

PEDIATRIC IMPLICATIONS OF NORMAL INSULIN-GH-IGF AXIS PHYSIOLOGY

Peter Bang, MD, PhD, MSci, Linköping University, Faculty of Medicine and Health Sciences, Dept of Biomedical and Clinical Sciences, Div of Children's and Women's Health, SE-58185 Linköping, Sweden. peter.bang@liu.se

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ABSTRACT

Understanding the involvement of the insulin-GH-IGFaxis in the different phases of human growth, development, and metabolism is the key to understanding human pathophysiology. The normal physiological actions of the axis optimize human growth and metabolism to impact adult height by approximately one third. IGF binding proteins modulate access of circulating IGF-I to the tissues and modulate IGF-I and -II access to the type 1 IGF receptor (IGF1R) at the cellular level. Complete lack of IGF1R signaling is most likely not compatible with a viable human fetus, while allelic haploinsufficiency impairs brain development and causes severe short stature. Lack of insulin receptor signaling in Leprechaunism may result in the rare event of an alive but severely small for gestational age baby that will only survive if treated with recombinant-IGF-1 to substitute inulin receptor signaling with IGF1R signaling via their common intracellular pathways. IGF-I gene defects result in mental retardation and severe fetal and postnatal growth failure with GH hypersecretion and marked insulin resistance. Likewise, *IGF2* gene defects or imprinting defects cause severe fetal growth failure but somewhat less adverse effects on postnatal growth, more variable effects on brain development, and an absence of marked metabolic effects. GH fine-tunes insulin and IGF-I signaling with no impact on IGF-II expression and has a minor impact on fetal development and growth. GH effects on lipolysis are established in the newborn and ensure gluconeogenesis and prevents hypoglycemia after birth. The complete absence of GH expression such as in GHRHR or GH1 gene defects

or absence of GH signaling in GHR or STAT5B gene defect leads to an adult height of 120-130cm if untreated, and has severe metabolic consequences. Even excess of insulin, GH, IGF-I and IGF-II signaling are associated with severe metabolic disease and excess growth and/or obesity. Malnutrition or malabsorption causes decreased insulin signaling which reduces GHR expression and blocks the GH signaling pathway leading to IGF-I expression (GHR uncoupling), while GH's metabolic actions on lipolysis and gluconeogenesis are unaffected. GH signaling attenuate insulin actions on glucose metabolism which causes insulin resistance and hyperinsulinemia or may precipitate diabetes. However, insulin signaling pathways that enhances GHR function or suppress IGFBP-1 or SHBG production are still intact and promote anabolism, optimize growth, enhance androgen actions and play a mechanistic role in premature adrenarche and PCOS. Long-term nutritional deprivation compromises growth, while from a developmental perspective, decreased insulin signaling (leading to GHR uncoupling) prolongs life (at least in some experimental animal models) which ensures that fertile age is reached, and survival of the species is ensured. For the health of the general population, the subtle changes in insulin, GH and IGF-I signaling associated with gene polymorphisms or epigenetic changes programmed during fetal and early postnatal life and affecting gene expression are important. They determine growth and pubertal development in childhood and predispose the individual for developing the metabolic diseases and malignancies in adult life, as predicted by the Barker hypothesis. As the roles of the insulin-GH-IGF-axis in

growth and metabolism, often discussed separately, are intimately linked they will be described jointly here.

EARLY WORK DEFINING THE INSULIN-GH-IGF-AXIS

Daughaday realized that the mitogenic effect of GH in the growth plate was not direct but mediated by Insulin-like Growth Factor-I (IGF-I), at that time named sulphation factor or somatomedin C (1). Another effect of IGF-I was insulin-like and not inhibited by insulin specific antibodies (2,3,4) and therefore it was named non-suppressible Insulin-like activity (NSILA). Hall and Van Wyk purified IGF-I from human muscle extracts (5,6) and realized that these biological activities originated from the same molecule. They also identified significant quantities in blood (7). The primary structure of IGF-I and Insulin-like Growth Factor-II (IGF-II) was discovered by Froesch and coworkers as a result of their persistent work to characterize the metabolic activity of NSILA (8,9). Soon after, the mitogenic activity of the sulphation factor or somatomedin C as well as somatomedin A was shown to be identical to IGF-I (10). Rechler and Nissley demonstrated that IGF-II was identical to

multiplication stimulating activity, a factor known to stimulate DNA synthesis in chick embryo fibroblasts (11).

The concept that binding proteins existed for peptide hormones like the IGFs, similar to those for steroid and thyroid hormones, were suggested by studies from Zapf and Froesch (12) and by Hintz (13), demonstrating that NSILA was present in high molecular weight complexes in serum. The binding was exclusive to IGFs and did not apply to insulin or proinsulin despite their structural similarities. High molecular weight IGF-I complexes with IGFBPs were GH dependent (14) and formed a ternary complex composed of IGFBP-3 (15), the Acid Labile Subunit (ALS) (16) and IGF-I or IGF-II. Low molecular weight complexes contained IGF-I or IGF-II bound to an insulin regulated liver derived protein IGFBP-1 (17, 18), at first called the 28 kDa binding protein or PP12 (19). The existence of other IGF binding proteins, six in total, became clear when Hossenloop (20) developed Western ligand blotting as a technique to quantify these proteins. The components of the IGF-IGFBP-system are outlined in Figure 1.



Figure 1. The primary structures of IGF-I, IGF-II and insulin are similar. IGFs are produced by many differentiated cell types, and their bioactivity in the extracellular fluids or in the circulation are coordinated by six IGF binding proteins (IGFBP-1 through -6). IGFBP-3, the major binding protein in serum is stimulated by GH and it forms a large 150 kDa ternary complex with IGF-I or -II and the GH regulated acid labile subunit (ALS). IGFBP-5, an important supporter of bone tissue formation, also forms ternary complexes with IGF-I or -II and ALS. IGFBP-1, suppressed by insulin, is one of several binding proteins in the smaller 50 kDa binary complexes with IGF-I or –II. IGFBP-2 has inverse association with insulin under many physiological conditions. In contrast, IGFBP-4, -5 and -6 do not appear to be directly regulated by GH or insulin and are important local regulators of IGF activity in bone and the CNS. The type 1 IGF receptor (IGF1R) is the mediator of the mitogenic, anti-apoptotic, differentiating and metabolic effects of both IGF-I and -II. The structural similarity of the IGF1R with the insulin receptor (IR) explains the formation of hybrid receptors in cells that expresses both receptors such as myocytes and preadipocytes. Cross reactivity among the ligands and the receptors have been demonstrated, although it has minor importance under physiological conditions but may cause non-islet-cell tumor hypoglycemia due to unprocessed pro-IGFs with markedly decreased binding affinity to IGFBPs. A second receptor, exclusively binding IGF-II, work as a scavenger receptor and is identical to the mannose-6-phosphate receptor, known to direct proteins for degradation in the lysosomes. A second level of control of IGF bioactivity is exerted by IGFBP proteases which release IGF-I activity after fragmentation of IGFBPs. Specific production and regulation of IGFBP proteases at the tissue level controls processes such as ovulation and atherosclerosis. Furthermore, interaction of IGFBPs and IGFBP proteases with the extracellular matrix modify the binding affinity for the IGFs and are involved in prolonging the actions of IGFs at the tissue level. Extracellular matrix also signals though integrin receptors on the cell surface

and modifies IGF-1R signaling. This figure also shows the existence of IGFBP-related proteins with markedly lower affinity for the IGFs and with physiological roles not related to their IGF binding.

ANIMAL EXPERIMENTS ESSENTIAL FOR THE UNDERSTANDING OF HUMAN INSULIN-GH-IGF AXIS PHYSIOLOGY

Insulin, IGF-I, and IGF-II and Their Receptors

Efstratiadis' series of knock-outs of the insulin-GH-IGF-axis in mice in the early 1990s clearly confirmed its importance in fetal and postnatal growth and metabolism (21). It also predicted the phenotype of experiment of nature in humans with gene defects in the axis yet to be discovered. The studies opened new insights, not least the equal importance of IGF-I and IGF-II in fetal growth, reducing birth weight by about 60 % in both lgf1 knock-out (lgf1ko) and lgf2ko animals and demonstrating that the previous perceived concept that there was a fetal (IGF-II) and a postnatal (IGF-I) form of IGF was incorrect. IGF-I and -II had actions through the type 1 IGF receptor (IGF1R) demonstrated by Igf1rko animals with 45% of wild type birth weight and no further effect when crossed with *lgf1*ko animals. While *lgf1*ko animals were viable depending on genetic background and were non-fertile, the *lgf1r*ko animals died from respiratory failure but with an absence of apparent malformations. Interestingly, crossing lgf2ko with *Igf1*rko resulted in further growth retardation indicating that IGF-II had actions through an additional receptor. Another new insight came from knock-out of the 'mysterious type 2 IGF receptor', identical to the mannose-6 phosphate receptor (M6P-R), specifically binding IGF-II and involved in internalization of proteins for lysosomal degradation. Knock-out of the Igf2r/M6pr resulted in increased serum and tissue levels of IGF-II and fetal overgrowth (140% of wildtype birth weight) (22). This receptor works to clear IGF-II and its presence in endothelial cells may, at least partly, explain the lack of endocrine actions of IGF-II due to its proteolytic lysosomal degradation (23). Thus, IGF-II effects on fetal growth are paracrine/autocrine actions mediated by the IGF1R. Knock-out of the Igf2r/M6pr gene combined with

Igf2ko/Igf1rko could partly rescue growth retardation, a finding that was explained by IGF-II actions via the insulin receptor (INSR). The formation of heterodimers. more commonly named hybrid receptors, between type A or B isoforms of the insulin receptors (INSR_A or INSR_B) and the IGF1R of which IR_A-IGF1R are highly expressed in the fetus (and in malignant cells) and activated by IGF-II, may further point to the importance of IGF-II during the fetal period. INSR_B-IGF1R hybrids comprises up to 30 % of INSR and IGF-I receptors in muscle due to high expression of both and this hybrid predominantly responds to IGF-I (less to insulin) and explains the important role of IGF-I in growth and metabolism in skeletal muscle.

Postnatally, the *lgf1*ko mice continued to grow poorly, resulting in an adult weight 30% of wild-type and with poor gonadal function and delayed bone development. Knock-out of GH or its receptor (GHR), both expressed in the mouse fetus, did not affect birth size, indicating that the *lgf*1 gene is not under GH control during the fetal period. The actions of GH and its receptor on growth in mice were obvious from postnatal day 15 and largely slowed growth resulting in a 50 % reduction of wild-type adult weight. On the other hand, double Ghrko/Igf1ko resulted in further postnatal growth retardation relative to lgf1ko mice completely obstructing further weight gain after postnatal day 15 and supporting previous studies suggesting that progenitor cells in the growth plate require direct GH actions (24).

IGFBPs and IGFBP Proteases

Like the above attempts to pinpoint the role of important ligands and receptors in the axis, steps to assess the role of IGF binding proteins involved in modulating IGF-I and IGF-II bioactivity were taken (reviewed by Pintar (25)). In contrast to the pronounced phenotypes caused by mutations in receptors and their ligands, the growth phenotypes of the various IGFBP knock-out animals were far less pronounced as were the metabolic changes observed (26,27,28). It was argued that there is a large degree of redundancy among the functions of the IGFBPs which to some extend contradicts their specialized functions in various tissues (29). However, this idea was to some extent supported by the finding of somewhat more pronounced phenotypes in double and triple knock-out animals (30). This is largely in accordance with the absence of reports of IGFBP gene defects causing growth retardation in humans. The most affected phenotype identified was that of *lgfbp4*ko mice who were growth retarded at birth and displayed poor postnatal growth (30). No such mutation has been identified in humans. IGFBP-4 is specifically degraded by the metalloproteinase PAPP-A (Pregnancy Associated Plasma Protease -A) produced by the placenta as well as bone and ovary. In Pappa knock-out animals a 20-30% reduction in body weight was reported (31). Interestingly, the growth restriction phenotype of mice null for Pappa could be rescued by disruption of IGF-II imprinting during embryonic development (32).

Endocrine Versus Paracrine Autocrine IGF-I

One of the controversies of the area has been the relative contribution to linear growth of circulating endocrine IGF-I largely produced by the liver versus peripherally produced IGF-I with major paracrine/autocrine actions on local tissues. The major importance of paracrine/autocrine IGF-I was demonstrated by liver specific *lgf1*ko mice (*Ligf1*ko) with largely unaffected longitudinal growth (33). Circulating levels were 20% of wild-type with compensatory elevation of GH, insulin resistance and hyperinsulinemia. With age the animals developed type underlining 2 diabetes. the metabolic consequences of largely elevated GH combined with circulating IGF-I deficiency (34). Somewhat unexpectedly, this animal model closely resembles children and adolescents with type 1 diabetes, as further elaborated on below.

Another model to assess the relative importance of endocrine versus paracrine/autocrine IGF-I is the liver-

specific *Ghr*ko mouse. It produces a similar phenotype but with more specific hepatic consequences of absent GH signaling (35).

INSULIN-GH-IGF AXIS PHYSIOLOGY, A PEDIATRIC PERSPECTIVE

Insulin-GH-IGF-Axis and Human Fetal Growth

IGF-I controls the pace of the cell cycle from early on in human embryogenesis. INSR and IGF1R is expressed in human pre-implantation blastocysts already from the 8-cell stage, while IGF-II is expressed already in the oocyte (36). After implantation, IGF-I is produced in the human embryo (37), but until then the source of IGF-I is thought to be the female reproductive tract, and it is known that the availability of the IGF-I ligand is important for blastocyst growth in human in vitro fertilization - IVF (38). IGF-I production is controlled by nutritional factors in the early embryo and even later during human fetal development (39). Circulating endocrine IGF-I increases with gestational age (40) and is strongly correlated with fetal growth in the second part of gestation (41,42). However, little has been reported concerning the regulatory control of the IGF1 gene in the human fetus. IGFBPs can be identified in the human fetal circulation (40), and recently the role of IGFBP-5 in regulating fetal growth was suggested by fetal growth retardation in the absence of a specific IGFBP-5 protease, PPAP-A2 (43). Insulin continues to be permissive for IGF-I production even after GH is established as the major pituitary stimuli controlling endocrine as well as paracrine/autocrine IGF-I, as described below.

Fetal Growth Restriction and Programming of the Insulin-GH-IGF-Axis Setpoint

Insulin resistance has been developmentally advantageous for mankind until very recent decades of excess food and sedentary life style. It was proposed by Barker et al (44) in his 'fetal and infant origin of adult disease' hypothesis that intrauterine restriction of growth compensated by excessive postnatal catch-up growth results in an increased risk of developing disease entities of the metabolic syndrome later in life. In his early epidemiological studies, he demonstrated that there is a U-shaped relationship between birth weight and risk of obesity, insulin resistance, type 1 diabetes, hypertension, dyslipidemia and ischemic heart disease, with lower birth weight (within the normal range) imposing a risk. Notably, at higher birth weights this risk rises again which may represent genetic risks of obesity and type 2 diabetes. The concept was that poor fetal nutrition would lower fetal IGF-I and program the child to a low IGF-I setpoint and slower postnatal growth, an epigenetic phenomenon that could be preserved over a few generations (45). At the same time, small for gestational age (SGA) babies becomes insulin resistant (46) and this trait is enforced by a low endocrine IGF-I setpoint (47), creating the best physiological circumstances for the storage of fat during short times of food availability in a world with limited access to food. However, in a world of plenty, this advantage would turn into a disadvantage and give fast increases in body weight, hyperinsulinemia, and the development of metabolic syndrome problems early on (reviewed by Dunger et al (48). New information even suggests that the parental nutritional state can impose epigenetic metabolic changes in the fetus (49).



Figure 2. In the fetus (insert) IGF-I increases with gestational age toward birth. Endocrine circulating IGF-I is strongly nutritionally dependent and correlated with birth size. Pituitary GH control of IGF-I production is not fully established during the first year of human life. The ability of serum IGF-I levels to increase during childhood is dependent on the shift from binary complexes of IGF-I with short half-life to a complete dominance of IGF-I bound in a stable ternary complex with the GH dependent proteins IGFBP-3 and ALS. Both these proteins increase, when pituitary GH control of the axis is established. During pubertal development, sex steroids change the set-point of negative IGF-I feedback and allow a

peak of IGF-I in mid-puberty. Total IGF-I levels decline to low levels in senescence. Serum IGF-I reference values based on Juul (50).

Postnatal Establishment of Pituitary GH Control of IGF-I Production

In humans, full GH control of the IGFI gene, as well as the IGFBP3 and Acid Labile Subunit (ALS) genes, is developmentally regulated and established not until the first year of life. IGF-I and IGFBP-3 levels increase slowly from birth until a more rapid increase and peak during puberty, which is followed by a decline toward low levels in senescence (50, 51) (Figure 2). The late establishment of pituitary GH control of the axis is strongly supported by animal data from GHR KO mice reviewed above, and by a new model of Laron syndrome (GHR defect) in pigs (52). In accordance, newborns with mutations in the GH Releasing Hormone Receptor (GHRHR) gene resulting in an isolated GH deficiency (GHD) phenotype was associated with normal birth weight in one cohort (53) and slightly subnormal birth weight in another (54). In a subgroup of 12 children with congenital isolated GHD, birth weight $(1.1 \pm 0.8 \text{ SD})$ and length (0.5 ± 1.3) SD) was not affected (Mehta A). GH1 mutations appear to be slightly more affected with mean birth weights of -1.0 ± 0.9 (54). Studies of common polymorphisms in GH1 demonstrate dose effects of 150 and 100 grams in term newborns of normal and low birth weight, respectively (55). Somewhat contradictory to the observations in animals, Savage et al (56) reported 27 prepubertal children with severe GH insensitivity syndrome (GHIS or Severe Primary IGF Deficiency (SPIGFD)) to have a median (range) birth weight SDS of -0.72 (1.75 - (-3.29)) and birth length SDS of -1.59 (0.63 - (-3.63)). SPIGFD in these patients were defined by phenotypic and biochemical and they were characteristics treated with recombinant human (rh)IGF-1 in one of the clinical trials leading to approval of this therapy, as described later. There was no complete genetic characterization of these patients and 7 patients had a normal serum GH Binding Protein (GHBP) suggesting that the extracellular part of GHR was not affected. In a monograph, Professor Zvi Laron (57), who gave his

name to this syndrome, reported that birth weight is unaffected while birth length is slightly on the shorter side. In summary, human fetal growth is only marginally affected by GH. GH is detectable and the GHR is expressed in the human fetus and the metabolic effects of GH on lipolysis are essential to maintain normal levels of glucose in the newborn.

Given this critical role of GH in adjusting metabolism to the fasting condition, it is plausible that the metabolic effects of GH are required for optimal linear growth of the human fetus and that this explains marginal effects on linear growth of the human fetus. However, strict GH control of IGF-I in the human fetus would predict severe growth retardation of the abovementioned genetic defects comparable with the birth size observed in defects in the IGF-I gene. And that is not observed: Children with IGF1 defects suffer from far more severe fetal growth retardation with birth weight SDS around -4 and birth length SDS of -5 to -6 (reviewed in (58). In the study by Mehta et al (Mehta A), children with congenital isolated GH deficiency demonstrated growth retardation as already at 6 months of age (-2.6 ± 1.0 SD and -2.2 ± 1.3 SD in weight and length, respectively) prior to starting treatment. This suggests that the developmental GH control of IGF-I production is established early after birth. It is in accordance with data from Jensen RB and Juul A et al (Jensen RB) suggesting that low IGF-I is a marker of GH deficiency early in life. Children with combined pituitary hormone deficiencies were even more growth retarded at 6 months (Metha A).

GHR Signaling Pathway to *IGF1* Gene Transcription

The important cell signaling steps associated with GH stimulated *IGF1* gene activation, transcription and IGF-I production are detailed in the Endotext chapter 'Normal Physiology of Growth Hormone in Adults'. Briefly, GH binding to preformed dimeric GHR - JAK2 complexes introduces structural changes in the

receptor complex that separates JAK2 inhibitory and kinase active sites and enables trans-phosphorylation of the two JAK2 molecules (reviewed in (59). The GHR belongs to the class 1 cytokine receptors which uses STAT as one of its principal secondary messengers, and the subsequent phosphorylation of two STAT5b molecules results in a phospo-STAT5b homodimer which translocate to the cell nucleus, binds to STAT5b recognition sites on the *IGF1* gene promoter, and initiates transcription (Figure 3).

Primary and Secondary IGF-I Deficiency

IGF-I deficiency may be the result of low or inadequate production of GH. This condition is known as GH deficiency (GHD) but in analogy with other pituitary deficiencies leading peripheral to hormone deficiencies the term secondary IGF-I deficiency was proposed (Rosenfeld et al). GHD is severe in GH1 or GHRH-R gene defects and in some children with congenital GHD as well as after treatment of brain tumors with radiation therapy. However, these conditions are rare and most children treated with rhGH has less severe GHD or an indication not associated with GHD. Disorders of GH in childhood is outlined in the Endotext chapter Disorders of Growth Hormone in Childhood by Murray and Clayton (132)

Primary IGF-I deficiency or GH insensitivity is severe in homozygous genetic defects in genes including the GH receptor (Laron syndrome) gene, STAT-5b gene and IGF-I gene. Less severe growth retardation is reported in children with homozygous genetic defects in the ALS gene. Treatment with rhGH do not improve growth in these cases while rhIGF-1 is efficient in SPIGFD and approved by FDA and EMA. In many patients with severe primary IGF-I deficiency (SPIGFD) defined by low IGF-I (less than – 3 SDS or the 2.5th percentile), severe short stature (Height SDS less than – 3), normal GH secretion and absence of acquired insensitivity to GH (discussed below) genetic defects may be absent. Still treatment with rhIGF-1 may be as efficient as in patients with confirmed homozygous GHR defects (131).

Insulin Enhancement of GHR Signaling to *IGF1* Gene Transcription

Insulin signaling enhances the GH signaling pathway to enable IGF-I production in the fed state and promotes linear growth and other anabolic responses (60, 61, 62). Moreover, GH signaling to elicit IGF1 gene transcription is blocked in the absence of appropriate insulin signaling, a phenomenon also known as un-coupling, and resulting in growth arrest (60, 61, 63, 64) (Figure 3). This is partly a result of insulin's effects on hepatic GHR expression, and partly a post-receptor signaling effect as unraveled by extensive animal studies (reviewed in (60). In obese individuals with hyperinsulinemia, hepatic GHR expression is enhanced as indicated by elevated GHBP levels reflecting proteolytic cleavage of highly expressed surface GHR and release of the extracellular part to the circulation (65). This allows obese individuals to maintain normal serum IGF-I levels despite markedly diminished GH secretion (66, 67). Consequently, obese individuals have attenuated GH responses to GH secretagogues (68).



Figure 3. Multiple, partly identical, pathways have been described to be activated by the GHR, the INSR and many other hormone kinase receptors not shown on the slide. Limiting this cartoon to the GHR and INSR, still the complexity is very high and the potential candidate hubs for crosstalk are numerous. The crosstalk that, following activation of the GHR, leads to resistance to specific signaling events from the INSR (related to glucose metabolism) is more well established and describe in detail in the Endotext chapter 'Normal Physiology of Growth Hormone in Adults'. In the current review the focus is on the crosstalk that is executed by activation of the INSR and results in enhanced signaling from the GHR leading to gene activation of IGF1 and other GH dependent genes such as IGFBP3 and ALS. There are basically no studies addressing this crosstalk on the cellular level despite the strong evidence for INSR signaling being permissive for IGF1 transcription. Given that mTORC1 and mTORC2, downstream the INSR, are essential hubs for substrate and energy sensing and thus controlling the switch between cell anabolism and catabolism, they appear to be strong candidates to determine whether IGF1 should be on or off. A further argument for their candidate role is the so far limited evidence of mTORC1 and mTORC2 involvement in branched chain amino acid sensing directly enhancing IGF1 transcription. The unique role of the Jak2, STAT5b pathway in connecting the GHR with IGF1 gene activation has not been challenged and is thus the major candidate pathway to be affected by INSR signaling crosstalk. It is less clear which of the signaling pathways from the GHR that results in enhanced lipolysis although the STAT5b pathways has been implicated. This is particularly interesting given that GHR induced lipolysis does not require INSR signaling crosstalk. The reader is encouraged to seek specific information regarding other GHR and INSR pathways depicted in the cartoon but not further discussed in this review.

While short-term fasting decreases serum IGF-I but does not affect GHBP (69), the triad of IGF-I deficiency, poor growth and pubertal delay/arrest in long term nutritional deficiency such as in anorexia nervosa is associated with low GHBP that is partly restored with weight gain (63). Also, circulating IGF-I deficiency due to hepatic under-insulinization in type 1 diabetes is associated with low GHBP levels. Normal circulating IGF-I and GHBP are fully restored only after experimental intra-peritoneal (70) or intraportal (71) insulin delivery.

Functional/Acquired IGF-I Deficiency -Uncoupling of GHR Signaling to *IGF1* Gene Transcription and Maintained GHR Metabolic Signaling Due to Insulin Deficiency

Increased GHR signaling in obese children does not generally result in elevated IGF-I, due to negative feedback inhibition of GH. In contrast, impairment of GH signaling due to insulin deficiency cannot generally be compensated by GH hypersecretion. This is true in fasting children (63) and adults (62). The exact mechanisms by which insulin and GH signaling crosstalk on the post-receptor level is not yet understood (Figure 3). More recent data suggesting that FGF21 plays a role in mediating these events needs further confirmation (72). Interestingly, GH signaling leading to activation of lipolysis in adipose tissue and increased hepatic glucose production via both glycogenolysis and gluconeogenesis in the liver, is not affected by the absence of insulin crosstalk (reviewed in (72)). This has important implications in securing substrate mobilization and gluconeogenesis during fasting and explains the cardinal hypoglycemic symptoms in GHD and GHIS newborns in the absence of intrauterine growth retardation (IUGR). GH signaling leading to lipolysis is thought to involve STAT5b. Most information comes from animal models and involves GH signaling in the liver, but in mouse adipose tissue GHR KO downregulates beta-3 adrenergic receptor expression and inhibits lipolysis (73). GH effects are lost if STAT5b signaling is blocked (74), gene transcript profiles of GHR KO and STAT5b KO animals overlap largely, and STAT5b controls key regulator enzymes involved in lipid metabolism (75). However, if STAT5b mediates both metabolic signaling and IGF-I production it still needs to be understood where the two pathways diverge, and why GH metabolic signaling is not blocked in the absence of insulin crosstalk. In humans, recent studies have identified new GH signaling responses involving GH downregulation of fat-specific protein (FSP27), a negative regulator of lipolysis. MEK/ERK activation and inhibition of peroxisome proliferator-activated receptor-y (PPARy) are involved, and this offers an alternative signaling pathway from the GHR (76).

Interactions Among Endocrine Axes

The activity of the insulin-GH-IGF-axis is dependent on the other endocrine axes which have permissive actions on GH stimulated IGF-I expression and affect IGFBPs and proteases (Figure 4). For example, thyroxine is needed to enhance GH effects on endocrine IGF-I expression and a normal GH-IGF-IGFBP-axis is needed for optimal thyroid hormone production (77). Sex steroids further enhance the function of the GH-IGF-axis, most likely by attenuating pituitary and hypothalamic sensitivity to IGF-I negative feedback (78). The pivotal role of sex steroids on the setpoint of the axis is reflected by the peak circulating levels of IGF-I and IGFBP-3 reached in mid-puberty (50,51). On the other hand, GH via its stimulation of local IGF-I is important for testicular production of testosterone and spermatogenesis (79), and the local IGF-IGFBP-axis is involved in selection and growth of the primary follicle in the ovary, estradiol production and ovulation (80, 81). Finally, cortisol has impact on the actions of the GH-IGF-axis on growth by blocking IGF1R signaling leading to apoptosis (82) despite normal endocrine IGF-I levels (83).



Figure 4. Hypothalamic GH releasing hormone and somatostatin establish the pulsatile pituitary GH secretion that is established as the main regulator of endocrine and paracrine/autocrine IGF-I production during the first year of life in humans. Insulin is permissive for this regulation by modulating GHR signaling, and normal beta-cell release of insulin is required for normal liver derived endocrine IGF-I levels measured in serum (blue insert) that in most cases is a good marker of the local production and actions of IGF-I. During fasting the GH regulation of IGF-I is uncoupled, resulting in decreased IGF-I (and catabolism) and elevated GH secretion and maintained lipolytic signals securing gluconeogenesis and preventing hypoglycemia. Apart from insulin, the endocrine thyroid axis is important for normal GH induced IGF-I production and during pubertal development sex steroids from the gonads enhance the performance of the GH-IGF-axis presumable by relaxation of the negative IGF-I feedback on GH secretion allowing a higher set-point of the axis. Whether this is an estradiol effect is not fully elucidated but it is suggested by the fact that non-aromatizable androgens such as oxandrolone do not affect IGF-I levels. The actions of the adrenal axis are most likely local and involve actions on IGF1R signaling leading to apoptosis of stem cells in the growth plate and thus irreversible loss of height. Cortisol excess leaves endocrine IGF-I and GH levels largely unaffected.

Discordance Between Endocrine and Paracrine/Autocrine IGF-I

An important example of metabolic and mitogenic consequences of an unbalanced endocrine versus autocrine/paracrine insulin-GH-IGF-axis comes from observations in children and adolescents with type 1 diabetes (Figure 5). They suffer specifically from insulin deficiency in the hepatic portal circulation as a result of the subcutaneous delivery of insulin (reviewed by Dunger (64)). This attenuates their endocrine production of circulating IGF-I despite excessive GH secretion (84). Circulating IGF-I deficiency and GH hypersecretion induce insulin resistance which is further augmented by insufficient suppression of hepatic glucose output. To overcome this, higher subcutaneous insulin doses are needed to maintain glycemic control, and this results in

aggravated systemic hyperinsulinemia. The importance of local tissue hyperinsulinemia and GH hypersecretion in generating high paracrine/autocrine IGF-I production and promoting mitogenic vascular events leading to diabetic long-term complications should not be underestimated. Based on this insight. a promising new drug targeting the alphaVbeta3 integrin affecting IGF-I signaling in smooth muscle cells has been found to inhibit the development of atherosclerotic lesions in diabetic pigs (85). Another consequence of a compensatory increased in local IGF-I activity is the finding of normal childhood and pubertal linear growth despite endocrine IGF-I deficiency in type 1 diabetes (86). It is interesting that the endocrine and paracrine/autocrine changes in the insulin-GH-IGF-axis observed in children with type 1 diabetes closely resembles those observed in liver IGF-I KO mice which eventually leads to diabetes in the KO mice. Given that portal delivery of insulin, which has the potential to completely restore IGF-I levels in type 1 diabetes (70, 71, 87), remains an experimental treatment, rhIGF-1 treatment to restore circulating IGF-I and suppress GH and decrease insulin needs appears to be the most feasible approach to take (88). In a 6-month clinical trial of a single daily injection of rhIGF-1 improved glycemic control in adolescents with type 1 diabetes were found (89). Long-term studies on diabetic vascular complications have yet to be performed.

If paracrine/autocrine IGF-I production is lost in addition to liver-derived IGF-I, the metabolic consequences become obvious. This situation was first reported in a boy with a deletion of exon 4 of the IGF-I gene (90) resulting in severe fetal and post-natal growth arrest, poor brain development and extreme insulin with resistance compensatory hyperinsulinemia and acanthosis nigricans. A short trial of treatment with rhIGF-1 resulted in normalization of circulating IGF-I, suppression of GH hypersecretion and a markedly decreased insulin response to a meal tolerance test (91). In this example and in type 1 diabetes, it has been discussed whether the normalization of glucose metabolism following rhIGF-I therapy is most importantly associated with insulin-like effects of IGF-I on glucose uptake in muscle or suppression of GH hypersecretion? Although most studies support the importance of GH suppression, prolonged actions of IGF-I similar to that of long-acting insulin analogs in type 1 diabetic patients are important. IGF-I is equipotent with insulin in stimulating glucose uptake in human muscle but has less effects in fat and liver (92). Reports that newborns with a complete lack of insulin effects due to inactivating defects in the INSR gene can now survive for extended time into adolescence when treated with recombinant IGF-I, that stimulate glucose uptake via the IGF1R sharing common signaling pathways with the INSR, support an important direct role of IGF-1 signaling on metabolism (93).



Figure 5. Changes in liver derived endocrine IGF-I measured in the circulation and paracrine/autocrine IGF-I are in most cases concordant. In the absence of practical and validated methods to measure IGF-I at the tissue site of action, paracrine/autocrine IGF-I activity is assessed by determining known physiological actions of IGF-I such as growth or glucose metabolism. Type 1 diabetes is a condition with discordant changes in endocrine vs. paracrine/autocrine changes in IGF-I that in many ways resembles those reported in a mouse model of conditional knock-out of IGF-1 expression in the liver. In type 1 diabetes, insulin deficiency in the liver, caused by a systemic rather than a portal insulin replacement therapy, results in a functional GHR signaling defect to IGF-I transcription (uncoupling). Low endocrine IGF-I production decreases circulating IGF-I and results in decreased negative pituitary feedback and GH hypersecretion. The lack of direct IGF-I effects on glucose uptake in muscle and the diabetogenic effects of GH (including maintained signaling to lipolysis) decreases insulin actions on glucose metabolism (known as insulin resistance). The portal insulin deficiency also fails to suppress hepatic glucose production. In other to maintain glycemic control, the increased insulin requirement can only be met by more subcutaneous insulin leading to systemic hyperinsulinemia. There is no direct information about local paracrine/autocrine IGF-I activity, but there are several indications that tissue hyperinsulinemia and GH hypersecretion results in a compensatory increase of tissue IGF-I activity. Firstly, linear growth is not impaired in children and adolescents with Type 1 Diabetes despite of their low endocrine IGF-I (comparable to levels in short stature children), indicating a compensatory upregulation of local IGF-I activity (IGF-I being the most important stimulator of longitudinal growth). Secondly, it is plausible that increased local IGF-I activity contributes to diabetes complications known to be tightly associated with increased rather than decreased IGF-I activity. While type 1 diabetes is not generally associated with increased risk of cancer, the increase in local production of IGF-I in obesity and type 2 diabetes may contribute.

Liver Disease and Endocrine Versus Paracrine/Autocrine IGF-1 Production

In children with severe liver disease, there may be similar discrepancies between circulating endocrine levels of IGF-I and IGF-I activity in peripheral levels contributed by paracrine/autocrine secretion of IGF-I (94). However, less is known about peripheral IGF-I activity and it is possible that there are more secondary metabolic and nutritional issues that could lower local IGF-I production and impact on linear growth. Particularly in liver cirrhosis associated with thalassemia major, which concomitantly can impair pituitary GH secretion, there is no secondary upregulation of local IGF-I and linear growth failure is common (95). Recently, increased IGF-I expression was reported in obese children with non-alcoholic fatty liver disease (NAFLD) and it was combined with upregulation of IGF1R (96), not expressed in the normal liver but involved in liver repair, such as after liver resection in a mouse model (97).

GH and Cytokine Crosstalk

STAT5b phosphorylation is also mediated by activation of other members of the cytokine receptor family and has an impact on immunological function: this is evident from the finding of immune deficient symptoms in children with STAT5b genetic defects (98) but these are not found in GHD and GHIS children.

Negative Control of GHR Signaling

Control of the GH signaling cascade is also under inhibitory control, principally by two mechanisms. Firstly, tyrosine phosphatases including PTP dephosphorylate GHR associated molecules. In Noonan's syndrome genetic defects in the PTPN11 gene may affect this pathway and has been implicated in poor growth and poor response to GH therapy, although reports are conflicting (99, 100). In addition, the SOCS gene is activated by GH signaling and works as a short intracellular negative feedback loop which rapidly down-regulate GHR activity by internalization and receptor ubiquitinoylation resulting in lysosomal and proteasomal degradation (101).

Other Nutritional Signals to the GH-IGF-Axis

Nutritional supplementation increasing dietary protein intake from cow's milk increases endocrine IGF-I, while an equal intake of animal protein from meat does not (102). It is possible that the amino-acid composition that differs depending on the dietary source may contribute, and there are other constituents in skimmed milk not present in meat. More likely, however, it is explained by a higher carbohydrate intake in the milk group (while fat content was higher with meat supplementation) and the finding that fasting insulin levels doubled (103), in accordance with the essential regulatory role of insulin on GHR signaling discussed above. There is, however, direct evidence for a regulatory role of amino-acids on IGF-I production that is independent of insulin. Branched chained amino-acids (BCAA) are known to stimulate cell growth by the activation of mTORC2, a protein complex that controls protein synthesis in cells by sensing nutrient and energy availability and is also one of the main signaling pathways of IGF-I and insulin (Figure 3). The role of BCAA has been studied in rats given a restricted diet containing high levels of BCAA, compared to a group given low levels of BCAA (104).

The availability of nutrients in the circulation might also have a direct effect on the production of IGF-I. Human breast adipocytes cultured in high glucose levels have been found to produce more IGF-I compared to adipocytes cultured in low glucose levels (105).

IGF-II

IGF-II is a paracrine/autocrine hormone which is as essential for fetal growth as IGF-I (21). IGF-II may also contribute considerable to postnatal growth although the absence of endocrine effects makes it difficult to study in humans. Less is known about the metabolic actions of IGF-II. Growth promoting actions of IGF-II is via the IGF1R and IGF1R-INSR hybrid receptors which has preference for IGF-II actions if the A isoform of the INSR receptor - expressed in the fetus and in malignantly transformed cells - pairs with the IGF1R in the hybrid (106). The human IGF2 gene is an imprinted gene (107) exclusively expressed from the paternal allele in certain tissues (reviewed by Rossignol (108). The imprinted promoter region is found in a complex configuration with the H19 gene on chromosome 11 and shares two important imprinting regions with this gene. Methylation of the imprinting region ICR1 results in expression of the paternal IGF2 allele, while H19 gene expression is suppressed. Correct imprinting should lead to expression of the paternal allele only and sufficient expression of IGF-II for normal growth. Loss of methylation of the ICR1 on the paternal allele results in the phenotype of Silver Russell syndrome (SRS) which may also arise from other genetic aberrations that have not yet been linked to the IGF-II production or signaling cascade including maternal uniparental dyssomnia of chromosome 7.

SRS is characterized by proportional IUGR with severe SGA at birth, relative sparing of the brain with close to normal head circumference at birth, severe feeding difficulties in infancy and childhood (which in contrast to Prader-Willi syndrome does not rebound into feeding obsession later in life), postnatal growth retardation and body asymmetry. As indicated by the SRS phenotype, IGF2 gene transcription of some organs are not controlled by imprinting. The relative normal development of the brain and the relative macrocephaly of SRS is explained by the lack of imprinting control of IGF-II expression in the brain. Children with genetic mutations in the expressed paternal allele of the IGF2 gene, were reported to have an SRS phenotype. They had somewhat more pronounced psychomotor developmental problems compared with the SRS phenotype, which has increased risk of autism spectrum defects including attention deficit hyperactivity disorder. In SRS, there is a normal postnatal expression of the IGF2 gene in the liver leading to normal levels of circulating IGF-II.

Interestingly, the normal endocrine levels of IGF-II do not overcome the postnatal growth restriction. A similar lack of endocrine IGF-II effects on growth and metabolism was reported in the IGF-I deficient child mentioned previously with a loss of exon 4 of the IGF1 gene. He had compensatory increased GH, IGFBP-3, ALS as a result of lack of negative IGF-I feedback and secondary to the increased IGF binding capacity, increased IGF-II (see also the chapter on IGF-binding proteins below). Another observation in favor of this view is the lack of a correlation between newborn cord levels of IGF-II and birth size (41). This contrasts with a strong positive correlation between cord blood IGF-I concentrations and birth size. The role of IGF-II should be viewed in the light of Efstradiadis series of knockout experiments in mice (21) where the lgf2ko mice had the same degree of fetal growth retardation as the *iqf1*ko which demonstrates that IGF-II is a paracrine/autocrine and not an endocrine hormone.

It is possible that circulating IGF-II after release from the ternary complex is cleared from the circulation by binding to the IGF2R – identical to the mannose-6phosphate receptor – which is associated with lysosomes and results in degradation of IGF-II in endothelial cells (23). In the elegant mouse KO experiments by Efstradiadis et al (21), KO of the IGF2R resulted in fetal overgrowth. However, the largely elevated IGF-II serum levels in that model are more likely a secondary finding, while the lack of clearance of paracrine/autocrine IGF-II is the explanation for the excessive growth.

IGF Binding Proteins

Six IGFBPs bind IGF-I and IGF-II inside and outside the circulation and has impact on IGF bioactivity (reviewed by Clemmons (109). The IGFBP-related proteins share some structural similarities with the six IGFBPs but have no relevant impact on IGF bioactivity. GF-I passes the endothelium intact primarily via IGF1R mediated transcytosis and this process is essential for endocrine actions of liver derived IGF-I (110). Limited experimental evidence from animal and tissue cultures suggest that IGF-I complexed with IGFBP-1 and -2 may leave the circulation, although the extent and importance is unclear (Bar 1990). After endothelial passage IGF-I redistributes to soluble IGFBPs in the extravascular fluids or IGFBPs bound on extracellular matrix or cell surfaces (111). The concentration of unbound IGF-I in the circulation is likely to be proportional to unbound IGF-I concentrations in the tissues, but they are not equal and may have different relationship in different target tissues with differentially-expressed IGFBPs.

As pointed out earlier in this review, transgenic animal studies disrupting one or more IGFBPs have not suggested that a marked growth phenotype should be expected in children and no IGFBP mutations causing growth retardation in children had been reported to the best of my knowledge. Interestingly, as predicted by the Igfbp4-ko and the pappa-ko animal models described previously, a human PAPP-A2 gene defect with growth phenotype was recently reported as detailed below (43).

Free IGF-I Assays

Assays claiming to measure free circulating IGF-I have been developed, but it is unclear to what extent different techniques are influenced by redistribution of IGF-I among IGFBPs associated with the assay procedure (112). Anyway, the fact that IGF-I redistribute among extravascular IGFBPs after passing the endothelium is likely to affect the local tissue bioavailability even more. Moreover, the fact that most data in the literature originate from one assay technique established in one single laboratory has resulted in a lack of confirmatory reports. In a few cases, measurements with different free IGF-I assays have been reported from the same study/experiment with large differences in results (113). The bottom line is that measurements of free IGF-I have not been demonstrated to better predict different physiological or pathophysiological conditions in humans and do therefore not provide any clinically important contributions (114, 112). Techniques to assess IGF-I at the tissue site of action pose practical and methodological challenges. Attempts to establish and

validate a method to determine local tissue levels by microdialysis have been reported in adolescents with type 1 diabetes, where endocrine levels are a poor marker of local IGF-I activity (115).

Ternary Complex Formation

The developmental establishment of GH control over the IGF1, IGFBP3 and IGFALS genes in early childhood initiates the dominance of the ternary complex formed by IGF-I or IGF-II and IGFBP-3 (or IGFBP-5) and ALS as the quantitatively most important circulating form of IGF-I and IGF-II (reviewed by Baxter (116). In the human fetus and newborn, serum IGFBP-3 and ALS concentrations are low and ternary complex formation is absent (117). Although IGF2 gene expression is not under GH control, the circulating levels of IGF-II are largely influenced by GH status since IGF-II (as well as IGF-I and IGFBP-3) is rapidly cleared from the circulation if not bound in the ternary complex. This can be observed in children with SPIGFD, who are deficient in IGF-I as well as IGFBP-3 and ALS, and in whom sc injected rhIGF-I displays a very fast serum clearance rate (118). As mentioned, formation of the ternary complex also governs the circulating levels of IGFBP-3 which under physiological conditions is present in a 1:1 molar relationship with IGF-I plus IGF-II. ALS is a large glucoprotein that under physiological conditions are present in a two-fold molar excess (16).

Immunometric IGFBP-3 assays have been claimed to be more predictive of GH status in very young children; however, the support for that is weak. It is rather a misconception related to problems of commercial IGF-I assays at the lower end of IGF-I detection. Moreover, IGFBP-3 has been claimed to provide information about IGF-I bioavailability from calculating the molar ratio of total IGF-I to IGFBP-3. Given that both IGF-I and IGFBP-3 are rapidly cleared from the circulation if unbound, using the IGF-I/IGFBP-3 ratio and disregarding IGF-II concentrations (that are 2-3-fold those of IGF-I on a molar basis) does not make any sense. During puberty, for example, IGF-I bioactivity is increased (114). This is dependent on the 3-4-fold increase in total IGF-I (50), which consequently results in an increase in unbound IGF-I, even if the increase is matched with the same absolute molar increase in IGFBP-3 (and complexed with ALS in a ternary complex). A common view is that increased IGF-I bioactivity depends on a higher IGF-I/IGFBP-3 molar ratio during puberty. However, the increase in molar ratio is entirely explained by the fact that IGF-I and IGFBP-3 increase with the same number of moles per liter, but with a larger relative increase in IGF-I than IGFBP-3 and with IGF-II molar concentrations being unchanged (112).

IGFBP Proteolysis and Physiological Consequences

The fact that proteolytic cleavage of IGFBP-3 is common, and may result in falsely elevated IGFBP-3 immunoactivity, is the most likely reason for observing a low IGF-I/ IGFBP-3 ratio. Under certain physiological conditions first described in pregnancy (119, 120), specific proteases cleave IGFBP-3 into several proteolytic fragments of which each may retain immunoactivity and thus give rise to signals in an immunometric assay (121). This will lead to overestimations of the IGFBP-3 immunoreactivity in pregnancy, which is already truly increased due to increased placental GH tonus. It may also lead to the erroneous conclusion that IGF-I bioactivity is decreased. On the contrary, IGF-I bioactivity is increased in the maternal circulation resulting from increased total serum IGF-I and decreased binding affinity of fragmented IGFBP-3 (122). There is strong experimental evidence that IGFBP proteolysis results in lower IGF binding affinity. The finding that partial IGFBP-3 proteolysis, such as in pregnancy, does not disrupt the ternary complex, has questioned its significance. However, evidence for increased IGF-I bioactivity in a ternary complex with fragmented IGFBP-3 exists (123). IGFBP-3 proteolysis has also been described in insulin resistant states such as fasting, obesity and type 1 and 2 diabetes (62, 124, 125). While several known proteolytic enzymes such as those involved in blood clotting (126) and cancer metastasis (127, 128) have been identified as IGFBP-

Recently, a human gene mutation of PAPP-A2, a circulating and tissue protease with IGFBP-5 (and to some extent IGFBP-3) as its primary substrate (Gaudamauskes et al), was demonstrated to have a marked growth phenotype involving fetal and postretardation in children natal arowth in а consanguineous family (43). Largely elevated levels of circulating IGF-I but as a result of absence of proteolysis of IGFBP-5 and IGFBP-3, necessary for disruption of ternary complex formation, IGF-I bioactivity in serum is low and presumably tissue bioactivity of IGF-I (and IGF-II) is low. Functionally, this is a state of severe primary IGF-I deficiency (despite of elevated total serum IGF-I) and pharmacokinetic studies suggested that sc. Injections of rhIGF-1 resulted in a fraction of unbound IGF-I in serum despite the impaired proteolysis of IGFBP-5 and IGFBP-3 (133). Attempts to improve linear growth by rhIGF-I treatment has been reported to result in some improvements in a few affected children but not all (134, 135)

It is beyond the scope of this chapter to review the overwhelming evidence from cell biology experiments demonstrating the important role of IGFBPs in modulating IGF bioactivity and the role of IGFBP proteases and their actions at the cellular level. Furthermore, IGFBPs other than IGFBP-3 may play a role in the access of IGF-I to various tissues (129).

SUMMARY

In the present review the pivotal role of nutrition and insulin in determining the regulation and actions of the GH-IGF-axis is reviewed. For the pediatrician, caring for patients in a phase of rapid growth and development, it is important to refer to normality and understand the requirements for a normal insulin-GH-IGF-axis in order to succeed in this task. In the complex work-up, treatment and management of growth disorders a thorough understanding of the normal physiology of the axis is essential in taking the right actions (130, 131). From the normal physiology of this axis, it is possible to understand the consequences of various genetic defects and disorders that affect its regulation and function. The most severe conditions associated with defects in the axis may cause a loss of adult height of approximately 1/3 and may cause severe developmental and neurological deficits and compromise pubertal

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maturation and fertility. Minor changes in the setpoint of the axis caused by programming of the fetus exposed to intra-uterine growth retardation may predispose the individual for poor linear growth and later metabolic disease, insights that the pediatrician should be aware of and consider in order to improve health and prevent later disease.

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