in utero, leading to lung hypoplasia and entailing neonatal death.

IGF-I regulates growth, development and survival of multiple cell types by binding to the tyrosine kinase receptor IGF-1R. From E13.5 onward, IGF-1R interacts with both IGF-I and IGF-II [36]. Null mutants for the IGF-1R gene die invariably at birth of respiratory failure and exhibit severe growth deficiency (45% normal size). Several lines of evidence suggest an active role of IGF-1R signaling in lung development. IGF signaling induces alveolar and vascular maturation of fetal lung development and IGF-1R signaling is also involved in vascularization and angiogenesis of human fetal lungs [37]. The present study focused for the first time on lung structure and function in IGF1R mutant mice at different stages of development, showing that whereas animals with IGF-1R knockdown have normal lung morphology and breathing, IGF-1R knockout animals show marked delay in cell differentiation and arrest in the canalicular stage of prenatal respiratory organ development. These experimental findings are consistent with the description of a lung hypoplasia in patient with deletion of the distal long arm of chromosome 15, where the IGF-1R gene maps [38].

New paradigms

Circulating insulin-like growth factors may contribute substantially to insulin receptor isoform A and insulin receptor isoform B signaling

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Background: Serum contains a higher amount of total insulin-like bioactivity than immunoreactive insulin, and anti-insulin antibodies only block a small portion of the total serum insulin bioactivity. In the

human body, due to alternative splicing of exon 11 of the insulin receptor (IR) gene, two IR transcripts are generated, resulting in IR isoform A (IR-A) (lacking exon 11) and in IR isoform B (IR-B) (full length). IR-A is the predominant isoform in fetal tissues and cancer cells, while the IR-B is the classical receptor for insulin with metabolic effects in muscle, liver and adipose tissues. IGF-I and IGF-II bind to both IR-A and IR-B, but the respective contribution to the total insulin-like activity is still uncertain. The aim of this study was to determine the potential biologic actions of serum on both IR-A and IR-B and to assess the relative contribution of circulating IGFs in this respect.

Methods: Cell-based kinase receptor activation (KIRA) assays were used, one specific for the human IR-A and one specific for the human IR-B. Since there are no specific antibodies available for the two isoforms, specificity was determined by transfecting human embryonic kidney (HEK) cell line with either the IR-A or the IR-B. The principle of these two assays is based on quantification of phosphorylated tyrosine residues within the IR after in vitro stimulation with serum. Transfected HEK IR cells were stimulated with either serial dilutions of insulin, IGF-I or IGF-II or human serum.

Results: Analysis of serum samples showed that there was a significant positive correlation between serum insulin-like and immunoreactive insulin concentrations (IR-A: $r = 0.56$, $p = 0.01$, IR-B: $r = 0.68$, $p = 0.001$). The addition of IGF-I or IGF-II antibodies to human serum samples substantially decreased the endpoint signal in both KIRA assays.

Conclusions: IGF-I and IGF-II present in human serum may contribute substantially to IR-A and IR-B signaling in vitro. This KIRA-based approach may help to gain more insight into the roles of IGF-mediated IR-A and IR-B activation in health and disease.

IGF-I and IGF-II primarily activate the IGF-I receptor (IGF-IR), but they can also activate the IR. The impact of IR-mediated IGF actions on metabolism is still uncertain, although in vitro and in vivo studies suggest that both IGFs exert an insulin-like activity in specific tissues thus contributing to the regulation of metabolism [39]. The present study shows that IGF-I and IGF-II present in human serum may contribute substantially to IR-A and IR-B signaling in vitro. It is noteworthy that physiological fasting insulin levels are around 50 pmol/l, whereas IGF-I concentrations are around 20 nmol/l and IGF-II around 80 nmol/l. As free IGFs are around 2% of the total IGF concentration, the total amount of free circulating IGFs is around 2,000 pmol/l. Although the affinity of insulin for the IRs is 10- to 100-fold higher than of IGF-I and IGF-II affinity, the insulin-like activity attributable to free IGFs is about 40-fold compared to insulin. The translation of this notion based on circulating levels of the molecules into clinical aspects is not easy as multiple mechanisms intervene in the tissues to reduce IGF bioactivity such as locally produced IGF-binding proteins (IGFBPs) and IGFBP proteases which modulate the IGFBP affinity for IGFs. Nonetheless, clinical examples of the potential importance of this IGF-mediated insulin-like activity have been reported. The extrapancreatic tumor hypoglycemia syndrome has been related to the secretion of big insulin-like growth factor (IGF)-II by the tumor [40]. Furthermore, IGF-I improves glucose and lipid metabolism in type 2 diabetes [41] and reduces hyperglycemia in patients with extreme insulin resistance [42].

New perspective

Longitudinal infusion of a complex of insulin-like growth factor-I and IGF-binding protein-3 in five preterm infants: pharmacokinetics and short-term safety

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Background: IGF-I is essential for normal growth and development of fetus. After preterm birth, serum concentrations of IGF-I fall rapidly below normal intrauterine levels. These low levels of IGF-I are associated with impaired brain growth and retinopathy of prematurity (ROP). Similarly, low serum IGFBP-3

levels in preterm infants are associated with the development of ROP. Treatment with IGF-I/IGFBP-3 may therefore be beneficial for brain development and may reduce the risk of ROP. The aim of this study was to determine the pharmacokinetics and short-term safety of continuous infusion of rhIGF-I administered with its binding protein-3 (rhIGFBP-3) in a small group of very preterm infants.

Methods: The authors performed a phase II pharmacokinetics and safety study in 5 very preterm infants with a median (range) gestational age of 26wk+6d (26wk+0d to 27wk+2d) and birth weight of 990 (900–1,212) g. Continuous intravenous infusion of recombinant human (rh)IGF-I/rhIGFBP-3 was initiated during the first postnatal day and continued for a median (range) duration of 168 (47–168) h, in doses between 21 and 111 µg/kg/24 h.

Results: The concentrations of IGF-I and IGFBP-3 in serum increased during infusion of an equimolar preparation of rhIGF-I/rhIGFBP-3. The predicted dose of the rhIGF-I/rhIGFBP complex required to establish circulating levels of IGF-I within the reference intrauterine range in a 1,000-gram infant was between 75 and 100 µg/kg/day. None of the infants developed hypoglycemia, severe ROP or any degree of cerebral intraventricular hemorrhage, or any evidence of intracranial hypertension. None of the infants developed signs of tonsillar hypertrophy.

Conclusions: The infusion of rhIGF-I/rhIGFBP-3 during the first week of life seems to be an effective and safe treatment to increase IGF-I levels in extremely preterm infants.

Decreased levels of IGF-I during postnatal development in preterm infants have been associated with impaired brain growth (as determined by lower head circumference and magnetic resonance imaging at term age), and with severe ROP [43–45]. However, replacement therapy with rhIGF-I alone is unlikely to be effective due to the short half-life (<1 h) of circulating IGF-I in preterm infants. This rapid clearance of IGF-I from circulation is probably secondary to the inability of preterm babies to form the ternary complex [46]. Therefore, the infusion of a combined rhIGF-I/rhIGFBP-3 preparation could represent a successful strategy to induce a sustained increase of circulating IGF-I in preterm neonates. In this study, the continuous intravenous infusion of recombinant human (rh)IGF-I/rhIGFBP-3 in preterm infants normalized IGF-I circulating levels without significant side effects. Unfortunately, after completion of 7 days of infusion, serum IGF-I levels fell below intrauterine concentrations for corresponding gestational age, indicating that infusion should be continued for more than 7 days to maintain physiological IGF-I concentrations during the early postnatal period. The study was performed in only 5 preterm neonates, nonetheless these preliminary results are promising and warrant future studies aimed at evaluating the optimal treatment duration, and assessing the efficacy of prolonged continuous administration of rhIGF-I in preventing brain alterations and ROP in very preterm infants.

Food for thought

Effects of breastfeeding on body composition and maturational tempo in the rat

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Background: Weaning from lactation is the hallmark of the infancy-to-childhood transition (ICT). ICT is itself responsive to sex, stress and other environmental cues that are presumed to inform the developing organism about risks and opportunities in its current and future environment. The aim of this study was to experimentally test the hypothesis that the age of weaning affects growth, maturation, and developmental tempo and litter size. The effects of weaning were investigated across four generations.

Methods: Length and body mass index (BMI), as well as physiological development and sexual maturation, were repeatedly measured in rat pups weaned early (d16), normally (d21) or late (d26). Males were bred to females of the same weaning age group for four generations.

Results: The weaning age influenced the length and adiposity of rats from infancy through adulthood, with short lactation resulting in a thin/long phenotype and long lactation in a short/heavy phenotype. The thin/long habitus of the d16-weaned group was associated in adulthood (d80–d90) with greater glucose tolerance and insulin sensitivity. The offspring of early-weaned d16 parents, compared to d26 offspring, had greater body fat mass and smaller lean body mass. Whereas F1 animals showed no difference across the original age-of-weaning groups, F2 offspring of early-weaned parents accelerated their pre-weaning infantile development as compared to offspring of late-weaned parents. This difference in developmental tempo was sustained for F3 to F4 pups. The early-weaned F2 females showed earlier vaginal opening and estrus and early-weaned males showed earlier onset of testicular growth and attainment of maximal testicular volume compared to late-weaned rats. These traits were enhanced from F2 to F4 generations in both female and male rats. In generations 3 and 4, early-weaned rats bear larger litter sizes and heavier newborn pups.

Conclusions: The duration of infancy, as indexed by weaning age, predicts and perhaps programs growth, body composition, and tempo of physiological development and maturation, as well as litter size and parity and, thereby, reproductive strategy.

Multiple genetic and environmental factors determine how and when the organism grows and matures. The time window particularly susceptible to environmental cues is intrauterine and early postnatal life, which is characterized by a high degree of plasticity that enables the organism to adapt to the current and, perhaps, to the future environment. The ICT, marked by the weaning from breastfeeding, demarcates the transition from maternal provision, protection and support to greater independence. This intriguing study shows that the duration of infancy is closely related to the developmental tempo and pubertal maturation. These data suggest that early weaning age influences the subsequent development in a manner consistent with an insecure, fast, life-history strategy, as indicated by accelerated growth, development and maturation, long/thin stature and, in subsequent generations, large litter size. On the contrary, a prolonged lactation would influence the development in a manner consistent with a secure, slow, life-history strategy, as suggested by the slower growth, development and maturation, short/overweight stature, and, in subsequent generations, small litter size. The duration of infancy/breastfeeding would therefore represent another modality of programming to better adapt the organism to the present and predicted future environment. Although fascinating, these results obtained in rodents appear, at the moment, hardly transferable to humans whose growth and development outcomes are influenced by many further potential determinants such as lifestyle, diet, epigenetics and psychological factors. Nonetheless, longitudinal studies in humans are warranted to investigate the existence of a similar programming in our species.

Mechanism of the year

Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells

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Background: IGF-1 is a key factor not only in the acquisition of peak bone mass (PBM), but also in the maintenance of bone mineral density (BMD). IGF-I is the most abundant growth factor in bone matrix and stimulates the differentiation of mesenchymal stem cells (MSC) in vitro. The aim of this study was to investigate the effects of bone matrix released IGF-I on bone architecture and mass.

Methods: Mice were generated with knockout of the IGF-1 type 1 receptor (*Igf1r*) gene in osteoprogenitor cells committed to the osteoblast lineage.

Results: The knockout of *Igf1r* impaired bone formation in adult mice by reducing the number of mature osteoblasts. IGF-1 was shown to activate mTOR through the IRS1-PI3K-Akt pathway to regulate the

osteoblast differentiation of MSCs for bone formation. IGF-1 released from the bone matrix induced the osteoblast differentiation of MSCs recruited by TGF- β_1 in the process of coupling bone resorption with bone formation. The concentrations of IGF-1 in the bone matrix and marrow of aged rats were lower than in those of young rats and directly correlated with the age-related decrease in bone mass. Likewise, in age-related osteoporosis in humans, bone marrow IGF-1 concentrations were 40% lower in individuals with osteoporosis than in individuals without osteoporosis. Finally, the combined administration of IGF-1+IGFBP-3 attenuated bone loss.

Conclusions: IGF-1 regulates new bone formation by acting as a differentiation factor on the osteoblast differentiation of MSCs through activation of the mTOR signaling pathway. As the pool of IGF-1 in the bone matrix decreases during the aging process, the induction of IGF-1 deposition in the bone matrix could potentially be a therapeutic approach to delay or prevent osteoporosis.

IGF-1 plays a key role in bone formation and maintenance [47]. The maintenance of bone mass is accomplished by the coordinated activities of osteoblasts and osteoclasts which determine the skeletal remodeling. This experimental mouse knockout study shows that IGF-1 induces differentiation of MSCs into osteoblasts through the activation of mTOR, thus contributing to generate an osteogenic microenvironment. mTOR-mediated signaling contributes to both the whole organ and to cellular energy metabolism in response to nutrient availability and environmental stimuli [48]. The downregulation of mTOR pathway increases lifespan and stem cell homeostasis in diverse species including mammals [48]. The aging-associated decline in local IGF-1 is clearly associated with impairment of bone remodeling thus exposing the bone to demineralization and ultimately leading to osteoporosis. Consistent with this, in humans, skeletal IGF-1 declines by almost 60% between the ages of 10 and 60 years [49]. Interestingly, the administration of IGF-1 in combination with IGFBP-3, but not IGF-1 alone, was able to increase the local pool of IGF-1 and stimulate bone formation. This finding is consistent with the results of a trial in humans showing beneficial effects of an IGF-1/IGFBP-3 preparation on bone mass, muscle strength, and functional ability [50]. Therefore, this study provides the rationale for further clinical trials aimed at assessing the effects of IGF-1/IGFBP-3 on bone health in aging as well as in pediatric conditions associated with altered bone remodeling, such as osteogenesis imperfecta or osteoporosis secondary to neuromuscular diseases, chronic illnesses, glucocorticoid treatments, chemotherapies and radiotherapies [51].

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