Growth and Growth Factors

Elena Inzaghi^a and Stefano Cianfarani^{a,b}

^aDPUO 'Bambino Gesù' Children's Hospital – 'Tor Vergata' University, Rome, Italy and

^bDepartment of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

The majority of articles selected for this chapter refer to novel pathophysiological mechanisms that have recently been elucidated. Levels of expression and methylation as well as specific polymorphisms in the *IGF2* gene were shown to play a key role not only in fetal growth but also in early postnatal weight gain. IGFBP-4 was reported to be a key mediator between nutrition and adipose tissue expansion. FGF21 was shown to exert anti-inflammatory and antifibrotic actions in adipose tissue of *db/db* mice and to improve functional and morphological features of kidneys in diabetic nephropathy. IGF-I was revealed to stabilize the atherosclerotic plaque by enhancing the synthesis of collagen type 1. GH, through the activation of EGF receptor, was described to restore the liver regeneration capacity impaired by steatosis. Interplay between SIRT1 and the GH/IGF-I axis was reported to represent a crucial adaptive response to fasting in the liver. GH was even demonstrated to block the progression to type 1 diabetes in NOD mice by favorably modulating the immune response. Finally, the mechanism of the year is undoubtedly the elucidation of JAK2 activation by the GH receptor, which represents a molecular model common to all class I cytokine receptors. All of these novel mechanisms and actions were identified in animal and/or cellular models, thus making their translation into human physiology as well as clinical utility a working hypothesis to be tested in the near future. Nevertheless, this body of experimental knowledge about mechanisms directly or indirectly involved in many human pathologies may be considered a significant leap forward for the development of new therapeutic strategies based on the modulation of growth factors.

Important for clinical practice

Paternally expressed, imprinted insulin-like growth factor-2 in chorionic villi correlates significantly with birth weight

Demetriou C, Abu-Amero S, Thomas AC, Ishida M, Aggarwal R, Al-Olabi L, Leon LJ, Stafford JL, Syngelaki A, Peebles D, Nicolaides KH, Regan L, Stanier P, Moore GE

Fetal Development and Growth Research Group, Clinical and Molecular Genetics Unit, Institute of Child Health, University College London, London, UK

gudrun.moore@ucl.ac.uk

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Background: The complex interaction between genes and environment leading to appropriate fetal growth and development has not yet been elucidated. Whereas animal models have revealed the important role played by the IGF system in regulating fetal growth, in humans the results are still conflicting. *PHLDA2* (pleckstrin homology-like domain family A member 2) has been implicated in the regulation of growth in utero. This study aimed at investigating whether the expression levels in early gestation of *IGF1*, *IGF2*, their receptors (*IGF1R* and *IGF2R*), and of *PHLDA2* are associated with birth weight (BW).

Methods: 260 chorionic villus samples (CVS) were collected at 11–13 weeks of gestation. mRNA expression of *IGF1*, *IGF2*, *IGF1R*, *IGF2R* and *PHLDA2* was assessed by PCR. The statistical correlation with term BW was corrected for various confounding factors.

Results: Transcript levels of *IGF2* and *IGF2R* (but not *IGF1*, *IGF1R* and *PHLDA2*) were significantly associated with BW. Consistently, small for gestational age (SGA) newborns showed lower *IGF2* expression compared to appropriate for gestational age (AGA) newborns.

Conclusions: This study suggests that *IGF2* expression at 12 weeks of gestation is predictive of fetal growth. *IGF2* may represent a marker for very early detection of SGA risk.

Low BW is associated with higher risks of perinatal morbidity and mortality as well as cardiometabolic diseases in adulthood [1]. 'In utero programming' has been proposed to explain the association between intrauterine growth restriction and metabolic risk in postnatal life. Reduced nutrient transfer from mother to fetus may affect fetal growth and development, by influencing production and function of insulin-like growth factors (IGFs). The role of the IGF system in fetal growth has been widely established in experimental mouse models. In humans, *IGF2* is a maternally imprinted gene and therefore a paternal-expression driven growth promoter. Its encoded protein has well-known effects on fetal growth. *Igf2* knockout mice show severely impaired fetal growth [2]. In humans, disordered *IGF2* expression results in syndromes of either overgrowth or restricted growth. *IGF1* and *PHLDA2* (pleckstrin homology-like domain family A member 2) also play key roles in fetal growth [3]. *IGF-I* levels are reduced in placental tissues of SGA babies [4].

Previous studies of human placental expression of *IGF1*, *IGF2*, and the type 1 (*IGF1R*) and type 2 IGF receptor (*IGF2R*) genes have been confined to samples obtained at birth. This study collected villus samples in the first trimester of pregnancy and provides the first evidence for an early role of *IGF2* in the regulation of fetal growth. The expression of *IGF2* and the ratio of *IGF2* to *IGF2R* were closely related to birth weight. Moreover, SGA newborns had reduced expression of *IGF2*. Interestingly, *IGF2R* was also directly related to birth weight though less strongly than *IGF2*. *IGF2R* encodes a non-functional receptor which mainly acts as IGF-2 scavenger. The authors speculate that *IGF2* and *IGF2R* may exert opposite effects on fetal growth, reflecting a closely regulated growth process. When *IGF2* expression increases, the levels of *IGF2R* also increase, resulting in greater clearance of IGF-2, thus maintaining 'optimal' levels of IGF-2 activity. Surprisingly, *IGF1* expression in chorionic villi was not associated with birth weight, which contradicts previous studies showing a key role of *IGF1* in the regulation of both pre- and postnatal growth [5]. This finding suggests that *IGF1* may be more relevant to later fetal growth. The clinical implication is that *IGF2* expression in early pregnancy may represent a marker of poor fetal growth and SGA at birth, thus leading to a closer pregnancy monitoring and, potentially, early therapeutic interventions.

Low incidence of pathology detection and high cost of screening in the evaluation of asymptomatic short children

Sisley S, Trujillo MV, Khoury J, Backeljauw P

Division of Pediatric Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Stephanie.sisley@bcm.edu

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Background: Short stature may represent either a normal variant of growth or may indicate underlying pathology. According to the guidelines of the consensus conference on the Diagnosis and Treatment of Children with Idiopathic Short Stature (ISS), ISS is defined as height shorter than -2 SD scores without any evidence of systemic, endocrine, nutritional, or chromosomal abnormalities. These guidelines recommend biochemical assessment in all patients 'for whom the history and physical exam do not suggest a particular diagnosis'. However, the cost-effectiveness of this approach has not been tested. The aims of this study were (1) to identify the incidence of pathologies found during routine screening of healthy short children, (2) to define the adherence to the current guidelines for diagnosis and treatment of children with idiopathic short stature (ISS) and ultimately (3) to determine the cost-effectiveness of universal height screening.

Methods: A retrospective review of 1,373 subjects referred for short stature was performed. 235 asymptomatic patients (normal history and physical examination) who met the consensus criteria for ISS were detected. Outcomes were the incidence of discovered pathologies, diagnostic group features, clinicians' adherence to testing guidelines, and screening costs.

Results: 98.7% (232) of short children had no pathology identified on laboratory/radiologic tests; of these, 23% (53) had familial short stature (FSS), 41% (95) had constitutional delay of growth and maturation (CDGM), and 36% (84) were considered ISS. Only 3 (1.3%) patients had an underlying pathology: 1 had celiac disease confirmed by biopsy, 1 had celiac disease unconfirmed by biopsy, and 1 had a possible *IGF1R* defect; these 3 patients had significantly lower height SDS than other groups. On average, each patient received 64.3% of the tests recommended by the guidelines; complete assessment was performed in only 2.1% of patients. The total screening cost was USD 315,321, or USD 105,107 per new identified diagnosis. If every patient had received all of the recommended tests, the total cost would have been USD 560,038 or USD 186,679 per new diagnosis.

Conclusions: The cost-effectiveness of current guidelines for screening for short stature in children is poor. Only positive findings on history and/or careful physical examination should indicate screening for pathology in otherwise healthy short children. An evidence-based update of the current guidelines is required for the management of children with short stature.

Short stature is the most common reason for referral to pediatric endocrinologists. As short stature can indicate various pathologies, several diagnoses have to be considered in assessment of these children. The 2008 ISS consensus guidelines recommended laboratory and radiological screening in otherwise healthy short children [6]. The merit of this retrospective analysis was to challenge for the first time the reliability, feasibility and cost-effectiveness ratio of these guidelines. All but 3 (1.3%) short children had normal variants of growth (23% familial short stature, 41% constitutional delay of growth and maturation, 36% idiopathic short stature). Interestingly, those patients with underlying pathology had more severe growth restriction, as noticed in a previous study [7]. The number of new pathologies discovered through screening of asymptomatic short children was smaller than previously reported [8]. This discrepancy may reflect the more conservative inclusion criteria used in this study. Notably, short children with severely impaired growth, as indicated by both height and height velocity, were excluded from the analysis.

This study raises doubts about the value of the recommended routine thorough investigation of asymptomatic short children. It also questions the feasibility of such an approach. Each patient underwent on average only 64% of all tests recommended. Finally, the cost-effectiveness of the recommended work-up was very high, more than USD 100,000 per new case identified. The clinical message of this highly original study is that careful clinical characterization, including history, physical examination and anthropometric assessment, should inform a personalized investigation panel for the short child. Although anthropometry taken alone may be misleading [9], evidence-based algorithms could be constructed to allow a rational step-by-step approach rather than a costly comprehensive laboratory screening. This was always obvious to 'old-fashioned' clinicians; it's time that we all get back to basics.

Clinical trials

Effects of recombinant human growth hormone for 1 year on body composition and muscle strength in children on long-term steroid therapy: randomized controlled, delayed-start study

Simon D, Alberti C, Alison M, Le Henaff L, Chevenne D, Boizeau P, Canal A, Ollivier G, Decostre V, Jacqz-Aigrain E, Carel JC, Czernichow P, Hogrel JY
Assistance Publique-Hôpitaux de Paris, Hôpital Robert Debré, Service d'Endocrinologie Pédiatrique, Paris, France
dominique.simon@rdb.aphp.fr

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Background: Long-term exposure to glucocorticoid (GC) excess may negatively affect body composition, muscle trophism and linear growth. Recombinant human growth hormone (rhGH) treatment is effective in ameliorating body composition and increasing growth in children receiving GC. This study investigated the effects of rhGH on muscle strength in children on long-term steroid therapy.

Methods: 30 short children with various diagnoses and ongoing chronic GC therapy were recruited for a randomized delayed-start controlled study. Patients were randomized in a 1:1 ratio to immediate rhGH initiation or delayed rhGH initiation after 6 months. After the first 6 months, the immediate-start group continued to receive rhGH for a further 6 months while the delayed-start group received rhGH therapy for 12 months; thus both groups received rhGH for 12 months. For both groups, the rhGH dose was 0.065 mg/kg/day for the first 6 months and was then adjusted to maintain serum IGF-I levels below +2 SDS for chronological age. The primary outcome was the change in composite index of muscle strength (CIMS) between the baseline visit and the 6-month visit; secondary outcomes were changes in height and body composition.

Results: rhGH therapy did not significantly improve CIMS or CIMS expressed as SDS for height in centimeters (CIMS SDS_{height}). rhGH treatment significantly increased height, lean mass (LM), and thigh muscle area (MA).

Conclusions: rhGH improves height, LM, and MA, but has no significant effect on muscle strength in children receiving ongoing chronic glucocorticoid therapy.

In GH-deficient adults, long-term GH therapy increases muscle strength [10]. In childhood, the majority of data on muscle outcomes is in patients with Prader-Willi syndrome [11]. In a study of SGA children, supraphysiological GH doses improved both muscle mass and strength [12]. Long-term GC therapy induces a chronic catabolic state responsible for growth retardation and muscle wasting. GCs can produce loss of thick-filament myofibrils and cause selective atrophy of fast-twitch type II muscle fibers, with relative sparing of type I fibers. Furthermore, chronic GC therapy is associated with glycogen accumulation, lipid deposits, and mitochondria aggregation, swelling, and vacuolization [13, 14].

This randomized controlled trial investigated the potential protective effect of rhGH therapy on muscle strength in children receiving long-term GC therapy. rhGH therapy did not improve muscle strength either after 6 or after 12 months. The study has the merit of being the first to evaluate the effects of rhGH on muscle strength in children with short stature receiving long-term GC therapy, however the wide heterogeneity in age and underlying diagnoses is its major weakness. Children with steroid-sensitive nephrotic syndrome, juvenile idiopathic arthritis, uveitis, Crohn's disease, immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, chronic granulomatous disease, and solid organ transplantation (liver and kidney) were included. As the underlying pathology may per se affect both muscle trophism and response to rhGH therapy, further studies on more homogenous cohorts are needed to confirm these negative preliminary results. Also, unfortunately the study omitted to examine the effect of rhGH on bone density damage induced by chronic GC treatment.

Safety, pharmacokinetics, and preliminary assessment of efficacy of mecasermin (recombinant human IGF-1) for the treatment of Rett syndrome

Khwaja OS, Ho E, Barnes KV, O'Leary HM, Pereira LM, Finkelstein Y, Nelson CA 3rd, Vogel-Farley V, DeGregorio G, Holm IA, Khatwa U, Kapur K, Alexander ME, Finnegan DM, Cantwell NG, Walco AC, Rappaport L, Gregas M, Fichorova RN, Shannon MW, Sur M, Kaufmann WE

Department of Neurology, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA
walter.kaufmann@childrens.harvard.edu

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Background: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder mainly affecting females, caused by mutations in the gene encoding the transcriptional regulator methyl CpG-binding protein 2 (MECP2). Animal models show that mecasermin (recombinant human IGF-I) may improve several features of RTT. This study reports findings on safety, tolerability, pharmacokinetics (PK), and preliminary assessment of efficacy of mecasermin in RTT.

Methods: 12 girls with *MECP2* mutations (9 with RTT) received mecasermin in two phases: phase 1, a 4-week multiple ascending dose (MAD) period using 40 up to 120 µg/kg twice a day, and phase 2, a 20-week open-label extension (OLE) at the maximum dose. MAD was completed by all subjects, while only 10 completed the whole study, due to two likely drug-related adverse events. Efficacy was evaluated by using automated cardiorespiratory measures, EEG, a set of RTT-oriented clinical assessments, and two standardized behavioral questionnaires.

Results: At the end of the MAD phase, IGF-I but not IGFBP-3 was increased both in serum and cerebrospinal fluid, following non-linear kinetics as distribution was mainly in the peripheral compartment. During the OLE phase, there were improvements in apnea and some neurobehavioral features such as anxiety and depression. EEG revealed reduced right frontal α band asymmetry, considered an index of anxiety and depression.

Conclusions: Recombinant human IGF-I is safe and well tolerated in patients with RTT and it may ameliorate certain breathing and behavioral abnormalities.

Rett syndrome (RTT) is a neurodevelopmental disorder characterized by normal early development followed by psychomotor regression, loss of purposeful use of the hands, distinctive hand movements, slowed brain and head growth, problems with walking, seizures, and intellectual disability. Patients may also present breathing disturbances and behavioral abnormalities. Mutations in *MECP2* on the X chromosome are found in more than 95% of individuals meeting diagnostic criteria for clas-

sical RTT [15]. *MECP2* encodes a nuclear multifunctional protein highly expressed in neurons, which is involved not only in transcriptional silencing but also in transcriptional activation, chromatin remodeling, RNA splicing and is relevant for normal development and maturation of the central nervous system. Anomalies in IGF-I/PI3K/AKT/mTOR signaling have been associated with RTT [16], and *MECP2* mutant neurons display impaired AKT/mTOR signaling activity. IGF-I is one of the most potent activators of the AKT signaling pathway. Administration of IGF-I restored dendritic spine dynamics in *Mecp2*-deficient mice and reversed, at least partially, many RTT relevant features including locomotor function impairment, breathing abnormalities, and heart rate irregularities [17]. Consistent with preclinical studies, this phase 1 trial indicates that systemic administration of IGF-I increases central nervous system IGF-I levels and is safe in girls with *MECP2* mutations. More importantly, IGF-I improved some clinical features of RTT such as breathing abnormalities (i.e. apnea) and abnormal behaviors (i.e. anxiety). By contrast, communication and motor function did not improve. The most common adverse effect of mecasermin, not observed in patients treated for primary IGF-1 deficiency, was the early onset of puberty. This side effect may be significant as early onset of puberty and early adrenarche are common in girls with RTT.

Current RTT treatments are focused on managing neurological symptoms (e.g. seizures, anxiety) and medical comorbidities (e.g. constipation, scoliosis), but have limited success. This study indicates for the first time that administration of IGF-I, an activator of the AKT signaling pathway, may be a promising treatment for RTT. The authors recognize that the small sample and the wide age range of patients represent major limitations. Nevertheless, this pioneering work may have a major impact on the understanding and treatment of RTT as well as other devastating neurodevelopmental disorders.

Skeletal muscle characteristics and motor performance after 2-year growth hormone treatment in adults with Prader-Willi syndrome

Lafortuna CL, Minocci A, Capodaglio P, Gondoni LA, Sartorio A, Vismara L, Rizzo G, Grugni G
Istituto di Bioimmagini e Fisiologia Molecolare del Consiglio Nazionale delle Ricerche, Milan, Italy
claudio.lafortuna@cnr.it

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Background: Impaired GH secretion in patients with Prader-Willi syndrome (PWS) is associated with higher fat mass and reduced lean body mass, involving skeletal muscle tissue, and is associated with reduced motor capacity. This study aimed to test whether GH treatment improves skeletal muscle (SM) mass and motor function in adults with PWS.

Methods: 15 obese PWS patients (9 males and 6 females, age 19–35 years) were assessed before and after 12 (GH12) and 24 (GH24) months of GH treatment. Computed tomography at lumbar and mid-thigh level allowed calculation of cross-sectional area (CSASM) of paraspinalis, quadratus lumborum and psoas muscles; attenuation of muscle tissue (ATTSM) was determined in the same slices. Maximal handgrip isometric strength (SHG) and isokinetic knee extension peak torque (PTKE) were measured. Indoor walking tests (WT) were used to assess motor performance and treadmill incremental test to exhaustion was performed to evaluate exercise endurance (EE).

Results: There were no significant differences between PWS patients with ($n = 6$) versus without GH deficiency in biochemical parameters, body composition, muscle characteristics, strength development, and exercise tolerance both at baseline and after GH treatment. Overall, GH treatment significantly increased LBM and decreased body fat percentage at GH12 and GH24, and also improved CASM and ATTSM in both lumbar and thigh muscles. SHG increased at GH12 (7%) and GH24 (13%). PTKE extrapolated at 0 angular velocity was remarkably higher at GH24, whereas EE before exhaustion was augmented by 13 and 17% at GH12 and GH24, respectively. WT was unchanged.

Conclusions: Independent of their GH secretory status, adults with PWS benefit from GH treatment in body composition, muscle size, strength performance in different muscle groups of upper and lower limb, and exercise tolerance.

Prader-Willi syndrome (PWS) is caused by either a microdeletion on the paternally-derived chromosome 15q11–13 or uniparental maternal disomy affecting the same region. In rare cases, PWS is due to an imprinting center mutation resulting in silencing of normally active genes on the paternally-derived 15q11–13. 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, characterized by high fat mass (FM) but low lean body mass (LBM), energy expenditure and resting metabolic rate, ultimately increasing metabolic disease risks [18]. Patients have considerably limited muscle strength and motor function, which may be relieved by GH treatment. A study in PWS children showed that early-onset GH therapy (started before age 2 years) improved their body composition, lipid profile, stature and motor function [19]. However, in PWS adults, only few conflicting reports on muscle size and function are available [20, 21].

This study of 15 PWS adults showed that 24-month GH therapy improved body composition, muscle size and strength, as well as exercise tolerance. Notably, the benefits of GH therapy were independent of baseline GH secretory status, with important implications for a wider eligibility of GH therapy in PWS adults. As GH has anti-insulin effects, long-term GH therapy has potential glucose altering side effects. In this regard, this study is reassuring as no significant effects of GH treatment on glucose, insulin or HbA_{1c} levels were found. Moreover, C-reactive protein, a well-known marker of inflammation and cardiovascular disease risk, decreased significantly during GH therapy, suggesting an additional benefit of GH in reducing obesity-related inflammation. Taken together, these findings suggest that GH therapy should be considered in PWS adults to improve body composition, muscle function and exercise tolerance, regardless of their GH secretory status. A longer follow-up in larger cohorts is needed to confirm the efficacy and safety of such a therapeutic approach.

Adult and near-adult height in patients with severe insulin-like growth factor-I deficiency after long-term therapy with recombinant human insulin-like growth factor-I

Backeljauw PF, Kuntze J, Frane J, Calikoglu AS, Chernausek SD
Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

philippe.backeljauw@cchmc.org

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Background: Recombinant human IGF-I (rhIGF-I) is the only effective treatment to stimulate linear growth in children with severe primary IGF-I deficiency (PIGFD). However, preliminary long-term results are poor, especially when compared to those in GH-deficient children treated with rhGH. This study aimed to evaluate the long-term effects of IGF-I therapy on adult or near-adult height in PIGFD.

Methods: In a predominantly open-label design, 21 children with PIGFD, all naive to IGF-I, received continuous IGF-I treatment until adult or near-adult height. Doses ranged from 60 to 120 µg/kg twice daily. Nine patients also received gonadotropin-releasing hormone (GnRH) analogue treatment for 2.9 ± 1.8 years.

Results: Mean duration of IGF-I treatment was 10 years. During the first year, height velocity increased from mean 3.1 to 7.4 cm/year. In subsequent years, height velocity progressively decreased but remained above baseline. Mean cumulative increase in height SD score at (near) adult height was +2 SDS. Mean total height gain from baseline was 13.4 cm. Nine patients showed increased heights $\geq +2.0$ SDS, whereas 12 patients showed increased heights $\leq +1.7$ SDS. Adult height was not different in patients who received rhIGF-I+GnRH versus those who received rhIGF-I alone. No unexpected side effects were reported.

Conclusions: Long-term IGF-I therapy effectively reduces the severe growth impairment of children with PIGFD. However, in most adults, height remains significantly lower than normal.

Primary IGF-I deficiency (PIGFD) is mainly caused by genetic defects in the GH-IGF-I axis and, according to European criteria, may be diagnosed in children with height ≤ -3 SDS, circulating IGF-I levels < 2.5 th centile, and normal or high stimulated GH responses [22]. Previous studies showed that IGF-I therapy stimulates growth in PIGFD children but the effect on adult height has been assessed in only few patients [23, 24]. The present study reports the efficacy and safety of long-term rhIGF-I in a relatively large cohort of PIGFD children, followed to (near) adult height. Consistent with previous data, the first year height responses were good but progressively declined in subsequent years [24]. Although most reached (near) adult heights taller than untreated patients (estimated using the Laron syndrome growth charts), IGF-I therapy was unable to normalize height. There was wide variability in the individual responses to IGF-I therapy: 11 improved their adult height by 15 cm or more, whereas in 10 adult height improved by only 11 cm or less. These results confirm that the efficacy of long-term rhIGF-I in severe PIGFD is far less good than rhGH therapy in GH-deficient children, who

often achieve normal adult heights. What are the reasons for this limited efficacy? First, IGF-I has both endocrine and paracrine/autocrine actions. Subcutaneous IGF-I administration restores the endocrine component but not the local autocrine/paracrine component, which is crucial for linear growth. Second, some treated patients with PIGFD still lack the direct effects of GH on target tissues, including the growth plate. Third, subjects with PIGFD have very low levels of IGFBP-3 and acid-labile subunit, which remain unaltered on IGF-I therapy. Therefore, the formation of the ternary complex, in which IGF-I is physiologically embedded in the circulation, is severely impaired and the clearance of exogenous IGF-I is accelerated.

As IGF-I may accelerate pubertal maturation [25, 26], a therapeutic option is the addition of gonadotropin-releasing hormone (GnRH) analogues. To date, no randomized controlled studies have been performed to assess such combined therapy. In this non-randomized study, combined treatment did not improve (near) adult height. However, a positive effect of GnRH analogue therapy may be possible if started at the onset of puberty [27]. This long-term study raised no new concerns about the safety of rhIGF-I treatment. One limitation is the wide dose range, from 60 to 120 µg/kg twice daily, with 2 patients even receiving up to 150 µg/kg twice daily. Another limitation is the reliance on a growth reference based on a small number of Laron syndrome patients to estimate the natural growth outcome in untreated PIGFD.

New mechanisms

Control of adipose tissue expandability in response to high-fat diet by the insulin-like growth factor binding protein-4

Gealekman O, Gurav K, Chouinard M, Straubhaar J, Thompson M, Malkani S, Hartigan C, Corvera S
Program in Molecular Medicine, Department of Medicine and Diabetes Center of Excellence, University of Massachusetts Medical School, Worcester, MA, USA
silvia.corvera@umassmed.edu

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Background: A surplus of caloric intake causes adipose tissue expansion through growth and proliferation of adipocytes that is paralleled by a simultaneous expansion of the stroma-vascular network. This study aimed to identify the molecular mechanisms involved in vascular and adipose tissue expansion in response to high-fat diet.

Methods: Male C57BL/6J mice were randomly assigned to a normal or high-fat diet (HFD) for 30 weeks. Rosiglitazone was administered for 2 weeks. Human adipose tissue samples were obtained from lower abdominal area in volunteers with BMI ranging from 25 to 36. An ex vivo angiogenesis assay was performed to measure adipose tissue expansion. cDNA microarrays were used to identify genes associated with insulin-stimulated adipose tissue sprouting.

Results: In the ex vivo angiogenesis assay, adipose tissue fragments formed sprouts consisting of endothelial, perivascular and various proliferative cells. Sprouting was directly stimulated by insulin. Pretreatment with the insulin-sensitizer rosiglitazone further increased sprouting. Exposure to HFD enhanced both basal and insulin-induced sprouting. Microarray analysis identified four genes positively correlated with insulin-stimulated sprouting (*Fads3*, *Tmsb10*, *Depdc6* and *Rasl12*) and four inversely correlated genes (*Asph*, *IGFBP-4*, *Ppm1b* and *Adcyap1r1*). Both IGFBP-4 expression and protein levels correlated inversely with adipose tissue expansion. IGF-I potently stimulated sprouting and immunostaining showed the presence of activated IGF-1 receptors in the vasculature. Sprouting from both mouse and human adipose tissue was suppressed by recombinant IGFBP-4.

Conclusions: Nutritional signals such as insulin regulate the local production of IGF-I/IGFBP-4 whose signaling plays a key role in adipose tissue expansion. This pathway may represent a potential target for interventions aimed at improving metabolic status.

Adipose tissue, previously considered merely as storage for excess energy under lipid form, is nowadays considered a true endocrine organ [28]. Compared to other organs, adipose tissue shows a greater and quicker ability to expand or contract. Adipose tissue expansion involves adipocyte hyper-

trophy as well as hyperplasia of precursor cells, and requires the development of an adequate stromo-vascular network. The precise sequence of events leading to adipose tissue expansion has not yet been elucidated. In humans, some components of the IGF system are present in adipose tissue [29]. This system consists of three ligands (IGF-I, IGF-2, Insulin), at least three surface receptors (IGF1R, IGF2R and insulin receptor), and six well-characterized binding proteins (IGFBP-1 to -6). IGFBP-4 is the most abundant IGFBP in adipose tissue. IGFBP-4 binds to IGF-I modulating its interaction with IGF1R. In this elegant work, based on an ex vivo angiogenesis assay, the authors identified roles of both IGF-I and IGFBP-4 in adipose tissue expansion. The findings suggest that excessive energy consumption elevates insulin, which in turn induces local IGF-I production and a parallel reduction in IGFBP-4, both leading to increased IGF-I bioavailability. IGF-I acts as a potent stimulator of adipose tissue sprouting, particularly stimulating endothelial and stromal cell proliferation. Factors that disrupt this coordinated expansion of adipocytes and adipose tissue stromo-vascular network in response to excess caloric intake are likely to enhance the metabolic risk.

Fibroblast growth factor 21 improves insulin resistance and ameliorates renal injury in *db/db* mice

Kim HW, Lee JE, Cha JJ, Hyun YY, Kim JE, Lee MH, Song HK, Nam DH, Han JY, Han SY, Han KH, Kang YS, Cha DR
Department of Internal Medicine, Wonkwang University, Gunpo, South Korea
cdragn@unitel.co.kr

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Background: Fibroblast growth factor 21 (FGF21) is a regulator of energy metabolism. Many types of fibroblast growth factor receptors (FGFRs) are expressed in adult as well as in developing murine kidneys. This study aimed to investigate the effects of exogenous FGF21 on renal function and morphology in *db/db* mice.

Methods: The animals were divided into three groups. The first group were *db/m* mice as non-diabetic controls, the second group were vehicle-treated diabetic *db/db* mice, and the third group were diabetic *db/db* mice given 25 µg/kg/day FGF21 by daily intraperitoneal injection for 3 months. RT-PCR was used to assess gene expression while Western blot analysis was used to evaluate protein production. Immunohistochemistry was applied to specimens of adipose tissue and kidney. Finally, cultures of mesangial cells were obtained from renal cortex.

Results: FGF21 improved insulin resistance and lipid profile, and normalized adipose tissue histology of *db/db* mice. Moreover, FGF21 reversed the upregulated pro-inflammatory and profibrotic adipokines in adipose tissue and the downregulated anti-inflammatory or adipocyte differentiation-related molecules in *db/db* mice. In the kidney, FGF21 ameliorated functional (urinary albumin excretion) and morphological glomerular abnormalities induced by chronic diabetic injury. Furthermore, FGF21 improved renal lipid metabolism and oxidative stress injury that have been proposed to play an important role in the progression of diabetic nephropathy. In cultured kidney, FGF21 was exclusively expressed in mesangial cells and knockdown of FGF21 expression further aggravated high-glucose-induced profibrotic cytokine synthesis in these cells.

Conclusions: FGF21 improves systemic insulin resistance and protects against nephropathy in diabetic *db/db* mice.

The fibroblast growth factor (FGF) family comprises 22 factors involved in the regulation of cell growth, angiogenesis and metabolism [30]. FGF21, a polypeptide mainly secreted by the liver, has positive effects on glucose metabolism (by increasing both glucose uptake and insulin sensitivity), lipid metabolism (by reducing triglyceride and LDL cholesterol levels and by increasing HDL cholesterol) and reduces body weight. Previous studies reported high levels of FGF21 in obese subjects, suggesting resistance to FGF21 [31, 32]. Serum FGF21 levels are elevated also in patients with renal dysfunction, and FGF21 is an early marker of subclinical diabetic nephropathy [33]. FGF21 has recently been proposed to reduce diabetes-induced kidney damage by decreasing renal lipid accumulation and suppressing inflammation, oxidative stress and fibrosis [34]. However, the mechanisms underlying the protective effect of FGF21 on renal function are largely unknown.

The present study confirms the protective role of FGF21 on renal damage associated with type 2 diabetes. FGF21 ameliorated obesity-induced increases in pro-inflammatory adipocytokines such as PAI-1 and TNF-α in adipose tissue and enhanced the production of anti-inflammatory and anti-dia-

betic molecules such as adiponectin and peroxisomal proliferator-activated receptor- γ . FGF21 also improved dyslipidemia and metabolic abnormalities and reduced the obesity-induced excess fat and liver weight. Furthermore, FGF21 decreased urinary albumin excretion as well as structural changes and oxidative stress in the diabetic kidney while no differences were found in terms of creatinine plasma levels. The finding of increased FGF21-related components such as β -Klotho, FGF receptor-1c (FGFR1c), and FGFR2c in diabetic kidney, might be explained by the presence of a FGF21-resistant status which was overcome by low-dose FGF21. Taken together, these experimental findings suggest that FGF21 may represent a novel therapeutic tool for the management of obesity and type 2 diabetes-related nephropathy.

Insulin-like growth factor-1 increases synthesis of collagen type I via induction of the mRNA-binding protein LARP6 expression and binding to the 5' stem-loop of COL1a1 and COL1a2 mRNA

Blackstock CD, Higashi Y, Sukhanov S, Shai SY, Stefanovic B, Tabony AM, Yoshida T, Delafontaine P
Heart and Vascular Institute and Department of Medicine, Tulane University School of Medicine, New Orleans, LA, USA

pdelafon@tulane.edu

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Background: The stability of atherosclerotic plaque is closely dependent on the content of collagen type I. Indeed, a deficiency in collagen type I expression increases plaque vulnerability and increases the risk of rupture. Insulin-like growth factor-I (IGF-I) stabilizes the atherosclerotic plaque by increasing the collagen content. The mechanisms underlying this protective action of IGF-I are unknown. This study aimed to investigate such mechanisms in both cellular and animal models.

Methods: Human aortic smooth muscle cells (SMCs) and *Apoe* knockout mice (*Apoe*^{-/-}) were studied.

Results: IGF-I increased the rate of collagen I synthesis, leading to enhanced extracellular collagen type I accumulation. Pro- α 1(I) expression was induced by IGF-I in human aortic smooth muscle cells and this was associated with increased expression of the mRNA binding protein, LARP6. Increases in both pro- α 1(I) and LARP6 were mediated by PI3K/Akt/p70S6K signaling. IGF-I increased the amount of COL1 α 1 and COL1 α 2 mRNA bound to LARP6 and this binding was crucial for the ability of IGF-I to induce synthesis of collagen type I. In vivo, IGF-1 infusion in *Apoe*^{-/-} mice increased expression of both LARP6 and pro- α 1(I) in aortic lysates, and SMC-specific IGF-1 overexpression augmented collagen fibrillogenesis in atherosclerotic lesions of *Apoe*^{-/-} mice.

Conclusion: IGF-I stabilizes the atherosclerotic plaque mainly by increasing the expression of LARP6 in vascular smooth muscle cells. LARP6 acts as an intermediary molecule ultimately leading to enhanced synthesis of collagen type I, thus favoring the stability of atherosclerotic plaque.

There is increasing evidence for a role of IGF-I on metabolic and cardiovascular diseases [35–37]. Experimental and clinical studies suggest that IGF-I levels are inversely related to carotid intima media thickness [38]. Both low and high IGF-1 concentrations have been associated with angiographically documented coronary artery disease [39, 40]. However, whether IGF-I exerts either a positive or a negative effect on vessels is still debated. LARP6, a member of the La-related family of RNA-binding proteins, has yet poorly defined actions but is involved in the regulation of collagen type I translation. This study confirms previous evidence that IGF-I infusion increases plaque collagen content. In addition, it has the merit to further clarify the mechanisms underlying IGF-I-dependent plaque stabilization, pointing out the role of LARP6 in mediating the increased synthesis of collagen type I in vascular smooth muscle. This elegant biochemical study opens avenues for future clinical development of new therapeutic strategies to stabilize atherosclerotic plaques.

GH administration rescues fatty liver regeneration impairment by restoring GH/EGFR pathway deficiency

Collin de l'Hortet A, Zerrad-Saadi A, Prip-Buus C, Fauveau V, Helmy N, Zioli M, Vons C, Billot K, Baud V, Gilgenkrantz H, Guidotti JE
INSERM, Institut Cochin, Paris, France
helene.gilgenkrantz@inserm.fr or jacques-emmanuel.guidotti@inserm.fr
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Background: Liver steatosis has been correlated with impairment of the growth hormone (GH) pathway. In animal models, GH receptor inactivation severely downregulates epidermal growth factor receptor (EGFR) expression which is associated with a deficiency in liver regeneration capacity. This study tested the hypothesis that deregulation of the GH/EGFR pathway is a generalized mechanism underlying liver regeneration deficiency.

Methods: The authors studied two contrasting mouse models, *ob/ob* mice, a genetic model associated with hyperphagia and obesity but no steatohepatitis, and mice on a methionine- and choline-deficient (MCD) diet, which induces steatosis and steatohepatitis associated with weight loss. Furthermore, 32 obese patients with different levels of steatosis underwent hepatic biopsies to quantify liver EGFR expression.

Results: The authors observed an inverse correlation between liver EGFR expression and degree of steatosis in both humans and mice. GH/EGFR downregulation was observed in the steatosis murine models, and was related to liver regeneration impairment. After partial hepatectomy (PH), *ob/ob* mice showed a more severe deficiency of liver regeneration capacity compared to MCD diet fed mice. This difference may be due to a decrease in STAT3 phosphorylation observed 32 h after PH. GH administration reversed the liver EGFR expression and ameliorated hepatocyte proliferation subsequent to PH.

Conclusion: Downregulation of the GH/EGFR pathway may be a common mechanism in the hepatic regeneration defect associated with steatosis. GH treatment may have potential to reverse this defect.

Liver steatosis is a widespread health problem in Western countries, mainly due to the outbreak of obesity. Liver steatosis may progress to non-alcoholic steatohepatitis (NASH), and eventually liver fibrosis and cirrhosis. Recent studies show that liver steatosis is associated with hepatocyte proliferation defect [41] and downregulated GH pathway [42]. Epidermal growth factor (EGF) is a key regulator of cell survival and proliferation. Its receptor (EGFR) is activated by specific ligands but also by other growth factors such as GH. The GHR knockout animal model has diminished EGFR expression and concomitant impaired EGF signaling, whereas GH overexpression increases EGFR activation [43]. During liver regeneration, EGFR is a critical regulator of hepatocyte proliferation and an essential mediator in the GHR pathway [44].

The present study elucidates the mechanism underlying the impaired hepatocyte regeneration in fatty liver, showing the key role played by GH/EGFR pathway. The authors found GH/EGFR pathway defects both in the mouse model of steatosis and in human liver biopsies from obese adults. Interestingly, GH administration partially rescued the liver regeneration deficiency associated with fatty liver. The potential clinical implication of these results is the translation into pilot clinical trials of GH therapy in patients either with steatosis or undergoing hepatic resection.

SIRT1 regulates adaptive response of the growth hormone – insulin-like growth factor-I axis under fasting conditions in liver

Yamamoto M, Iguchi G, Fukuoka H, Suda K, Bando H, Takahashi M, Nishizawa H, Seino S, Takahashi Y
Division of Diabetes and Endocrinology and Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan
takahash@med.kobe-u.ac.jp
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Background: Survival under fasting conditions implies a critical and difficult adaptation process involving both metabolic and endocrine responses. Starvation and malnutrition lower circulating IGF-I levels despite elevated GH secretion, indicating a condition of GH resistance. Sirtuin 1 (SIRT1) is a protein deacetylase, which acts as an important regulator of energy homeostasis in response to nutrient avail-

ability. This study aimed to examine the role of SIRT1 in the regulation of GH-dependent IGF-I production in the liver under fasting conditions.

Methods: SIRT1 knockdown in vivo was performed in mice and HepG2 cells were used for in vitro experiments.

Results: Whereas under fasting conditions serum IGF-I levels decreased in mice, knockdown of SIRT1 in the liver increased both IGF-I serum levels and mRNA expression, thus suggesting that SIRT1 negatively regulates GH-induced IGF-I production in the liver. Overexpression of SIRT1 inhibited GH-induced IGF-I mRNA production in HepG2 as well as in rat primary hepatocytes. This inhibitory effect was mediated by the inhibition of GH-induced Tyr phosphorylation of signal transducer and activator of transcription (STAT)5. SIRT1 induced deacetylation of STAT5B by direct interaction. SIRT1 knockdown increased the acetylation and GH-induced tyrosine phosphorylation of STAT5, as well as the GH induced interaction of the GH receptor with STAT5.

Conclusions: This study reveals the interplay between SIRT1 and GH-IGF-I axis, necessary to the adaptive response under fasting. SIRT1 reduces GH-induced tyrosine phosphorylation on STAT5 in hepatocytes both in vitro and in vivo. This inhibition results in GH resistance with consequent reduced IGF-I production.

Under starving conditions several metabolic and endocrine adaptive mechanisms promote survival [45]. IGF-I levels decrease despite an increase in GH secretion, indicating GH resistance [46]. GH resistance induced by fasting represents an adaptive response that, by inhibiting growth, conserves energy for survival. The elevated levels of GH induce the release of free fatty acids by adipose tissue whereas the reduced levels of IGF-I blunt growth and decrease insulin sensitivity. Sirtuins are a group of protein deacetylases, members of the silent information regulator 2. In mammals, SIRT1 regulates gluconeogenesis and lipolysis during fasting.

This study provides evidence of the involvement of SIRT1 in the regulation of GH-dependent IGF-I production in the liver. SIRT1 reduced IGF-1 expression by inhibiting GH-induced STAT5 phosphorylation in the liver according to the following cascade of events: under fasting conditions, activated SIRT1 directly interacts with and deacetylates STAT5. The deacetylated STAT5 has a reduced ability to interact with phosphorylated Tyr residues in GHR and thereby decreases its phosphorylation and effect on transcriptional activity. These findings indicate that SIRT1 negatively regulates the GH-dependent recruitment of STAT5 to GHR through the deacetylation of STAT5. This work contributes to clarify the physiological mechanisms underlying the adaptive responses to fasting, pointing out a central role of the GH-IGF-I axis for adaptation to malnutrition.

New paradigms

Growth hormone prevents the development of autoimmune diabetes

Villares R, Kakabadse D, Juarranz Y, Gomariz RP, Martínez-A C, Mellado M

Department of Immunology and Oncology, Centro Nacional de Biotecnología, consejo Superior de Investigaciones Científicas, Madrid, Spain

mmellado@cnb.csic.es

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Background: Growth hormone (GH) receptors are expressed in many lymphocyte subsets and GH is implicated in the regulation of several immune functions, though its role in autoimmune pathologies is still debated. This study aimed to investigate the effect of GH overexpression in a murine model of type 1 diabetes (T1D).

Methods: The authors studied a NOD (non-obese diabetic) mouse strain transgenic for GH, characterized by circulating GH levels constantly above ~5 µg/ml.

Results: Transgenic animals were resistant to the development of diabetes. GH protected the β cells from apoptosis and stimulated their proliferative capacity. GH altered the cytokine environment, favored anti-inflammatory macrophage (M2) polarization, maintained activity of the suppressor T-cell population and limited Th17 cell plasticity.

Conclusions: Sustained GH expression reduced the pancreatic inflammatory phenotype and blocked the progression to overt diabetes in a murine model of T1D.

Increasing evidence suggests a close link between the immune and neuroendocrine systems. Immune cell functions are regulated by neuroendocrine hormones and immune cells are able to produce neuroendocrine hormones. This cross-talk between the two systems is highly complex and still largely unexplored [47]. Growth hormone (GH), beyond its known effects on growth and metabolism, also exerts actions on the immune system, by regulating T- and B-cell proliferation, immunoglobulin synthesis, human myeloid progenitor cell maturation, and Th1/Th2 ratio [47]. Almost all immune cells present both GHR and IGF-IR on their surface [48].

This study reports a protective effect of GH on the development of T1D in a murine model. GH modulated the immune system, reduced the aggressiveness against pancreas islets, ultimately preserving β -cell survival and function and promoting their proliferation. The efficacy of GH to protect β cells was evident during the first phases of T1D development, when pancreas damage was still limited. This work has the merit to show the GH ability to influence the immune mechanism involved in the pathogenesis of T1D. Although the NOD mouse model of T1D does not completely mimic the human disease, it is tempting to speculate that GH or its analogues may represent in the future a potential therapeutic option to arrest T1D development. However, whether the observed protective effect was due to a direct GH action or mediated by local IGF production was not investigated.

New perspective

Insulin-like growth factor-I and -II levels are associated with the progression of non-alcoholic fatty liver disease in obese children

Cianfarani S, Inzaghi E, Alisi A, Germani D, Puglianiello A, Nobili V

Department of Pediatrics, University Hospital, Bambino Gesù Children's Hospital, Tor Vergata University, Rome, Italy
stefano.cianfarani@opbg.net

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Background: In adults IGF-I is a suggested marker of disease progression in non-alcoholic fatty liver disease (NAFLD). This study aimed to examine the correlations between circulating levels of IGF-I, IGF-II and IGFBP-3 with clinical, biochemical, and liver histological features in obese children with biopsy-proven NAFLD.

Methods: Anthropometry, biochemical assessment, IGF system evaluation, and liver biopsy were performed in obese children (42 females, 57 males, mean age 8.73 ± 1.98 years). Liver histology was graded according to the NAFLD Activity Scoring System. The degrees of steatosis, inflammation, ballooning, and fibrosis were calculated.

Results: 14 patients presented non-alcoholic steatohepatitis. Among all variables, IGF-I was the strongest predictor of both ballooning and NAFLD activity score, IGF-I/IGFBP-3 ratio was the strongest predictor of liver inflammation, while IGF-II was the strongest predictor of liver fibrosis. Children with low IGF-II levels had higher waist circumference, apoB concentrations, fasting glucose and insulin values, and HOMA-IR.

Conclusions: Both IGF-I and IGF-II levels are associated with histological progression of liver steatosis suggesting that these IGF system components might be used as markers of liver damage progression in obese children.

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease worldwide, both in adults and children. NAFLD encompasses a wide spectrum of severity ranging from simple steatosis to the necro-inflammatory form of non-alcoholic steatohepatitis (NASH). There are increasing data involving the GH-IGF-I axis in the development and progression of NAFLD/NASH. IGF-I has been proposed as a marker of both fibrosis and steatosis in adult patients with NAFLD [49]. A recent study proposed that IGF-I exerts an anti-inflammatory action: an imbalance between IGF-I and cytokines would favor NAFLD development and progression [50].

This cross-sectional study, conducted in almost 100 obese children, related the main components of the IGF system to progression of NAFLD. Serum IGF-I levels were inversely correlated with histological severity, IGF-II predicted liver fibrosis, and the IGF-I/IGFBP-3 ratio predicted liver inflammation. These results differ slightly from those reported in adults, in whom IGF-I levels correlated with fibrosis. This difference might be due to a lower rate of fibrosis in the pediatric population [51]. Since liver is the main site of IGF-I production, progressive liver damage may lead to lower IGF-I levels. However, none of the children had clinical or biochemical features of hepatic insufficiency, and no correlation between IGF-I and albumin levels was found. A possible pathogenic role of IGF system in NAFLD development and progression is suggested by these findings, however, molecular confirmation in liver tissue specimens is required. The observed association between IGF-II and fibrosis is novel. Interestingly, lower IGF-II levels were also associated with a worse metabolic profile. Increasing evidence suggests that IGF-II may play a key role in the regulation of protein, lipid and carbohydrate metabolism [52].

To date, liver biopsy is the gold standard test to distinguish simple steatosis from NASH. The identification of serum markers of patients at high risk of progressing to fibrosis would be useful for the clinical management of obese children.

Relationship between insulin-like growth factor I levels, early insulin treatment, and clinical outcomes of very low birth weight infants

Beardsall K, Vanhaesebrouck S, Frystyk J, Ogilvy-Stuart AL, Vanhole C, van Weissenbruch M, Midgley P, Thio M, Cornette L, Gill B, Ossueta I, Iglesias I, Theyskens C, de Jong M, Ahluwalia JS, de Zegher F, Dunger DB; NIRTURE Study Group
Department of Pediatrics, University of Cambridge, Addenbrooke's Hospital NHS Trust, Cambridge, UK
kb274@cam.ac.uk

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Background: Endogenous insulin stimulates hepatic IGF-I production. In preterm infants both low levels of IGF-I and hyperglycemia are associated with increased morbidity and mortality. This study aimed to assess the impact of early insulin treatment in very low birth weight (VLBW) infants on IGF-I levels and morbidity.

Methods: The authors analyzed data from 283 VLBW infants (BW <1,500 g) enrolled in a multi-centre randomized controlled trial. The treatment group received continuous insulin infusion (0.05 U/kg/h) with additional intravenous 20% dextrose started within 24 h of birth and continued for 7 days. Blood samples were taken on days 1, 3, 7 and 28.

Results: Early insulin treatment did not change IGF-I levels during the first week of life. IGF-I increased significantly between day 7 and day 28 (post-intervention). In the first week of life, IGF-I levels were lower in newborns with early hyperglycemia. At day 28, lower IGF-I levels were independently associated with a higher risk of chronic lung disease: each 1-μg/l lower IGF-I was associated with a 3.23-fold higher risk of chronic lung disease (95% CI 1.09–9.10). Higher IGF-I levels on days 7 and 28 were positively associated with all growth parameters.

Conclusions: Early intervention with insulin has a significant impact on IGF-I secretion between days 7 and 28 in VLBW infants. In turn, higher IGF-I levels were associated with reduced morbidity and better growth outcomes.

Hyperglycemia is common in VLBW infants and is associated with high mortality and morbidity. Hyperglycemia is thought to be due to insulin resistance related to the acute stress response and also to relative insulin deficiency. In preterm infants, low IGF-I levels are associated with high risk of retinopathy of prematurity (ROP), slow weight gain, poor head growth and low brain volume. As insulin regulates IGF-I liver production, these authors hypothesized that early insulin treatment could reduce the risk of hyperglycemia meanwhile increasing IGF-I levels, and eventually reduce morbidity and mortality. Consistent with previous reports [53], a close relationship between low IGF-I levels and early hyperglycemia was observed. Unlike a previous report [54], early insulin treatment did not increase serum IGF-I during the first week of life. However, more than one third of infants of the control group had insulin therapy started at median day 3 for severe hyperglycemia. Secondly, low protein intake received by all newborns may have affected IGF-I production. Thirdly, exogenous peripheral insulin administration might have downregulated endogenous portal insulin

levels, which is the most potent stimulator of hepatic IGF-I secretion. However, this explanation is not consistent with the late increase in IGF-I observed between days 7 and 28. The finding of an independent relationship between hyperglycemia and low IGF-I in the first weeks of life suggests that IGF-I is regulated by insulin and may be a marker of either insulin resistance or relative insulin deficiency.

Food for thought

DNA methylation of IGF2DMR and H19 is associated with fetal and infant growth: the generation R study

Bouwland-Both MI, van Mil NH, Stolk L, Eilers PH, Verbiest MM, Heijmans BT, Tiemeier H, Hofman A, Steegers EA, Jaddoe VW, Steegers-Theunissen RP

The Generation R Study Group, and Department of Obstetrics and Gynecology, Department of Clinical Genetics, Erasmus Medical Centre, Rotterdam, The Netherlands
r.steegers@erasmusmc.nl

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Background: Fetal growth likely depends on epigenetic changes of embryonic growth genes. This study aimed to investigate the associations between DNA methylation, genetic variants in *IGF2DMR*, *H19* and *MTHFR* and both fetal and infant growth.

Methods: 69 small for gestational age (SGA) and 471 control (AGA) children were selected from a population-based prospective birth cohort in the Netherlands (Generation R Study). Fetal growth was assessed by serial ultrasound measurements. Postnatal weight gain was assessed at 3 and 6 months. DNA methylation of *IGF2DMR*, *H19* and *MTHFR* (methyltetrahydrofolate reductase) and genetic variants in *IGF2/H19* were assessed on DNA extracted from umbilical cord white blood cells.

Results: Hypomethylation of *IGF2DMR* but not *H19* was found in SGA children. *IGF2DMR* methylation was inversely associated with weight gain in the first 3 months after birth ($\beta = -0.53$, 95% CI -0.91 , -0.16 , $p = 0.005$). *MTHFR* was hypomethylated in both SGA and AGA groups. Genetic variants in the *IGF2/H19* locus were associated with both higher (rs3741205, rs2251375) and lower (rs2067051) *IGF2DMR* methylation. A *IGF2* SNP (rs3741205) was associated with birth weight and risk of SGA.

Conclusions: DNA methylation and genetic variants of *IGF2DMR* and *H19* are associated with both prenatal and postnatal growth.

Epidemiological studies report associations between exposures to adverse in utero environments, and intrauterine growth retardation (IUGR), and the later development of insulin resistance, type 2 diabetes, hypertension, hyperlipidemia, and cardiovascular disease in adult life [1]. Metabolic programming is the suggested underlying mechanism, whereby environmental cues transferred from mother to fetus during critical periods of embryo-fetal life induce permanent changes in metabolic and endocrine functions of the offspring. Epigenetic mechanisms are involved in gene silencing, genomic imprinting and transcriptional regulation of tissue-specific genes during cellular differentiation and allow a wide range of phenotypes to develop from a single genotype. Multiple mechanisms are involved, including methylation-demethylation of cytidine-guanosine (CpG) sequences in gene promoter regions. One of the best characterized epigenetically regulated loci is *IGF2*, which is maternally imprinted and only paternally expressed due to regulation by differentially methylated regions. *IGF2* encodes a growth factor with a critical role in fetal growth and development [2]. Previous studies reported conflicting results on the association between *IGF2* methylation and birth weight [55, 56].

The present study found that SGA infants have lower *IGF2* methylation in cord white blood cells, and this is also associated with a higher weight gain during the first 3 months after birth. Notably, rapid early weight gain in the first 3 months after birth is in turn associated with higher risk of the metabolic syndrome in adults [57]. Taken together, these findings support the concept that in utero epigenetic modifications of *IGF2* may contribute to both fetal growth restriction and cardiometabolic

risk in adult life. Although fascinating, this observation is not fully consistent with the recognized inhibitory effect of methylation on gene expression. Although lower *IGF2* methylation was positively associated with early weight gain, consistent with the expected higher *IGF2* expression, the association between lower *IGF2* methylation with restricted fetal growth does not fit this model and remains unexplained.

Mechanism of the year

Mechanism of activation of protein kinase JAK2 by the growth hormone receptor

Brooks AJ, Dai W, O'Mara ML, Abankwa D, Chhabra Y, Pelekanos RA, Gardon O, Tunny KA, Blucher KM, Morton CJ, Parker MW, Sieriecki E, Gambin Y, Gomez GA, Alexandrov K, Wilson IA, Doxastakis M, Mark AE, Waters MJ
The University of Queensland, Institute for Molecular Bioscience (IMB), St Lucia, QLD, Australia
m.waters@uq.edu.au or a.brooks@uq.edu.au
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Background: The growth hormone receptor (GHR) is a member of class I cytokine receptor family, as are the prolactin, thrombopoietin and erythropoietin receptors. GHR signaling is mainly mediated by the transcription factors JAK (Janus kinases) and STAT (signal transducers and activators of transcription). However, the mechanism whereby GHR initiates signaling remains elusive. This study aimed to define the molecular mechanism responsible for JAK2 activation induced by GHR.

Methods: The ToxR assay was used to test the interaction of transmembrane domains (TMD). Fluorescence resonance energy transfer (FRET) was used to monitor the movements of GHR and JAK2. Cysteine cross-linking data were used to the faces of the transmembrane helices in contact in the basal state. Culture cells were used to assess the proliferative signals mediated by GHR.

Results: In the basal inactivated state, GHR is mainly found at the cell surface as a dimer, held together by its transmembrane helices that are aligned in parallel. The binding of GH induces a conformational change; the transmembrane helices switch from a parallel to a left-hand crossover state that leads to separation of the helices at the lower transmembrane boundary and separation of the JAK2 binding motifs. This separation removes the pseudokinase inhibitory domain of one JAK2, which blocks the kinase domain of the other JAK2, and vice versa. This cascade of conformational changes eventually leads to JAK2 activation and starts intracellular signaling.

Conclusions: This study clarifies the molecular mechanism responsible of GHR-mediated JAK2 activation. This model may be applied to the other class I cytokine receptors and may be instrumental to design specific inhibitors with potential clinical applications.

The growth hormone receptor (GHR) exerts growth and metabolic functions, either directly or via IGF-I induction [58]. This receptor lacks catalytic activity inside its intracellular domain, and signal transduction is mainly mediated either by cytoplasmic tyrosine kinase JAK2 or by activation of Src family of proto-oncogene tyrosine-protein kinases. The main intracellular mediator of GH signaling is STAT5 (signal transducer and activator of transcription 5). Previous studies had shown that GHR on the cell surface is predominantly constitutively dimerized in the absence of the ligand (GH). GHR activation by GH binding induces a conformational change including a rotation of one GHR subunit. However, the mechanisms by which the conformational changes result in JAK2 activation remained elusive until the present study. These authors found an intrinsic dynamism of GHR involving the transmembrane helices that leads to the removal of the pseudokinase inhibitory domain from the kinase domain of JAK2, ultimately leading to JAK2 activation and switch-on of intracellular signaling. This molecular model may generalize to the other class I cytokine receptors. These findings provide the basis for understanding the oncogenic JAK2 mutations responsible for polycythemia vera and other hematologic disorders and may also represent the premise for designing cytokine receptor-based therapeutics.

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