ENDOCRINE REVIEWS

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The Role of Liver-Derived Insulin-Like Growth Factor-I

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IGF-I is expressed in virtually every tissue of the body, but with much higher expression in the liver than in any other tissue. Studies using mice with liver-specific IGF-I knockout have demonstrated that liver-derived IGF-I, constituting a major part of circulating IGF-I, is an important endocrine factor involved in a variety of physiological and pathological processes. Detailed studies comparing the impact of liver-derived IGF-I and local bone-derived IGF-I demonstrate that both sources of IGF-I can stimulate longitudinal bone growth. We propose here that liver-derived circulating IGF-I and local bone-derived IGF-I to some extent have overlapping growth-promoting effects and might have the capacity to replace each other (= redundancy) in the maintenance of normal longitudinal bone growth. Importantly, and in contrast to the regulation of longitudinal bone growth, locally derived IGF-I cannot replace (= lack of redundancy) liver-derived IGF-I for the regulation of a large number of other parameters including GH secretion, cortical bone mass, kidney size, prostate size, peripheral vascular resistance, spatial memory, sodium retention, insulin sensitivity, liver size, sexually dimorphic liver functions, and progression of some tumors. It is clear that a major role of liver-derived IGF-I is to regulate GH secretion and that some, but not all, of the phenotypes in the liver-specific IGF-I knockout mice are indirect, mediated via the elevated GH levels. All of the described multiple endocrine effects of liver-derived IGF-I should be considered in the development of possible novel treatment strategies aimed at increasing or reducing endocrine IGF-I activity. **(*Endocrine Reviews* 30: 494–535, 2009)**

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Abbreviations: AD, Alzheimer's disease; ALS, acid-labile subunit; AR, androgen receptor; BMD, bone mineral density; CRF, chronic renal failure; DLP, dorsolateral prostate; EGF, epidermal growth factor; GHS, GH secretagogue; IGFBP, IGF binding protein; KO, knockout; LIP, liver IGF-I producer; MUP, major urinary protein; rhIGF-I, recombinant human IGF-I; SHP, SH domain containing protein tyrosine phosphatase; STAT5b, signal transducers and activators of transcription protein 5b; VP, ventral prostate; VSMC, vascular smooth muscle cell.

I. Introduction

This review examines the physiological role of liver-produced IGF-I, a controversial topic addressed or touched upon in several earlier articles in *Endocrine Reviews* (1–5). We aim to summarize present knowledge regarding the role of liver-derived IGF-I in different organ systems, focusing on information that has appeared in the literature since the previous review in 2001 (4).

Although there are several biological effects of IGF-I, they essentially are related to either its status as a growth factor or the structural similarity between the IGF-I and insulin systems. Comparisons of the chemical structures of IGF-I and proinsulin show large (about 40%) amino acid sequence homology. The type I IGF receptor (subsequently referred to as the IGF-I receptor), which mediates the biological effects of IGF-I, has a 60% amino acid sequence homology with the insulin receptor (5, 6). Indeed, the IGF-I receptor and insulin receptor can form heterotetramers consisting of one α- and one β-chain from the IGF-I receptor complex and one α- and one β-chain from the insulin receptor (5, 6). The secretion of both IGF-I and insulin is stimulated by food intake and inhibited by fasting, a major biological similarity between these systems. This review covers the metabolic effects of liver IGF-I on carbohydrate and fat metabolism as well as its possible effects on body fat accumulation.

In addition to its metabolic and partly insulin-like effects, IGF-I is a growth factor and a prime mediator of the growth-promoting effects of GH. This review discusses the role of liver-derived IGF-I for the anabolic and growth-promoting effect of GH, and especially considers the relation between hepatic and extrahepatic IGF-I production. We also discuss the origin and effects of circulating IGF-I and describe the interaction between IGF-I and GH in the classic negative feedback loop formed by the hypothalamus-pituitary and liver, in relation to the well-known pulsatility of GH secretion (7).

The effect of IGF-I is modulated by its association with six IGF binding proteins (IGFBPs) (8, 9). IGFBPs regulate the biological actions of IGF-I (and also IGF-II) in several ways. They transport IGFs from the circulation to peripheral tissues (*e.g.*, IGFBP-1, -2, and -4), maintain a reservoir of IGFs in the circulation (mainly IGFBP-3), potentiate or inhibit IGF action, and may also exert IGF-independent effects (9). Furthermore, in addition to binary complexes with IGFBPs in biological fluids, IGF-I is sequestered into ternary complexes of one molecule each of IGF-I, IGFBP-3 (or IGFBP-5), and an acid-labile subunit (ALS) (10). The present review discusses mouse models with knockout (KO) of liver-derived or locally produced IGF-I as well as models with KO of liver-derived IGF-I combined with KO of total ALS and/or total IGFBP-3.

There are some limitations on the content of this review. We do not provide a detailed discussion of the effects of IGF-I on development and prenatal growth or the effects of the IGF system on tumor growth and metastasis, a topic recently described in *Endocrine Reviews* (5). Furthermore, we do not discuss the effects of IGF-II in detail because it is mainly important in prenatal and tumor growth (although it is produced in large quantities in humans, but not rodents, after birth) (5). In the mouse models used to investigate the effects of liver-derived IGF-I, no evidence suggests compensatory effects by IGF-II (11, 12). However, IGF-II may be of importance for kidney growth during adulthood (see *Section VI.C*).

II. Liver-Derived IGF-I and Longitudinal Bone Growth

A. Background, including the original somatomedin hypothesis

Although several hormones and nutritional factors participate importantly in normal postnatal longitudinal bone growth, it is generally accepted that GH is the most important hormone in this respect (3, 13–15). However, GH is not required for normal intrauterine growth, a finding supported by evidence that GH deficiency and/or GH insensitivity do not associate with reduced birth size (15–18). Mouse models with GH deficiency or GH insensitivity suggest that the major effect of GH on postnatal growth does not occur until after 2 wk of age (15, 19, 20). In contrast, KO studies in mice and case reports of patients with inactivating mutations of IGF-I or the IGF-I receptor have established clearly that IGF-I is a major regulator of intrauterine growth (19, 21–25). Evidence that birth weight and size associate with polymorphisms in the IGF-I promoter region further supports a role of IGF-I for intrauterine growth (26–28). Importantly, the stimulatory role of IGF-I on intrauterine growth is independent of GH. An important role of IGF-I in body growth is supported by evidence that low serum IGF-I associates with low body weight in dogs (29, 30), and a single IGF-I allele is a major determinant of small size in dogs (31).

A widely discussed question involves whether the stimulatory effect of GH on postnatal growth acts directly on tissues or is mediated by a liver-derived growth factor, initially called sulfation factor but later renamed somatomedin and subsequently shown to be identical to IGF-I. According to the original somatomedin hypothesis by Daughaday *et al.* (1, 32, 33), GH stimulates skeletal

growth by stimulating liver production of IGF-I, which in turn stimulates longitudinal bone growth in an endocrine manner (Fig. 1A).

During the 1980s and 1990s, the original somatomedin hypothesis was challenged by some key findings. The first experiments suggesting a role of locally produced IGF-I came from studies of D'Ercole *et al.* (34, 35), who showed that multiple tissues produce IGF-I and that GH treatment of hypophysectomized rats increased IGF-I content of many organs. Several studies have confirmed a stimulatory role of GH on IGF-I expression in multiple nonhepatic tissues (36–40). Consistent with the idea that GH may mediate its effects in part by increasing local production of IGF-I, we demonstrated that local injection of human GH directly into the cartilage growth plates of the hind limbs of hypophysectomized rats produced significantly increased length of injected limbs compared with the control, contralateral limbs (41). Other investigators have confirmed our results, clearly establishing a direct effect in the growth plate region of GH on bone growth (2, 42–46). Furthermore, the findings that simultaneous infusion of IGF-I antiserum and GH blocked the local effect of GH on cartilage growth (47) and that local administration of GH stimulated linear growth of hypophysectomized rats and GH-deficient mice with little or no increase in serum IGF-I provided indirect evidence that GH's effect on cartilage growth may be mediated in part via increased local IGF-I production and/or action (2, 3, 46). In addition, the original somatomedin hypothesis was challenged by the finding that treatment of GH-deficient patients with GH resulted in a better growth response than treating GH-insensitive patients with recombinant human IGF-I (rhIGF-I) (48, 49). These results indicated that GH has local effects that may be independent of increased levels of the circulating "endocrine" form of IGF-I, thereby introducing an alternative to the original somatomedin hypothesis. We proposed that the local effect of GH included both an effect that could not be replaced by IGF-I and an effect mediated by increased local IGF-I (Fig. 1B) (2, 3). In addition, although believed to be of minor importance, a role for circulating liver-derived IGF-I could not be excluded (Fig. 1B). Importantly, until a decade ago, techniques were not available to evaluate the relative importance of liver-derived endocrine IGF-I *vs.* locally produced IGF-I for the regulation of bone growth.

An important study by Lupu *et al.* (19) established an IGF-I-independent effect of GH on body growth. They compared the body length of mice with GH receptor inactivation (GHR KO), IGF-I inactivation (IGF-I KO) and IGF-I GHR double inactivation (IGF-I + GHR DKO; Fig. 2). Importantly, GHR-IGF-I double KO mice had a more severe reduction in body length than IGF-I KO mice, demonstrating that GH exerts IGF-I-independent effects on body growth (Fig. 2). The authors concluded that GH and IGF-I promote postnatal growth by both independent and common functions because the growth retardation of double GHR-IGF-I KO is more severe than that observed with either class of single mutants (19). Although it is clear that IGF-I is the major regulator of intrauterine growth, our comparison of GH-deficient and total IGF-I KO mice revealed that GH contributed more to longitudinal bone growth than IGF-I during postpubertal growth (50).

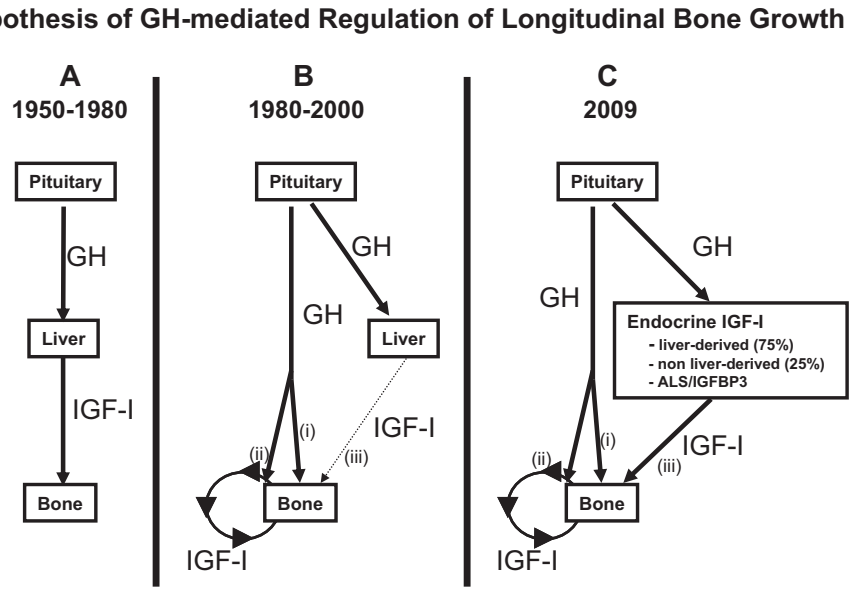
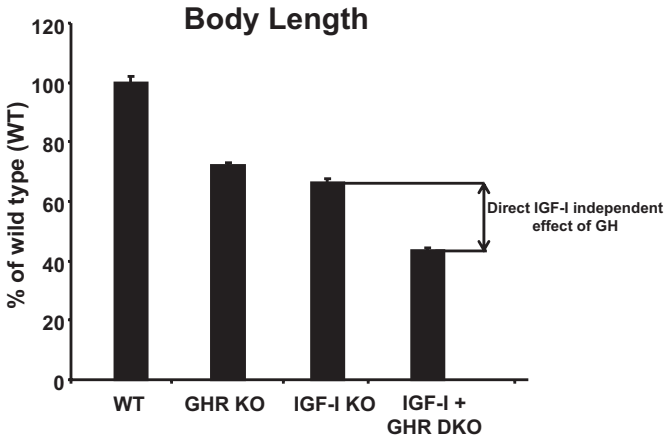


FIG. 1. Hypotheses of GH-mediated regulation of postnatal longitudinal bone growth. The different hypotheses of the mode of action for GH on longitudinal bone growth are described in detail in the text of *Section II*. A, Hypothesis proposed 1950–1980; B, hypothesis proposed 1980–2000; and C, currently proposed hypothesis.



Effects of gene deletion of growth hormone receptor (GHR KO), IGF-I (IGF-I KO) and the combination of GHR and IGF-I (IGF-I + GHR DKO).

FIG. 2. GH exerts direct effects not dependent on IGF-I on body length. Comparison of body length in GH receptor KO (GHR KO), IGF-I KO, IGF-I/GHR double KO (IGF-I + GHR DKO), and wild-type (WT) mice at 130 d of age. The figure is adapted from Table 3 in Lupu *et al.* (19). Importantly, these data demonstrate that IGF-I and GH exert at least partly independent and additive stimulatory effects on body length. Values are given as percentage of WT and are means ± SEM.

B. Tissue-specific manipulation of IGF-I expression

Several elegant mouse models with tissue-specific manipulation of IGF-I expression have been developed and characterized during the last 10 yr to evaluate the relative importance of liver-derived endocrine IGF-I *vs.* locally produced IGF-I for the regulation of bone growth. The cre/loxP recombination system was used for all of these mouse models (51).

First, two mouse models with liver-specific IGF-I inactivation were developed in 1999 (Fig. 3B). Both models had exon 4 of the IGF-I gene completely inactivated in hepatocytes using either an albumin-Cre-mediated (12) or an inducible Mx-1-Cre-mediated (11) DNA excision. The growth phenotypes and most of the other phenotypes described during the last 10 yr (*i.e.*, bone parameters, GH secretion, and carbohydrate metabolism) of these two mouse models with liver-specific IGF-I inactivation are rather similar, which we believe strengthens the validity of the findings.

Second, to determine the role of bone-derived IGF-I for bone growth, we recently developed two different mouse models with inactivated IGF-I in chondrocytes and osteoblasts, respectively (52, 53). These two conditional KO models disrupt local but not circulating endocrine IGF-I action (Fig. 3D).

Third, Stratikopoulos *et al.* (54) recently presented a complex but elegant model in which they reexpressed IGF-I specifically in the liver of mice with a totally inactivated IGF-I [*i.e.*, liver IGF-I producer (LIP) mice]. The two mouse models with liver-specific IGF-I inactivation were aimed to determine whether liver-derived IGF-I is required for normal bone growth, whereas the experiments using the LIP mice were designed to determine whether liver-derived IGF-I has the capacity to stimulate bone growth in the absence of local bone-derived IGF-I and, therefore, should be regarded as complementary. Although each mouse model is informative, their growth phenotypes indicate that a full understanding of liver IGF-I action cannot be achieved by looking at only one model.

1. Lessons from liver-specific IGF-I inactivation (Fig. 3, B and C)

The role of liver-derived IGF-I for the regulation of longitudinal bone growth was evaluated, as described above, by using two different mouse models (11, 12). Both mouse models demonstrated that inactivation of liver-derived IGF-I resulted in an approximately 75% reduction in serum IGF-I with essentially unaffected body length (Fig. 3B) (11, 12). Based on these findings, it was proposed that GH stimulates longitudinal bone growth both via a direct effect not involving modulation of IGF-I and via local bone-derived IGF-I, but not liver-derived IGF-I (4, 11, 12, 55). A recent finding showing that mice with liver-specific (using albumin-driven Cre) GH receptor inactivation have normal body length and tibial length despite substantially decreased serum IGF-I levels supports the hypothesis that GH-stimulated growth does not require GH stimulation of IGF-I synthesis in the liver (56).

Because GH levels increased due to lack of IGF-I-mediated negative feedback in the mouse models with liver-specific IGF-I inactivation (for further details, see *Section IV* and Fig. 4), one might speculate that the growth of these mice could be compensated by enhanced growth stimulatory effect of GH on nonhepatic tissues (4, 11, 12, 55, 57). As investigated in several nonhepatic tissues, however, IGF-I mRNA levels did not increase in the two mouse models with liver-specific IGF-I inactivation (11, 12).

Another potential explanation for the lack of effect on body growth in mice with inactivated liver-derived IGF-I is that the remaining 25% of circulating IGF-I might be more bioavailable and, therefore, sufficient for skeletal growth because IGFBP-3 levels clearly decrease and ALS levels increase in these mice (11, 12, 58). In normal mice, 70–80% of IGF-I in the circulation exists in a ternary complex consisting of IGF-I, IGFBP-3, and ALS. This complex has a relatively long half-life ($t_{1/2}$ = 10–16 h). A smaller complex (50 kDa), comprising IGF-I and serum IGFBPs (mainly IGFBP-3), comprises about 15–20% of the circulating pool; the remainder (<5%) is free IGF-I (7.5 kDa), with an extremely short half-life (59). Analyses of mouse models with inactivated ALS and/or IGFBP-3 demonstrated that the total pool of circulating IGF-I depends on the amount of circulating ALS and IGFBP-3,

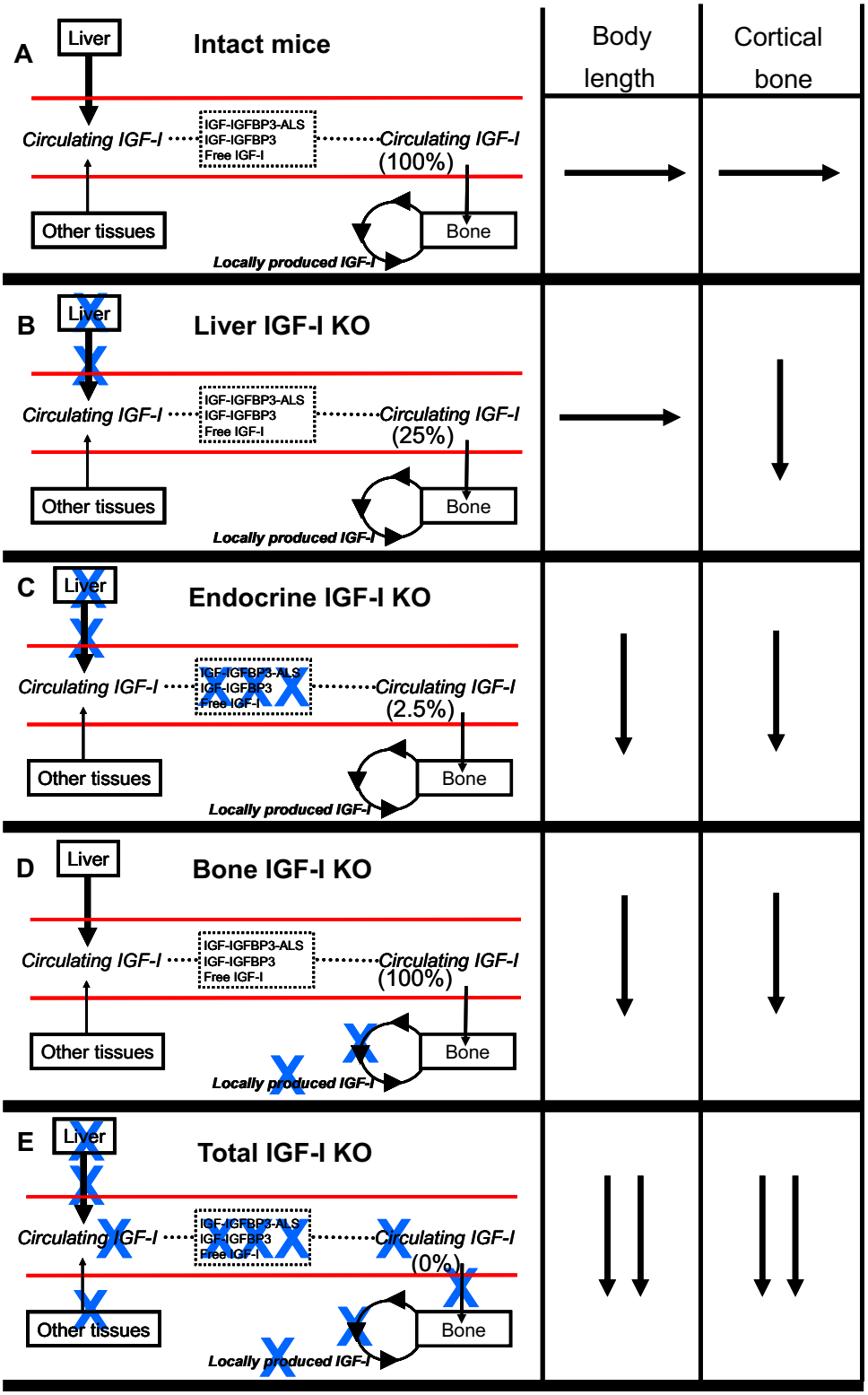


FIG. 3. The role of liver-derived IGF-I, the total pool of circulating endocrine IGF-I, and bone-derived IGF-I for body length and cortical bone mass. The bone length and the cortical bone mass in various IGF-I KO mouse models in relation to intact mice (A) and mice with total IGF-I inactivation (E, total IGF-KO) are summarized. The IGF-I KO models given in panels B–D are mice with liver-specific IGF-I inactivation (B, liver IGF-I KO); mice with dramatically reduced circulating endocrine IGF-I levels due to triple inactivation of liver IGF-I, total ALS, and total IGFBP-3 (C, endocrine IGF-I KO); and mice with bone-specific IGF-I inactivation (D, bone IGF-I KO). The total pool of circulating "endocrine" IGF-I in serum expressed as percentage of intact mice is given within *parentheses* for each mouse model. *Blue X* indicates inactivation/lack of this component. →, Unchanged compared with intact mice; ↓, reduced compared with intact mice but less reduced than in mice with total IGF-I KO; ↓ ↓, substantially reduced to the level seen in total IGF-I KO.

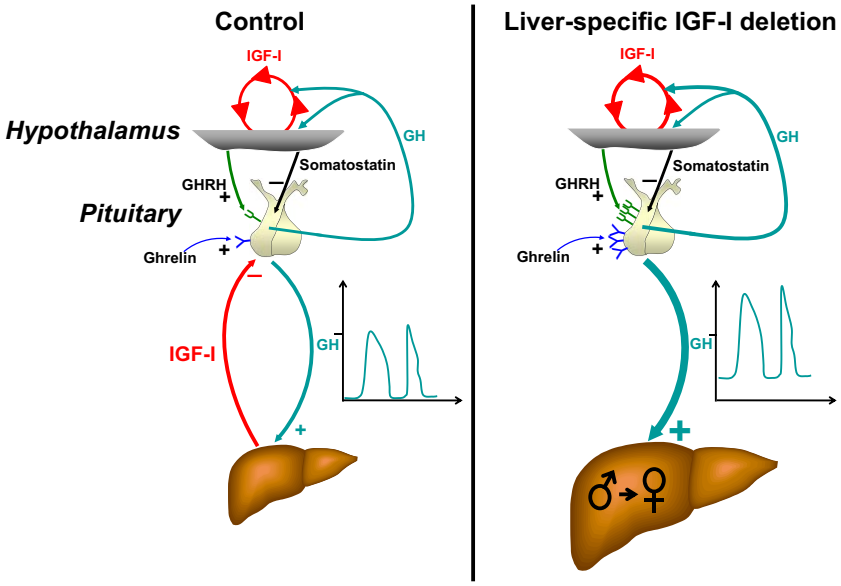


FIG. 4. Proposed model for hypothalamic-pituitary-liver feedback axis of GH secretion and how it is affected by liver-specific IGF-I deletion. Mice with liver-specific IGF-I KO (*right panel*) have increased GH secretion. Increased GH levels in turn enhance the liver weight. In male mice with depletion of liver-derived IGF-I, the enhanced GH trough levels feminize liver functions regulated by the sexual dimorphism of GH secretion in rodents. The mechanism by which lack of liver-derived IGF-I increases GH secretion seems to involve increased expression of GHRH and ghrelin receptors (*right panel*) and augmented responsiveness to these ligands at the level of the pituitary. There is no evidence that lack of liver-derived IGF-I enhances GH secretion via an effect on the hypothalamus, possibly because enhanced pituitary GH and local hypothalamic IGF-I secretion partly counteract the effects of lack of liver-derived IGF-I on the hypothalamus.

which contributes to the stability of circulating IGF-I (60, 61). In addition, circulating IGFBPs (mainly IGFBP-3) might either reduce or enhance the biological activity of IGF-I in the target tissues (in this case, bone).

Yakar *et al.* (61, 62) investigated body growth in mice with very low circulating IGF-I levels resulting from double KO of liver-derived IGF-I and total ALS (62) and of triple KO of liver-derived IGF-I, total ALS and total IGFBP-3 [= endocrine IGF-I KO, Fig. 3C, (61)]. Both mouse models have very low serum IGF-I levels (−97.5% in triple KO mice), most probably as a combined result of deficient IGF-I secretion from the liver and reduced stability of the remaining IGF-I in the circulation. In contrast to mouse models with isolated liver-specific IGF-I KO (Fig. 3B), both the double and triple KO mice displayed a modest but significant reduction in total body length (Fig. 3C) (61, 62), clearly demonstrating that endocrine IGF-I is indeed essential for normal body growth. Based on a comparison between the essentially normal body length in mice with liver-specific IGF-I KO, with approximately 25% serum IGF-I remaining, and the reduced body length in the double and triple-inactivated mice, with 2–10% remaining serum IGF-I, we propose that a serum IGF-I threshold in the range of 10–25% of normal serum IGF-I exists, below which serum IGF-I levels associate with body growth (11, 12, 61, 62). In contrast, normal body growth is seen above this serum IGF-I threshold.

Importantly, reduced body length in the triple KO mice (−6%) with extremely low circulating IGF-I levels is much lower compared with mice with total IGF-I inactivation (−40%; Table 1), suggesting that a substantial proportion

TABLE 1. Magnitude of skeletal changes in mice lacking total, local bone, liver-derived, and endocrine IGF-I actions

		Local bone IGF-I KO			
	Total IGF-I KO[a]	Chondrocyte IGF-I KO[b]	Osteoblast IGF-I KO[c]	Liver IGF-I KO[d]	Endocrine IGF-I KO[e]
Serum IGF-I	100% ↓	Normal	Normal	75% ↓	97.5% ↓
Length	40% ↓	7% ↓	14% ↓	Normal	6% ↓
Cortical bone width	38% ↓	7% ↓	20% ↓	9% ↓	18% ↓
Cortical vBMD	30% ↓	4% ↓	5% ↓	7% ↓	11% ↓
Trabecular BV/TV	55% ↑	ND	20% ↓[f]	10–20% ↓	8% ↓[f]
Bone formation rate	70% ↓	ND	48% ↓	ND	ND
Mineral apposition rate	40% ↓	ND	38% ↓	ND	ND

ND, Not determined; vBMD, volumetric BMD; BV/TV, bone volume/ total volume.

[a] Data for length, bone width, and cortical vBMD were derived from Mohan *et al.* (50), whereas data for trabecular BV/TV, bone formation rate, and mineral apposition rate were derived from Bikle *et al.* (153).

[b] Data for chondrocyte IGF-I KO mice were derived from Govoni *et al.* (52).

[c] Data for osteoblast IGF-I KO mice were derived from Govoni *et al.* (53).

[d] Data for liver-specific IGF-I KO are summaries of the main findings from several studies using mice with liver-specific IGF-I KO (11, 12, 58, 61, 62, 155).

[e] Data for length and bone width of endocrine IGF-I KO were derived from triple KO mice lacking liver-derived IGF-I, total IGFBP-3, and total ALS (61), whereas data for cortical vBMD and trabecular BV/TV were derived from double KO mice lacking liver-derived IGF-I and ALS (62).

[f] Not significant.

of the body growth depends on local IGF-I expression. Although the mouse models with combined liver IGF-I KO and total ALS KO/IGFBP-3 KO, with extremely low serum IGF-I, have been very informative in elucidating the role of endocrine IGF-I, one must consider that both ALS and IGFBP-3 might exert effects other than contributing to the stability of circulating IGF-I. Although the liver is the principal source of circulating IGFBP-3 and ALS, others have reported expression of both IGFBP-3 and ALS in bone and other nonhepatic tissues (60, 61, 63). Furthermore, IGFBP-3 acts independently of IGF-I to regulate growth, apoptosis, and metabolism of target cells (64–69). Therefore, the expression of ALS and IGFBP-3 in nonhepatic tissues and possible IGF-I-independent effects of IGFBP-3 should be considered while interpreting findings using mice with combined liver IGF-I KO and ALS KO/IGFBP-3 KO.

2. Lessons from IGF-I inactivation in bone (bone IGF-I KO; Fig. 3D)

Several different types of cells in the bone microenvironment might contribute to local autocrine/paracrine produced IGF-I in bone. However, the relative contribution of IGF-I produced by various cell types has been unclear. To determine whether IGF-I produced locally by chondrocytes exerts a significant role in regulating bone growth, we used the Cre/loxP approach to disrupt IGF-I production in cells that express collagen type II α-1 chain because type II collagen is predominantly expressed in chondrocytes (= chondrocyte IGF-I KO) (52). In these studies, we found that conditional disruption of IGF-I in chondrocytes led to significant reductions in body length (-7%). Interestingly, postnatal gain in body length (between 4 and 12 wk of age) decreased by as much as 27%. Because disruption of IGF-I in type II collagen producing cells did not influence liver IGF-I expression or circulating levels of IGF-I, these data clearly establish a role for local chondrocyte-produced IGF-I in regulating bone growth. However, skeletal growth of the chondrocyte-specific IGF-I KO mice was clearly less affected compared with mice with total IGF-I inactivation (-40%; Table 1), demonstrating that IGF-I from either the circulation or from other cells in the bone microenvironment can maintain, at least partly, body growth in the absence of chondrocyte-derived IGF-I.

To further explore the role of autocrine/paracrine IGF-I for bone growth, we recently investigated the relative contribution of IGF-I produced by cells of osteoblastic lineage in regulating skeletal growth (53). Because both immature and mature osteoblasts express IGF-I, we used transgenic Cre mice in which Cre expression is driven by entire regulatory regions of the collagen type I α-2 chain for disruption of IGF-I gene in type I collagen producing mesenchymal cells (collagen type I α-2 IGF KO). IGF-I expression in bone and muscle but not liver and brain decreased significantly in the conditional mutants. Importantly, disruption of IGF-I in type I collagen-producing mesenchymal cells did not influence circulating levels of IGF-I, whereas body and femur length decreased by 14–15%, thus supporting the notion that local IGF-I is essential for normal bone growth. However, similar to the chondrocyte-specific IGF-I KO mice, skeletal growth was clearly less affected than in mice with total IGF-I inactivation (-40%; Table 1). These data indicate that local IGF-I from both chondrocytes and osteoblasts participates in the regulation of bone growth. If one assumes that deficient body length in mice with inactivated IGF-I in osteoblasts (-14%) is additive to that seen in mice with chondrocyte-specific IGF-I KO (-7%), combined inactivation would lead to 21% reduction in body length, more pronounced than that seen in mice with extremely low circulating endocrine IGF-I resulting from triple inactivation of liver-derived IGF-I, total ALS, and total IGFBP-3 (-6%; Table 1). However, validation of these theoretical calculations requires development of the combined osteoblast-chondrocyte IGF-I KO mouse model. Due to technical reasons, it remains complicated to inactivate IGF-I simultaneously in all different cell types in the bone microenvironment (not only including osteoblasts and chondrocytes) without affecting liver IGF-I expression, making it difficult to accurately determine the entire proportion of bone growth that is dependent on local IGF-I expression. Nevertheless, the two described mouse models with osteoblast/chondrocyte IGF-I KO with unaffected serum IGF-I levels have clearly established a role for local IGF-I in the regulation of longitudinal bone growth that cannot be replaced by endocrine IGF-I under normal physiological circumstances.

3. Lessons from reexpression of IGF-I specifically in the liver in mice with total IGF-I inactivation

The role of liver-derived IGF-I for bone growth was recently investigated further by the research group of Efstratiadis (54) using an advanced and elegant mouse model with reexpression of IGF-I specifically in the liver in mice with total IGF-I inactivation. They generated double transgenic mice carrying in an *Igf1* null background, 1) a *Igf1* cDNA placed downstream of a transcriptional "stop" DNA sequence flanked by *loxP* sites (floxed); and 2) a *cre* transgene driven by a liver-specific promoter (α-1 antitrypsin). The *Igf1* cDNA, which was inserted by knockin into the mutated and inactive *Igf1* locus itself to ensure proper transcriptional regulation, was expressed exclusively in the liver after Cre-mediated excision of the floxed block (= LIP mice).

The study demonstrated that approximately 30% of the body growth could be achieved by liver-specific IGF-I

reexpression, whereas a substantial proportion of the body growth also required nonhepatic IGF-I expression. Thus, this study clearly demonstrates that liver-derived IGF-I can enhance bone growth in the absence of local IGF-I but not sufficiently for normal bone growth. Further research is required to elucidate whether the mechanisms for the effects of liver-derived IGF-I and local IGF-I on longitudinal bone growth differ. However, due to breeding difficulties, only three LIP mice were evaluated and, therefore, the absolute magnitude of the growth-promoting capacity of liver-derived IGF-I should be interpreted with caution. Future studies using similar methodology for the reexpression of IGF-I specifically in bone in mice with total IGF-I inactivation will be useful to determine the proportion of body growth that can be achieved by local bone-specific IGF-I expression in the absence of liver-derived IGF-I.

A capacity of pharmacologically elevated IGF-I expression in the liver to stimulate body growth in mice with normal endogenous IGF-I expression in nonhepatic tissues is demonstrated by evidence that mice with transthyretin-driven liver IGF-I overexpression, resulting in increased serum IGF-I levels (50–60%), display slightly but significantly increased body length (70). Thus, genetically elevated liver IGF-I expression results in increased body growth in mice with no IGF-I expression in nonhepatic tissues (54) and also in mice with normal IGF-I expression in nonhepatic tissues (70), demonstrating that liver-derived IGF-I has the capacity to stimulate body growth.

4. Conclusions regarding regulation of body growth from the studies using mouse models with tissue-specific manipulation of IGF-I

The experiments using different mouse models with tissue-specific IGF-I inactivation alone or in combination with total ALS and IGFBP-3 KO clearly demonstrate that both endocrine IGF-I (Fig. 3C) and bone-derived IGF-I (Fig. 3D) are required for normal longitudinal bone growth (52, 53, 61, 62). Because the two mouse models with liver-specific IGF-I inactivation but with normal nonhepatic IGF-I expression have serum IGF-I levels above the threshold (<25% of serum IGF-I in intact mice) required for essentially normal body length, liver-derived IGF-I is not required for essentially normal body length in the presence of normal IGF-I expression in nonhepatic tissues (Fig. 3B) (11, 12). However, as demonstrated in the LIP mouse model, liver-derived IGF-I clearly can increase longitudinal body growth in the absence of nonhepatic IGF-I expression (54). Furthermore, the longitudinal bone growth of both the mouse models with endocrine IGF-I inactivation and the mouse models with IGF-I inactivation in bone is clearly less severely affected compared with mice with total IGF-I inactivation. We propose that all of these findings can be explained, because endocrine IGF-I and local bone-derived IGF-I to some extent have overlapping growth-promoting effects and might partly but not completely have the capacity to replace each other (= redundancy) in the maintenance of normal longitudinal bone growth.

C. Target cells for GH and IGF-I in the growth plate

As described in *Section II.A*, it is clear that both GH and IGF-I exert a direct growth stimulatory effect in the growth plate region (3, 41, 42, 45, 47). However, the primary target cells for GH and IGF-I in the growth plate during postnatal growth remain unclear. Based on *in vitro* studies of cultured growth plate chondrocytes performed 20 yr ago, we proposed earlier that GH within the growth plate acts primarily on growth plate precursors in the resting zone, followed by an IGF-I-mediated clonal expansion of chondrocytes in the proliferative layer of the growth plate (2, 3, 71, 72). However, we believe that the validity of our proposed mode of action remains unproven in animal studies *in vivo*. Some *in vivo* analyses have suggested that GH but not IGF-I can increase cell divisions within the resting zone of the growth plate and can also increase its size (73, 74). An effect of GH specifically in the resting zone is supported by the recent *in vivo* finding that GH induced rapid STAT5 phosphorylation in the resting zone but not in the proliferative or hypertrophic zones of the growth plate (75). However, a study by Hunziker *et al.* (76) indicated that not only GH but also IGF-I can increase cell divisions in the resting zone to some extent. Furthermore, Lupu *et al.* (19) suggested that it is not yet proven that cells in the resting zone are the immediate precursors of proliferative chondrocytes. When analyzing the growth plates of total IGF-I-inactivated mice with elevated GH levels, Wang *et al.* (74, 77) found that chondrocyte hypertrophy was affected, whereas chondrocyte numbers and proliferation were unaffected, suggesting that hypertrophic chondrocytes are important target cells for IGF-I. They also found that GH receptor-inactivated mice had a hypoplastic resting zone, whereas IGF-I-inactivated mice displayed an enlarged resting zone. Therefore, they proposed a dual role for GH in promoting longitudinal bone growth; an IGF-I-independent role in growth plate chondrocyte generation, and an IGF-I-dependent role in promoting chondrocyte hypertrophy (74). In contrast, Lupu *et al.* (19) found that both GH receptor-inactivated and IGF-I-inactivated mice had affected chondrocyte proliferation and that both IGF-I and the GH receptors are expressed in the proliferative layer of the growth plate, suggesting that proliferative chondrocytes are primary target cells for both GH and IGF-I. These conflicting *in vivo* results suggest that further studies are required to defi-

nitely identify the primary target cells for both GH and IGF-I in the growth plate region.

D. Human genetic disorders and skeletal growth

Several recent clinical case reports describing the growth phenotype of patients with genetic disorders affecting different GH/IGF-I related components have given important information about the effect of GH and IGF-I on skeletal growth (28). The growth phenotype of patients with genetic disorders, resulting in GH deficiency, GH insensitivity, IGF-I deficiency, IGF-I insensitivity, and ALS deficiency, are discussed below. In contrast, human mutations in any of the IGFBPs remain undescribed.

1. GH deficiency and GH insensitivity

Patients with genetic disorders resulting in GH deficiency (mutations in GH, GHRH receptor, Pit-1, or Prop-1) or GH insensitivity [mutations in GH receptor or signal transducers and activators of transcription protein 5b (STAT5b)] have normal birth size but a pronounced reduction of final height (18, 78–87). Patients with GH insensitivity have low IGF-I levels and, due to a loss of negative feedback, increased GH secretion (see *Section IV*). Because GH signaling induces IGFBP-3, these patients also have reduced IGFBP-3 levels (84–86). Treatment of GH-deficient patients with GH results in a more pronounced postnatal growth response than treatment of GH-insensitive patients with IGF-I, supporting the notion that GH exerts IGF-I independent effects on body growth (48, 49).

2. IGF deficiency as a result of IGF-I gene deletion/inactivating mutation

Woods *et al.* (25) first described a patient with a homozygous IGF-I gene deletion in 1996. Subsequently, a patient with inactivating mutation of IGF-I was presented by Walenkamp *et al.* (28, 88), and a patient with a mutation resulting in altered amino acid sequence for the E domain of the IGF-I precursor associated with very low IGF-I levels was presented by Bonapace *et al.* (89). In these three patients, IGF-I deficiency resulted in severe intrauterine and postnatal growth retardation as well as sensorineural deafness, microcephaly, and mental retardation. Interestingly, IGF-I haploinsufficiency resulted in subtle inhibition of intrauterine and postnatal growth (88). IGF-I treatment resulted in increased linear bone growth in the first patient with a homozygous IGF-I gene deletion (90).

3. IGF-I insensitivity

Abuzzahab *et al.* (24) first presented a patient with heterozygous mutation of the IGF-I receptor, resulting in intrauterine and postnatal growth retardation, in 2003. Subsequently, several patients with heterozygous IGF-I receptor mutations and growth deficiency have been presented, but the magnitude of growth deficiency has varied from modest to severe, probably because the different described mutations resulted in variable degrees of remaining IGF-I signaling (24, 91–94). Patients with IGF-I insensitivity are characterized by elevated IGF-I levels resulting from increased GH secretion due to a lack of IGF-I receptor-mediated negative GH feedback (28). In addition, they often have microcephaly. A gene dose effect of IGF-I receptor for human skeletal growth is supported by evidence that patients with IGF-I receptor haploinsufficiency resulting from terminal 15q deletion, including the IGF-I receptor, display intrauterine and postnatal growth failure (95, 96). In addition, trisomy of terminal 15q, resulting in duplication of the IGF-I receptor, associates with increased height (97). Thus far, no case has been found with a homozygous IGF-I receptor mutation, and observations in mice suggest that this defect is lethal (22, 94). In a patient with persistent postnatal growth retardation associated with haploinsufficiency of the IGF-I receptor due to a deletion of one copy of the gene, GH treatment yielded growth acceleration and resulted in normal adult height (96).

4. ALS deficiency

In 2004, Domené *et al.* (98–100) reported the first patient with a homozygous inactivating mutation of the ALS gene, followed by descriptions of several other cases of ALS deficiency (101–103). The first case had a minimal growth phenotype, but some later cases displayed a slightly more advanced growth phenotype (98–103). Importantly, follow-up of the first reported patient with ALS deficiency demonstrated normal growth spurt and final height (99). Because patients with ALS deficiency cannot form ternary complexes, they have very low IGF-I and IGFBP-3 levels, similar to those seen in ALS KO mice (60, 98–103). The extremely low circulating IGF-I levels associated with only a modest reduction of growth rates and final height in patients with ALS deficiency support the hypothesis of peripheral IGF-I and direct GH action (but not endocrine IGF-I) as the main promoters of longitudinal bone growth (103). Actually, the circulating IGF-I levels in patients with total ALS deficiency are comparable to the levels found in classical GH deficiency and GH insensitivity, conditions that cause much more severe growth deficiency than ALS deficiency (99).

In conclusion, human genetic disorders causing IGF-I insensitivity and total IGF-I deficiency associate with reduced intrauterine and postnatal growth, whereas GH deficiency and GH insensitivity specifically result in severe postnatal growth deficiency. The growth deficiency in patients with total IGF-I deficiency is severe, whereas pa-

tients with partial IGF-I resistance due to heterozygous mutations in the IGF-I receptor gene have a variable but often moderate growth deficiency (102). Patients with total ALS deficiency show modest growth deficiency that is clearly less pronounced compared with patients having total IGF-I deficiency and generally is less severe compared with patients having IGF-I receptor mutations. Compared with patients with total IGF-I deficiency, the much less pronounced growth deficiency in patients with total ALS deficiency, associated with extremely low circulating IGF-I levels, supports the hypothesis that bone-derived IGF-I can maintain an essentially normal postnatal longitudinal bone growth in situations with severe deficiency of endocrine IGF-I.

E. Mechanism of action for GH and IGF-I in the regulation of bone growth—an update

Figure 1C summarizes our current proposed mechanism of action for GH- and IGF-I-mediated regulation of longitudinal bone growth. Compared with previous hypotheses (Fig. 1, A and B), the proposed modifications/similarities are based mainly on new data presented after 2001 including: 1) the finding that double GH receptor/IGF-I KO mice have a more severe reduction in bone length than IGF-I KO mice clearly establishes a direct effect of GH not dependent on IGF-I (Fig. 1Ci) (19); 2) a key role of local bone-derived IGF-I for bone growth is established by the finding that mouse models with IGF-I inactivation in bone (52, 53), but with normal circulating IGF-I levels, display reduced longitudinal bone growth (Fig. 1Cii); and 3) a role of circulating endocrine IGF-I was demonstrated by the result that triple KO mice, devoid of the major part of the circulating endocrine IGF-I, displayed reduced bone length and by the result that reexpression of IGF-I in the liver in total IGF-I-deficient mice resulted in growth stimulation (Fig. 1Ciii) (54, 61).

Some previously proposed models for the mode of action of GH and IGF-I on longitudinal bone growth (Fig. 1, A and B) have considered modulation of liver IGF-I synthesis as identical to circulating endocrine IGF-I. Recent advances in the field have clearly shown that besides liver IGF-I secretion, the serum levels of ALS and IGFBP-3 have to be considered because these are of importance for the stability of IGF-I (Fig. 1C). Two independent mouse models with no remaining liver IGF-I expression showed that 75% of serum IGF-I is liver-derived, suggesting that 25% of circulating IGF-I derives from nonhepatic tissues; therefore, this nonhepatic contribution to circulating IGF-I levels should be considered as well. However, further studies are required to determine the exact origin and contribution of non-liver-derived IGF-I to serum IGF-I levels. Thus, the GH effect mediated via the circulating endocrine pool of IGF-I (Figs. 1C and 3C) is complex and might include modulation of: 1) liver IGF-I production; 2) IGF-I secretion from nonhepatic tissues to the circulation; and 3) the amount of ALS/IGFBP-3 in the circulation. Loss of one of these components (*e.g.*, in single liver-specific IGF-I KO or total ALS KO mice) does not essentially affect body length (11, 12, 60). However, when both liver-derived IGF-I and total ALS are inactivated, the remaining circulating IGF-I has a very short half-life, and the nonhepatic tissues consequently lack the capacity to maintain the total circulating IGF-I pool above a threshold level (around 10–25% of intact mice) required for normal longitudinal bone growth (61).

In conclusion, it is clear that GH stimulates longitudinal bone growth by both IGF-I-independent mechanisms (Fig. 1Ci) and IGF-I-dependent mechanisms (Fig. 1C, ii and iii). We propose that endocrine IGF-I (Fig. 1Ciii) and local bone-derived IGF-I (Fig. 1Cii) have some overlapping growth-promoting effects and can partly (= redundancy) but not completely replace each other in the maintenance of normal longitudinal bone growth.

III. Effects of Liver-Derived *vs.* Locally Produced IGF-I on Bone Mass

In addition to IGF-I from the circulation (see *Section III.D*), IGFs are available to skeletal tissues through *de novo* synthesis by osteoblasts and osteoclasts and also by release of stored IGFs from bone matrix during osteoclastic bone resorption (104–108). This section discusses the relative importance of bone-derived *vs.* liver-derived IGF-I for bone mass.

A. *In vivo* and *in vitro* studies on IGF-I expression and action in bone

We and others have shown that IGFs are the most abundant growth factors produced by bone cells and stored in bone matrix (104–109). The finding that 40–50% of basal osteoblast cell proliferation can be blocked by inhibiting the actions of endogenously produced IGFs in serum-free cultures of osteoblasts *in vitro* provides evidence that locally produced IGFs contribute importantly to basal bone cell proliferation (110, 111). In addition, osteoblast production of IGF-I is known to be regulated by agents that influence bone formation. For example, osteoblasts contain GH receptors, and GH treatment increases production of IGF-I in osteoblasts (3, 112, 113). The finding that the growth-promoting effects of GH on osteoblasts can be abolished by coincubation with IGF-I-neutralizing antibodies attests to the importance of IGF-I in mediating GH effects in osteoblasts (114). Thus, the effects of GH not only on growth plate cartilage but also on bone may involve local production and/or actions of IGF-I.

In vitro studies demonstrate that the regulatory effects of many systemic hormones (*e.g.*, PTH, thyroid hormone, glucocorticoids) and local effectors (bone morphogenetic proteins, mechanical strain) may mediate their effects on osteoblasts in part by controlling local production of IGFs (105, 107, 115–119). For example, PTH treatment increases both proliferation and matrix production in serum-free cultures of bone cells (120–122). Findings that PTH treatment also increases IGF-I expression and that biological effects of PTH on osteoblasts *in vitro* can be blocked by the addition of IGF-I-neutralizing antibodies suggest that PTH effects on osteoblasts may involve increased production and/or actions of IGF-I (123, 124). Accordingly, two independent studies have provided direct evidence that the anabolic effects of PTH on bone formation *in vivo* require IGF-I action in growing mice (125–127). Another study demonstrated that PTH administration increased bone density in the femur and vertebra of wild-type mice without altering the serum IGF-I levels and that the skeletal anabolic effects were lost in mice lacking insulin receptor substrate-1, a signal downstream of the IGF-I receptor (128). Thus, these data provide convincing evidence for a role of locally produced IGF-I in mediating anabolic effects of PTH on bone. However, using several different mouse strains with liver-specific IGF-I inactivation, global deletion of ALS, and both liver-specific IGF-I and total ALS inactivated genes, it was found that the PTH response on trabecular bone was genotype dependent (129).

Another important mediator of skeletal growth is thyroid hormone (130–132). Huang *et al.* (133) demonstrated that exogenous addition of T_3 increased indices of osteoblast cell activity in serum-free cultures and that neutralization of IGF-I action blocked the biological effects of T_3 on osteoblasts, thus suggesting that T_3 effects on bone may in part depend on local production of IGF-I in bone. In contrast to T_3, glucocorticoids exert negative effects on the skeleton by inhibiting bone formation and stimulating bone resorption (107, 134–141). Studies in several laboratories have shown that glucocorticoid actions on bone formation parameters involve regulation of production of IGF-I and/or their binding proteins both *in vitro* and *in vivo* (107, 134–141).

Mechanical strain is the only true negative feedback system regulating bone formation, and evidence suggests that mechanical strain's ability to increase bone formation is mediated, at least in part, by locally produced IGFs (107, 125). Compared with contralateral unloaded bone, mechanical loading resulted in rapid induction of IGF-I mRNA levels in the loaded limb within 4 h (142). Consistent with these data, a single 10-min episode of mechanical stimulation increased IGF-I expression in osteocytes of loaded bone within 6 h (143). In contrast to loading that increases IGF-I expression and bone formation, skeletal unloading yielded decreased proliferation of osteoblasts and their progenitors resulting from skeletal unloading-induced inhibition of the IGF-I signaling pathway. In terms of the mechanism by which mechanical strain and IGF-I interact to regulate osteoblast proliferation, studies have shown that IGF-I and mechanical strain interact synergistically to increase IGF-I receptor phosphorylation in an integrin-dependent manner involving recruitment of SH domain containing protein tyrosine phosphatase (SHP1) and/or SHP2 to IGF-I receptors and inhibition of SHP-mediated IGF-I receptor phosphorylation (144–146).

In conclusion, there is considerable experimental evidence to support a role for locally produced IGF-I in mediating the effects of systemic regulators on the bone formation process, and mechanical strain and IGF-I interact to regulate osteoblast proliferation.

B. Transgenic overexpression in bone

If locally produced IGFs are important regulators of bone formation, modulation of local IGF expression in bone should lead to corresponding changes in the bone formation process. Indeed, the following transgenic studies support a role for locally produced IGF-I in mediating bone formation changes (Table 2). To examine the influence of GH on bone deposition, Saban *et al.* (147) generated lines of transgenic mice expressing the GH gene driven by β-globin regulatory elements. GH synthesis in the transgenic lines has been shown to be erythroid-specific by RNAse protection experiments and by *in situ* hybridization of proximal tibia. Findings that serum levels of GH did not differ significantly in transgenic lines compared with control mice suggested that local but not systemic levels of GH increased in transgenic mice. Bone mineral density (BMD) was 30–40% higher in transgenic mice compared with control mice. Accordingly, histological cross-sections of tibia showed that adult transgenic mice had 20–45% increased cortical thickness compared with their controls. These findings suggest that GH released from erythroid cells increases bone deposition in part by stimulating local production of IGF-I in the bone marrow. In another study, Zhao *et al.* (148) created transgenic mice that overexpressed IGF-I specifically in mature osteoblasts by driving transgene expression using osteocalcin-specific promoter. In these studies, overexpression of IGF-I in its normal paracrine environment increased cancellous bone volume as determined by histomorphometric analyses. Consistent with these data, Jiang *et al.* (149) demonstrated that transgenic overexpression of IGF-I using 3.6 kb of 5′ upstream regulatory sequence and most of the first intron of the rat Col1a1 gene resulted in

TABLE 2. Genetically altered mouse models to evaluate local IGF-I actions in bone

Model	Promoter used	IGF-I alteration	Skeletal phenotype	Ref.
hGH transgenic	β-globin	Overexpression in erythroid tissue	Increased bone density and cortical thickness	147
IGF-I transgenic	Human osteocalcin	Overexpression in mature osteoblasts	Increased bone formation rate, trabecular and cortical bone volume	148
IGF-I transgenic	3.6-kb rat collagen 1α1	Overexpression in immature and mature osteoblasts	Increased femur length, cortical width and cross-sectional area; increased calvarial thickness	149
PAPP-A transgenic	2.3-kb rat collagen 1α1	Increased free IGF in bone microenvironment	Increased calvarial thickness and BMD; increased size of long bones	151
Chondrocyte IGF-I KO	3.0-kb mouse collagen 2α1	IGF-I disruption in chondrocytes	Decreased length, bone size, and BMD	52
Osteoblast IGF-I KO	Entire mouse collagen 1α2 gene	IGF-I disruption in cells of osteoblast lineage	Decreased length, bone size, and BMD	53

PAPP-A, An IGFBP-specific protease; hGH, human GH.

transgenic calvaria that were wider and had greater marrow and bone areas. These transgenic overexpression studies indicated that increased expression of IGF-I in cells of osteoblastic lineage or in bone microenvironment increased indices of bone formation presumably via increased autocrine/paracrine actions of locally produced IGF-I.

If locally produced IGFs in bone exert significant biological effects on cells of osteoblastic lineage, then inhibition of IGF action in the bone microenvironment should lead to inhibition of bone formation. To evaluate this prediction, Zhang *et al.* (150) produced transgenic mice with targeted expression of inhibitory IGFBP-4 using a human osteocalcin promoter to direct transgene expression specifically in osteoblasts. Their results showed that several indices related to bone formation decreased in the transgenic mice. Although the transgenic mice were of normal size and weight at birth, they exhibited striking growth retardation postnatally. In addition to the anticipated reduction in bone weights, there was also a modest reduction in the weight of other organs, raising the possibility that osteoblast-produced IGFBP-4 could have inhibited IGF actions in other tissues via paracrine and/or endocrine manner. In a recent study, Qin *et al.* (151) evaluated the consequence of transgenic overexpression of PAPP-A, an IGFBP-specific protease, on bone metabolism in mice and determined that overexpression of PAPP-A in osteoblasts using 3.6 kb of 5′ upstream regulatory sequence and most of the first intron of the rat type I collagen α1 gene produced anabolic effects on bone in mice. PAPP-A overexpression was found to increase IGF bioavailability by inducing proteolysis of inhibitory IGFBPs in bone microenvironment. Thus, the transgenic studies involving modulation of IGF action via increasing expression of IGFBP-4 or its protease, PAPP-A, exert opposite effects on bone formation, suggesting that locally produced IGF-I participates in bone formation.

In conclusion, several different transgenic overexpression studies affecting local IGF-I levels or bioavailability support a role of bone-derived IGF-I for bone formation.

C. Conditional knockout in bone

Although the transgenic studies provide evidence for the participation of locally produced IGF-I in regulating bone formation, one caveat with transgenic approaches is that the levels expressed by transgenic overexpression often are too high to represent normal physiological conditions. To overcome this drawback, the Cre/loxP approach has been used to specifically disrupt IGF-I or its receptor in bone cell types. Zhang *et al.* (152) generated osteoblast-specific KO of the IGF-I receptor gene by crossing IGF-I receptor loxP mice with transgenic mice in which Cre expression was driven by the human osteocalcin promoter. The mice carrying osteoblast-specific disruption of the IGF-I receptor were of normal size and weight but demonstrated a 24% decrease in cancellous bone volume at the distal femur caused by decreased trabecular number and increased trabecular separation as measured by microcomputed tomography. Histomorphometric analyses of bone formation indices at the epiphysis revealed significant decreases in bone formation rate, mineral apposition rate, and osteoblast number at 3 wk of age. The rate of osteoid mineralization decreased significantly in the mutant mice compared with control littermate mice. These findings suggest that IGF-I is essential for coupling matrix biosynthesis to sustained mineralization, particularly during pubertal growth spurt.

In contrast, neither cortical bone volume nor cortical thickness was significantly affected in the mutant mice. The lack of significant cortical bone phenotype in this

mouse model can be explained by the fact that the osteocalcin promoter, used to drive Cre expression in this study, disrupts IGF-I receptor expression in terminally differentiated mature osteoblasts only. Because IGF-I is expressed and acts on stromal cells as well as on immature and mature osteoblasts, it is not surprising that the observed skeletal changes in mice in which IGF-I receptor is disrupted only in mature osteoblasts is less pronounced compared with mice with conditional disruption of IGF-I in both immature and mature osteoblasts (see below, collagen type I α2 IGF-I KO mice) (53).

Because disruption of the IGF-I receptor in osteoblasts would eliminate not only the actions of locally produced IGF-I but also endocrine IGF-I, this model did not distinguish the role of endocrine *vs.* local IGF-I in regulating bone formation *in vivo*.

We recently found that conditional disruption of IGF-I in chondrocytes resulted not only in reduced bone length (see *Section II.B*) but also in reduced total body areal BMD (−5%) and bone width (−7%) in the femur and vertebrae (Table 1). Expression levels of PTHrP, Dlx-5, and Sox-9 decreased by 30–40% in the conditional mutants, suggesting that IGF-I produced by chondrocytes may regulate longitudinal growth and bone width, in part via regulating expression of one or more messenger molecules involved in chondrocyte proliferation and/or differentiation. Because disruption of IGF-I in type II collagen-producing cells did not influence circulating levels of IGF-I, these data establish a role for chondrocyte-produced IGF-I in regulating not only longitudinal bone growth but also bone width and bone mass accrual.

In addition, we investigated the relative contribution of IGF-I produced by cells of osteoblastic lineage in regulating bone mass accrual and bone metabolism (53). For these experiments, we used collagen type I α2 IGF-I KO mice (described in *Section II.B*), which have significantly reduced IGF-I expression in bone but normal levels of circulating IGF-I. Importantly, total body (−13%), femur (−41%), and vertebrae (−16%) areal BMD as measured by dual-energy x-ray absorptiometry decreased significantly in the mutant mice. Peripheral quantitative computer tomography analyses demonstrated a reduced cortical bone mass in both femur and vertebra. Histomorphometric studies revealed significant decreases in bone formation rate and mineral apposition rate in the conditional mutants, thus suggesting that loss of local IGF-I resulted in impaired differentiation and/or function of osteoblasts (Table 1). Three independent studies have now shown that disruption of the IGF-I receptor or IGF-I gene in osteoblasts resulted in a significant deficit in the mineralization rate (50, 152–154).

IGF-I effect on bone formation is dependent on the number and activity of osteoblasts. The finding that mineral apposition rate was significantly compromised in total IGF-I KO and osteoblast-specific IGF-I KO mice (Table 1) suggests that locally derived IGF-I contributes predominantly to osteoblast activity.

In conclusion, the studies using mice with conditional KO of IGF-I or the IGF-I receptor in osteoblasts, support the notion that locally produced IGF-I is critical for optimal bone development and subsequent mineralization.

D. Liver-derived IGF-I and bone mass

1. Cortical bone

To evaluate the relative contribution of endocrine IGF-I action in bone mass accrual, mouse models with disruption of liver-derived IGF-I (11, 12), total IGFBP-3 (61), and total ALS (60, 62) have been used in the past. As described in detail in *Section II*, the two mouse models with liver-specific IGF-I inactivation had largely unaffected bone length. In contrast, both mouse models displayed a significant reduction in cortical bone mass (58, 62), clearly establishing a role of liver-derived IGF-I for cortical bone mass (Fig. 3B and Table 1). However, the magnitude of the cortical bone deficit in these mice was less pronounced than that seen in mice with total inactivation of IGF-I (50). To rule out the possibility that the remaining 25% of circulating IGF-I in mice with liver-specific IGF-I KO is more readily bioavailable and thus sufficient for most of the bone mass accrual, Yakar *et al.* (61) generated triple KO mice lacking liver-specific expression of IGF-I, total ALS, and total IGFBP-3 (see *Section II*), resulting in a more than 97% reduction of total serum IGF-I levels (Table 1). Although the triple KO mice also exhibited a significant deficit in bone mass accrual, mainly resulting from reduced cortical bone mass, it was still less severe than that seen in mice with total IGF-I KO (Table 1). Thus, both liver-derived IGF-I and local IGF-I are required for normal accrual of cortical bone. Importantly, because both cortical bone mass and longitudinal bone growth were affected in the endocrine IGF-I KO mice (2.5% remaining serum IGF-I; Fig. 3C), whereas cortical bone mass but not longitudinal bone growth was affected in mice with liver-specific IGF-I inactivation (25% remaining serum IGF-I; Fig. 3B), we propose that the serum IGF-I threshold below which serum IGF-I influences cortical bone mass (>25% of normal IGF-I) is higher than the corresponding threshold for longitudinal bone growth (<25% of normal IGF-I).

2. Trabecular bone

Trabecular bone analysis by micro-computed tomography revealed a small but not statistically significant de-

crease in bone volume/total volume both in mice lacking endocrine IGF-I and in mice devoid of osteoblast-derived IGF-I. Similar analyses in mice with liver-specific IGF-I KO demonstrated a slight reduction of bone volume/total volume, which reached statistical significance in one study (155) but not in another study (61). In contrast, bone volume/total volume was 55% higher in mice with total disruption of IGF-I (Table 1). The reason for the differences in trabecular bone volume between total and osteoblast IGF-I KO remains undetermined. The complex role of IGF-I for trabecular bone is further illustrated by the results that mice with liver-specific IGF-I KO were protected from ovariectomy-induced trabecular bone loss (155).

In conclusion, comparisons of skeletal deficits of mouse models lacking total, liver-derived, endocrine, and bone IGF-I reveal the following:

a. Although disruption of endocrine or local IGF-I clearly resulted in reduced cortical bone mass, the magnitude of skeletal changes in mice lacking either of these IGF-I actions does not reach the severity seen in the total IGF-I KO mice. The mechanisms behind the more pronounced bone deficit in total IGF-I KO mice than in mice with endocrine or bone IGF-I KO might, in addition to lack of direct IGF-I effects on bone, include inadequate nutrition or altered serum levels of hormones such as vitamin D, PTH, and sex steroids. We have shown that serum levels of 1,25-dihydroxyvitamin-D were significantly reduced in the total IGF-I KO mice (154). Accordingly, serum calcium levels were slightly reduced in the total IGF-I KO mice, resulting in increased serum PTH levels. The elevated PTH levels in total IGF-I KO mice could be rescued by correcting IGF-I deficiency in these mice. Furthermore, a low calcium diet fed to these mice increased bone resorption and reduced bone formation to a greater extent than in wild-type mice. Because bone formation and bone resorption were uncoupled in total IGF-I KO mice, bone accretion took place at a reduced rate during the pubertal growth period. These data are consistent with the idea that lack of IGF-I could influence bone mass accrual in part via inducing changes in the PTH-vitamin D-calcium axis.

b. The serum IGF-I threshold (>25% of normal IGF-I) below which serum IGF-I influences cortical bone mass is higher than the corresponding threshold (<25% of normal IGF-I) for longitudinal bone growth. In general, it seems as if liver-derived/endocrine IGF-I affects cortical bone mass more than longitudinal bone growth (Table 1 and Fig. 3). In contrast, local IGF-I seems equally important for cortical bone and longitudinal bone growth (Fig. 3). Future studies to determine the mechanisms by which endocrine and local IGF-I regulate skeletal growth and potential interactions between these mechanisms will provide a better understanding of the mechanism of IGF-I action in bone.

IV. Liver-Derived IGF-I and GH secretion

Studies using mice with liver-specific IGF-I inactivation demonstrate that depletion of liver-derived IGF-I enhances GH secretion from the pituitary (57). This section reviews the importance of liver-derived IGF-I for the regulation of spontaneous pulsatile GH secretory pattern, especially in terms of the sexual dimorphism of the GH secretory pattern. Furthermore, the physiological importance of changes in GH secretory pattern induced by deficiency of liver-derived IGF-I will be discussed.

A. Pulsatile GH secretion

1. Biological effects of sexually dimorphic pulsatile GH secretion

GH secretion is pulsatile in rodents as well as humans. In rodents, there is also a clear sexual dimorphism (7, 156, 157). Male rats have episodic bursts of GH secretion and low GH levels between pulses, and female rats have higher basal interpulse GH levels and more frequent but lower amplitude pulses (157). The sexual dimorphism of GH secretion is best characterized in rats, from which repeated blood samples can be obtained to analyze the GH secretory pattern, but available results indicate similar gender differences in mice (7, 158). The GH secretion pattern in turn regulates several sexually dimorphic liver functions, including expression of P450 enzymes (159). Another liver function regulated by the GH secretory pattern relates to the number of TGFα/epidermal growth factor (EGF) receptors induced by a pulsatile plasma GH pattern in male rats (160–163). GH-regulated sexually dimorphic liver functions also include expression of major urinary protein (MUP) and prolactin receptors (7, 164).

The secretory pattern of GH is important for body growth in rats. A certain daily dose of GH induces larger body growth if it is given in a pulsatile manner than if it is given continuously (7, 165). A pulsatile GH secretory pattern induces IGF-I expression more effectively in rib growth plate and skeletal muscle, whereas there is no clear effect by GH pulsatility on liver IGF-I expression or serum IGF-I levels (36, 166).

2. Regulation of pulsatile GH secretion

GH secretion from the pituitary is regulated by two major hypothalamic peptides: GHRH, which induces GH secretion; and somatostatin, which inhibits GH secretion. Low basal GH levels between pulses in male rats probably

result from suppression of GH by surges of hypothalamic somatostatin, which may inhibit release of hypothalamic GHRH as well as its action on the pituitary (Fig. 4, *left panel*) (7, 167–169). Activation of the ghrelin receptor by treatment with analogs of the stomach-produced hormone ghrelin induces GH secretion (170, 171), involving direct stimulation at the pituitary level (172) as well as stimulation of GHRH release and possibly also inhibition of somatostatin release at the hypothalamic level (173–175). However, endogenous ghrelin may have little effect on GH secretion in mice, given that body growth and serum IGF-I levels are largely unaffected in ghrelin and ghrelin receptor KO mice (176, 177). This is in marked contrast to the profound effects of disruption of the GHRH system on GH secretion and body growth in mice (178, 179). On the other hand, selective lack of ghrelin receptor constitutive signaling in humans, due to ghrelin receptor mutations, may lead to a syndrome characterized by short stature (180). Furthermore, the ghrelin analog MK-677 has been shown to be effective in enhancing serum IGF-I levels in humans (181, 182). In conclusion, GHRH and somatostatin regulate GH secretion in humans and experimental animals, whereas endogenous ghrelin may be more important for growth promotion in humans than in rodents.

An additional level of regulation of GH secretion derives from the fact that GH can inhibit its own secretion via a short loop feedback effect at the hypothalamic level (183). The negative feedback effect of a spontaneous GH pulse in a male rat lasts about 3 h, causing the following GH trough (184, 185). Moreover, this effect seems to be dependent on the fact that each GH pulse with a certain time delay induces a somatostatin surge from the hypothalamus during the next GH trough (Fig. 5) (184, 185). Therefore, a dynamic interaction between the hypothalamus and the pituitary, and not only a biological clock function in the hypothalamus itself, could be of importance for the regular GH secretory pattern in male rats. This assumption also seems to be in line with mathematical predictions of how the GH secretory pattern is regulated (186).

Sexual dimorphism in rodents seems to be regulated by estrogen secretion in adult females and by androgen secretion neonatally and during adulthood in males. Essentially, estrogen increases and androgen decreases basal GH levels. These effects seem to be mediated by changes in hypothalamic release of somatostatin and GHRH (7, 187).

B. Negative feedback of liver-derived IGF-I on GH secretion

Specific depletion of liver-derived IGF-I causes a compensatory general increase in serum GH levels in mice (Fig. 4, *right panel*) (11, 12). This finding indicates that endogenous IGF-I secretion from the liver tonically suppresses GH secretion from the pituitary and concurs with earlier studies that pharmacological treatment with IGF preparations suppresses GH secretion (188, 189).

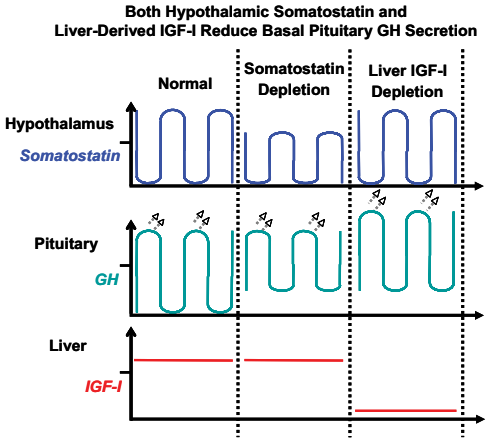


FIG. 5. Both hypothalamic somatostatin and liver-derived IGF-I reduce basal pituitary GH secretion. Proposed model is shown for the regulation of basal GH release from the pituitary by both intermittent somatostatin release from the hypothalamus and continuous IGF-I release from the liver in male rodents. The GH secretion in male rodents is intermittent, with low basal levels between pulses. In the normal situation (*left panels*), the low basal GH levels are due to suppression of GH release from the pituitary by pulses of hypothalamic somatostatin (*upper left*) that coincide with the low basal GH levels (*middle left*). In addition, basal GH levels are suppressed by continuous release of liver-derived IGF-I (*lower left*). Loss of either hypothalamic somatostatin (somatostatin depletion; *central panels*) or liver-derived IGF-I (liver IGF-I depletion; *right panels*) causes enhanced basal GH levels. Therefore, the effects of both somatostatin and IGF-I seem necessary to maintain low GH trough levels and thereby the masculinizing effect of pulsatile GH secretion in rodents. *Arrows* depict the effect of a GH pulse to initiate the somatostatin pulse during the next coming GH trough. Therefore, GH can inhibit its own secretion via a short loop feedback effect at the hypothalamic level, suggesting that the pulsatility of GH secretion is due to a reciprocal interplay between the hypothalamus and the pituitary and is not only due to an intrinsic rhythm of the hypothalamus itself.

Depletion of liver-derived IGF-I in male mice causes a feminization of some of the GH-regulated sexually dimorphic markers of liver functions (see *Section IV.A*), demonstrated by decreased levels of liver-derived MUP in urine and increased expression of prolactin receptors in the liver in male mice with liver-specific IGF-I KO (Fig. 4) (57). Due to the small blood volume in mice, it is difficult to measure the GH secretion pattern, but the indirect measurements of MUP and prolactin receptors indicate that the increased GH secretion in mice with liver-specific IGF-I KO results partly from more continuous GH secretion with increased baseline levels of GH. Thus, liver-derived IGF-I may tonically suppress basal GH secretion in male rodents and contribute to masculinization of various liver functions (Figs. 4 and 5). Both male and female mice with liver-specific IGF-I KO had increased relative liver

weights (Fig. 4) (57), probably due to increased GH levels (see *Section VI.D*).

To investigate the mechanism behind the increased GH levels in liver IGF-I-deficient mice, the mRNA levels of several regulatory factors that govern GH secretion were measured in the hypothalamus and the pituitary. Liver IGF-I-deficient mice showed increased expression of the receptors for GHRH and ghrelin/GH secretagogue (GHS) in the pituitary (Fig. 4). In accord with this finding, a GHRH antagonistic effect of IGF-I has earlier been demonstrated in rat pituitary cells *in vitro* (188). An inhibitory effect of IGF-I on GHRH receptor mRNA levels has also been reported *in vivo* after IGF-I replacement to the GH-deficient spontaneous dwarf rat (190). The increased pituitary GHRH and ghrelin/GHS mRNA levels in mice with liver-specific IGF-I depletion associated with increased responsiveness to systemically injected GHRH and ghrelin analog/GHS (57), suggesting an increase in the number of bioactive receptors in these animals (Fig. 4). As discussed above (see *Section IV.A*), endogenous GHRH likely is more important than endogenous ghrelin in stimulating GH secretion in mice (176–179), and the inhibition of GHRH effects by IGF-I may, therefore, be more important than that of ghrelin for GH secretion in rodents.

Depletion of liver-derived IGF-I or systemic IGF-I treatment to GH-deficient rats did not affect expression of hypothalamic GHRH, somatostatin, or neuropeptide Y, arguing against a negative feedback effect by endogenous liver-derived IGF-I at the hypothalamic level (57, 190). It has been reported that IGF-I can decrease hypothalamic GHRH expression and enhance somatostatin expression when given intracerebroventricularly, but not systemically (191). It was suggested that those results reflect a possible physiological effect by local IGF-I expression in the hypothalamus on GH secretion. Moreover, intracerebroventricularly administered IGF-I, at least when given in conjunction with IGF-II, seems to suppress GH secretion via effects on the central nervous system (192). IGF-I has been shown to have other effects in line with a regulation of GH secretion at the hypothalamic level, *e.g.*, enhanced somatostatin release *in vitro* (7, 169). However, these findings probably have implications for effects exerted by local hypothalamic IGF-I rather than by endogenous liver-derived IGF-I. In the liver-specific IGF-I KO mice, Wallenius *et al.* (57) observed a slight, probably GH-dependent, compensatory increase in hypothalamic IGF-I expression that partly might have reversed the effect of liver-derived IGF-I deficiency on GH secretion (Fig. 4).

In summary, loss of the feedback effect exerted by liver-derived IGF-I on the hypothalamic pituitary system results in increased GH secretion, including elevated baseline GH levels between pulses and increased expression and responsiveness of pituitary GHRH and ghrelin receptors. Therefore, the major site of action of endogenous liver-derived IGF-I in the regulation of GH secretion seems to be at the pituitary rather than at the hypothalamic level.

C. Liver-derived IGF-I and sexually dimorphic effects of GH

1. Liver

During the last decade, several studies have clarified the mechanisms mediating the effects of GH secretion on sexually dimorphic liver functions. The latent cytoplasmic transcription factor STAT5b is activated and translocated to the nucleus in rat liver in response to male pulsatile GH stimulation, but much less so after continuous GH treatment (193). Moreover, results from experiments with STAT5b KO mice indicate that STAT5b is responsible for the masculinization of the male liver (194, 195). The finding that liver-specific IGF-I KO feminizes GH-regulated liver functions (Fig. 4) (57) could be regarded as support for a mediator role of IGF-I in masculinization, as previously suggested (164). On the other hand, there are findings that do not support this hypothesis. For example, there was no difference in hepatic IGF-I mRNA expression after pulsatile and continuous GH treatment (36), and the levels of serum IGF-I were even somewhat higher after continuous GH treatment (166). This is in contrast to the observation that the established masculinizing factor STAT5b is activated more effectively by a pulsatile pattern (193). Moreover, there are few IGF-I receptors on the hepatocytes of the intact liver of male mice that could mediate a putative masculinizing effect (196–198). In conclusion, the most likely explanation for the finding that sexually dimorphic liver functions are feminized in male mice with liver-specific IGF-I KO (57) is that these mice exhibit enhanced basal GH levels. However, to finally elucidate the possible role of IGF-I as a hepatic mediator of masculinization, it is necessary to investigate whether GH-deficient liver-specific IGF-I KO mice are resistant to the masculinizing effect of pulsatile GH treatment in a way similar to that shown for STAT5b KO mice (199).

2. Body growth

There is emerging evidence that the GH-induced gender differences in body growth in rodents are mediated by nonhepatic, peripheral mechanisms involving STAT5b and local IGF-I. Global STAT5b KO markedly decreased body weight gain in male but not in female mice, whereas combined KO of STAT5b and the closely related STAT5a significantly reduced body weight gain in females and suppressed body growth more than KO of STAT5b alone in males (194, 200). Therefore, STAT5b may be important for male-specific body growth, whereas STAT5a partici-

pates importantly in body growth in both sexes. Muscle-specific STAT5a/STAT5b KO suppresses IGF-I mRNA levels, decreases longitudinal bone growth, and decreases body weight by 20% and 12% in male and female mice, respectively (201). Pulsatile GH causes a larger increase in muscle and rib cartilage IGF-I mRNA levels compared with continuous GH treatment (36), whereas liver IGF-I mRNA and serum IGF-I levels are stimulated at least as well by continuous as by pulsatile GH treatment (36, 166). Taken together, these data support the notion that larger body growth in male compared with female rodents could be due to effects exerted outside the liver, involving STAT5b activation and more effective stimulation of locally produced IGF-I, whereas liver-derived IGF-I is less important.

D. Human studies

GH secretion is pulsatile in all studied mammalian species, including humans (7, 169, 202). Additionally, sexual dimorphism of GH secretion occurs in humans, although it is less obvious than that in rodents (see *Section IV.A*). The results of several careful studies show that the GH secretory pattern is less regular in women than in men (202). Barkan and co-workers (203, 204) reported that trough GH levels are higher in young women than in young men, at least when the measurements are done during the follicular phase in the women, and the GH pulses are more frequent and the large nocturnal GH pulse is lower in women. Interestingly, the effects of IGF-I on GH secretion seem to be influenced by gender and sex steroids in humans. IGF-I suppresses both spontaneous and GHRH-stimulated GH secretion more effectively in men than in women, whereas men are less sensitive than women to the GH-suppressive effect of a GHRH antagonist (203, 204). Moreover, Veldhuis *et al.* (205, 206) observed recently that sex steroids influence the effects of IGF-I on GH secretion in older men and women. In addition, oral estrogens can suppress IGF-I production by the liver in humans (207). Therefore, there are indications that the regulation of GH secretion is sexually dimorphic and regulated by sex steroids in humans and that there are interactions between sex steroids and IGF-I in regulation of GH secretion in humans as well as in rodents.

In a clinical study, healthy adult subjects were treated with a selective GH receptor antagonist, which reduced plasma IGF-I levels by approximately 30%. Similar to liver IGF-I-deficient mice, the decrease in serum IGF-I was accompanied by an increase in mean GH levels in serum, due to augmented amplitude of GH secretory bursts as well as elevated basal GH release in both women and men (208). In that study, it was difficult to discern whether the enhanced endogenous GH secretion resulted from decreased liver IGF-I secretion and/or was caused by suppression of the short loop negative feedback at the hypothalamic level that has been described for GH (7, 184, 185).

A physiological function of IGF-I feedback on GH secretion was demonstrated by Hartman *et al.* (209), who showed that the enhanced GH secretion in humans during fasting is caused by a decrease in circulating IGF-I levels, even in the absence of decreased blood glucose, presumably due to decreased hepatic IGF-I production. Increases in GH production induced by low IGF-I production could be of importance for the adaptation to fasting with lipolysis and insulin antagonism. Interestingly, recent studies in rodents suggest that fibroblast growth factor 21, a hormone produced during fasting, may specifically induce GH resistance in the liver and decrease hepatic IGF-I production by specific inhibition of STAT5 (210).

In summary, IGF-I seems to interact with sex steroids in the regulation of GH secretion in humans, as also shown for experimental animals. Moreover, suppressed liver IGF-I production and enhanced GH levels seem to be of importance for the physiological response to fasting with lipolysis and insulin resistance in humans.

V. The Role of Liver-Derived IGF-I for Metabolism and Body Composition

A. Carbohydrate metabolism

Given the large homologies between the insulin and IGF-I systems, it is not surprising that IGF-I exerts profound effects on carbohydrate metabolism. IGF-I may induce insulin-like effects on glucose uptake directly in tissues with IGF-I receptors. Alternatively, IGF-I may enhance insulin sensitivity by suppressing GH release, via its well-known negative feedback (see *Section IV.B*).

Based on studies of liver-specific IGF-I KO mice, it has become clear that specific elimination of liver-derived IGF-I results in increased insulin levels and decreased insulin sensitivity (211, 212). Moreover, using the hyperinsulinemic-euglycemic clamp, insulin insensitivity was demonstrated in muscle, liver, and fat tissues (213, 214). The mice with liver-specific IGF-I KO also are more sensitive to streptozotocin-induced diabetes, due to decreased insulin sensitivity (215), or to loss of the antiapoptotic effect that IGF-I exerts on β-cells (216), as discussed below. Strong evidence suggests that decreased insulin sensitivity is secondary to decreased negative feedback by liver-derived IGF-I at the pituitary level, which in turn leads to enhanced secretion of GH, a hormone with well-known diabetogenic effects (Fig. 6) (214). However, it has been proposed that IGF-I has direct effects on glucose metabolism (217, 218), and therefore, it cannot be ruled out

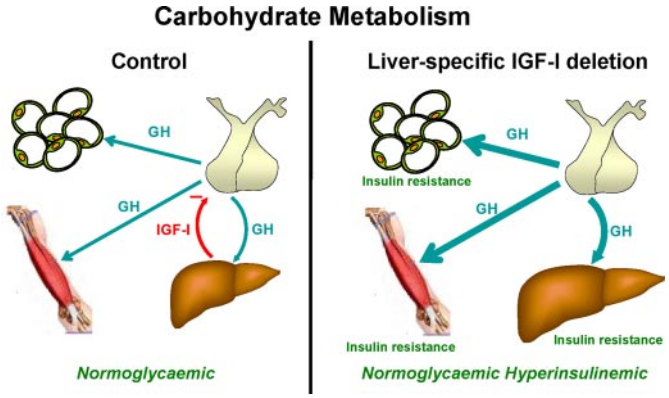


FIG. 6. Effects of depletion of liver IGF-I on carbohydrate metabolism. Lack of liver-derived IGF-I (*right panel*) causes enhanced GH secretion from the pituitary, which in turn results in insulin insensitivity in the liver, skeletal muscle, and adipose tissue. Therefore, more insulin is needed to prevent glucose secretion from the liver and to stimulate glucose uptake by skeletal muscle and adipose tissue. The mice with liver-specific IGF-I inactivation are normoglycemic but hyperinsulinemic.

fully that IGF-I also can have some effects of its own on glucose metabolism and insulin sensitivity (69).

The effects of ALS and IGF binding proteins on carbohydrate metabolism are complex. ALS KO does not seem to affect insulin sensitivity or GH secretion significantly (213). Surprisingly, combined KO of liver IGF-I and ALS resulted in improved insulin sensitivity compared with wild-type controls (213), despite a large increase in plasma GH levels in these double KO animals. One possible explanation is that these mice have increased levels of bioactive IGF-I in some tissues, high enough to affect insulin sensitivity directly but still not over the threshold level needed to have a substantial negative feedback effect on GH secretion. However, clarifying this issue will require further studies.

The KO of IGFBP-3, the third component of the ternary IGF-I:IGFBP-3:ALS complex that constitutes most of IGF-I in plasma, does not substantially affect insulin sensitivity or glucose metabolism (61, 219), and induction of supraphysiological levels of IGFBP-3 has variable effects on glucose metabolism (220). In a very recent thorough study, Yakar *et al.* (61) investigated the effect of triple KO of liver-derived IGF-I, total IGFBP-3, and total ALS, resulting in extremely low levels of circulating IGF-I. Serum insulin levels were higher in triple KO mice compared with controls, but if anything, the levels were lower than those in mice with liver-specific IGF-I KO only.

The above-mentioned data indicate that liver-derived IGF-I can modulate insulin resistance (probably largely mediated by increased GH secretion) and thereby cause compensatory changes in insulin secretion. However, other data suggest a direct effect of IGF-I on insulin release from pancreatic β-cells, although the importance of these effects for glucose metabolism in mice with liver-specific IGF-I KO is not fully clear. It has been demonstrated that β-cell-specific depletion of IGF-I receptors causes decreased glucose-stimulated insulin secretion but no changes in β-cell mass (221, 222). If IGF-I receptor deficiency in β-cells combines with insulin receptor depletion, the mice develop overt diabetes (223). Some evidence suggests that IGF-I, like insulin, can prevent apoptosis of β-cells via the insulin receptor substrate-2 pathway (216). In contrast, others have reported that pancreas-specific inactivation of the IGF-I gene leads to increased insulin secretion and islet cell mass, suggesting that pancreatic IGF-I may instead inhibit the islet cells (224).

In conclusion, liver-derived IGF-I seems to increase insulin sensitivity, largely by suppressing GH secretion from the pituitary. Consequently, lack of liver-derived IGF-I results in increased GH secretion and decreased insulin sensitivity in skeletal muscle, liver, and fat (Fig. 6). These effects of IGF-I can also be modulated by IGFBP-3 and ALS, opening the possibility that further research might identify methods to modulate the circulating levels of IGF-I system components for the treatment of diabetes (see *Section VII*).

B. Fat mass

According to several reports, liver-specific KO of IGF-I in mice enhances serum leptin levels at 2–4 months of age (61, 211, 214). High serum leptin levels could reflect increased fat mass after liver-specific IGF-I KO (61), but may also be secondary to the increased serum insulin levels discussed above (*Section V.A*) because insulin is known to stimulate leptin secretion (211, 225, 226). A third possible explanation is a direct effect by IGF-I on leptin production because it has been shown that injection of IGF-I acutely decreases leptin mRNA in fat as well as serum leptin levels in rodents (227, 228). Later in life, however, mice with liver-specific IGF-I KO are protected against the increase in body fat mass that occurs at an older age in wild-type mice (211, 229).

In accord with the finding that old mice with liver-specific IGF-I KO have reduced fat mass, it has been reported that mice overexpressing IGFBP-1, a well-known inhibitor of the metabolic effects of IGF-I, also become leaner (230). Although the reason for the fat-reducing effect of decreased levels of bioavailable IGF-I in different mouse models is unknown, these mice may acquire some protection from the age-related decrease in plasma GH levels, and GH in turn reduces fat mass. In support of this notion, it is well-known that liver-specific IGF-I KO increases serum GH levels (11, 12), and it has been proposed that the decreased GH secretion that occurs with age in both humans and rodents contributes to age-related obesity (231, 232). Alternatively, liver-derived IGF-I may be required for proliferation and differentiation of preadipo-

cytes, although the expression of IGF-I locally in fat seems unaffected in mice with liver-specific IGF-I KO (11). It has been shown that overexpression of both IGFBP-1 and IGFBP-2, the principal binding proteins of differentiating preadipocytes, decreases adipocyte differentiation in mice (230, 233). To our knowledge, no studies have examined the effect of liver-derived IGF-I on body fat mass in animals given a high-fat diet.

With respect to the elimination of endogenous binding proteins, body fat increases markedly in young ALS KO mice, but only slightly in young IGFBP-3 KO mice. As expected, young adult triple gene KO mice lacking liver-derived IGF-I, total ALS, and total IGFBP-3 also show increased adiposity (61). In contrast, triple gene KO of IGFBP-3, -4, and -5 resulted in decreased fat mass and markedly decreased adipocyte size (219).

In conclusion, lack of endogenous liver-derived IGF-I seemingly causes a transient increase in body fat and serum leptin levels in young mice, followed by decreased fat mass in older mice. It seems likely that the suppression of fat mass in older mice with liver-specific IGF-I KO results, at least partly, from enhanced GH secretion. Finally, as discussed in *Section IV.D*, decreased release of liver-derived IGF-I in response to absence of food intake may contribute to fasting-induced GH release in humans. This provides an important part of adaptation to fasting by inducing insulin resistance and lipolysis (209).

C. Lipid metabolism

There are few reports on the effects of liver-derived IGF-I on serum lipids. We observed enhanced levels of both total cholesterol and low-density lipoprotein cholesterol in male and female mice with liver-specific IGF-I KO (211). These findings concur with the observation that IGF-I treatment can decrease serum cholesterol in humans (234, 235). The role of GH regarding increased cholesterol levels in mice with liver-specific IGF-I KO remains unclear. GH deficiency associates with elevated serum cholesterol, and GH replacement therapy normalizes those levels (236–239). Moreover, GH treatment reverses the increase of serum cholesterol levels that occurs in old rodents (240). Therefore, although GH has been reported to increase cholesterol levels in young rodents (240), the increased cholesterol levels in mice with liver-specific IGF-I KO probably did not result from increased GH levels in these mice. Some, but not all, studies have reported that liver-specific IGF-I KO enhances serum triglyceride levels (211, 214). However, the accumulation of triglycerides in muscle and liver appears unchanged by lack of liver-derived IGF-I (214). Thus, further studies are needed to fully evaluate the role of circulating IGF-I on lipid metabolism.

VI. Liver-Derived IGF-I and Other Tissues

A. Brain

It is well established that IGF-I is of major importance for normal brain development, which will be briefly discussed. Importantly, during the last 5–10 yr, a series of studies have shown that liver-derived circulating IGF-I is an important regulator of adult brain function, which will be reviewed in this section.

1. Developmental effects of IGF-I

IGF-I receptors are widely distributed in the brain (241), and IGF-I is essential for the development of the brain (241–244). In contrast to GH overexpression, which results in a smaller increase in brain size than body size (245, 246), brain size is comparatively more increased than body size in transgenic mice with lifelong, global IGF-I overexpression (246). IGF-I overexpression increases myelination and the number of neurons and synapses (246–250), and overexpression of IGF-I protects granule neurons from apoptosis and improves ataxia in weaver mice (251). Inactivation of IGF-I or its receptor reduces brain size as well as the granule cell layer in the dentate gyrus and the numbers of oligodendrocytes and myelinated axons (252–254). Thus, IGF-I regulates the growth of new brain cells including neurons and glia, reduces developmental apoptosis, and increases synaptogenesis during early postnatal development.

2. Inactivation of the IGF-I receptor in the central nervous system

Using the cre-loxP recombinase system (nestin promoter), a mouse model with homozygous inactivation of the IGF-I receptor in neurons or glia was recently created (255). These mice were microcephalic and developed a complex phenotype involving severe growth retardation, infertility, and abnormal behavior (255). Mice with heterozygous inactivation of the IGF-I receptor in the central nervous system, including the hypothalamus, were also created (255). The phenotype in terms of central nervous function was not evaluated, but these mice were long-lived (255).

3. IGF-I affects behavior in adult mice

In adult life, IGF-I has been shown to be of importance for behavior. Intracerebroventricular administration of IGF ameliorated age-related behavioral deficits (256). In a later study in old mice with liver-specific IGF-I KO, both horizontal and rearing activity levels decreased when the mice were placed in a new environment (257). These results, combined with the compensatory high circulating GH in mice with liver-specific IGF-I KO (11, 57), indicate that liver-derived circulating IGF-I mediates at least some

of the effects of GH on exploratory activity previously observed in mice with overexpression of GH both globally (258) and in the central nervous system only (259).

4. Circulating IGF-I mediates exercise-induced effects on the adult brain

In older animals, it has been shown that an increase in circulating GH levels, by treatment with GH or GHRH, improves vascular microvasculature in the brain (260) and can prevent the age-related decrement in spatial learning and reference memory as assessed using the Morris water maze test (261). Studies have now investigated the extent to which these effects are mediated by the concomitant increase in circulating IGF-I. Circulating IGF-I mediates the exercise-induced increase in the number of new neurons in the adult hippocampus (262), and liver-derived IGF-I is a necessary factor in mediating the beneficial effects of exercise on brain vessel growth (263), anxiety, and spatial learning (264). The proangiogenic activity of liver-derived circulating IGF-I likely contributes to its neuroprotective actions by means of proper nutrient and oxygen supply to neurons (263).

5. Circulating IGF-I regulates brain amyloid-β (Aβ) levels in adult mice

A major finding is that liver-derived circulating IGF-I is one of the factors that regulates the clearance of brain Aβ (265). Systemic treatment with IGF-I can reduce the Aβ burden in Tg2576 mice overexpressing a mutant form of human amyloid precursor protein (APP695) (265). The mechanism underlying this increased clearance of brain Aβ by liver-derived circulating IGF-I, at the level of the blood-brain barrier, was speculated to involve enhanced transport of Aβ carrier proteins such as albumin and transthyretin into the brain (265). In a later study, the choroid plexus endocytic receptor megalin, a multicargo transporter known to participate in brain uptake of Aβ carriers, was identified as a potential mediator of IGF-I-induced clearance of Aβ and was also involved in IGF-I transport into the brain (266). Furthermore, treatment with IGF-I ameliorates cognitive dysfunction in double mutant amyloid precursor protein/presenilin 2 mice, counteracting vascular dysfunction by reducing vascular endothelial growth factor levels and endothelial cell proliferation and normalizing vascular density (267). IGF-I treatment also normalized abnormally elevated brain IGF-I receptor levels in amyloid precursor protein/presenilin 2 mice (267).

6. Circulating IGF-I regulates hippocampal function and spatial memory in adult mice

Using the Morris water maze test, mice with liver-specific IGF-I KO display reduced spatial learning and memory (268) (Fig. 7). Histochemical analyses revealed an increase in immunoreactivities but a decrease in mRNA levels of dynorphin and enkephalin in the dental gyrus of the hippocampus in old mice with liver-specific IGF-I KO (268). The intracellular accumulation of dynorphin and enkephalin might reflect decreased release of the peptide, probably related to reduced transcription of the two opioid peptides (268). Old mice with liver-specific IGF-I KO also displayed astrocytosis and increased metabotropic glutamate receptor 7a-immunoreactivity (268). These neurochemical disturbances suggest synaptic dysfunction and early neurodegeneration in old mice with liver-specific IGF-I KO (268). In addition to these characteristics, mice with liver-specific IGF-I KO exhibit disrupted long-term potentiation (a measure of synaptic plasticity) in the hippocampus, but not in cortex (269), and a reduction in the density of glutamatergic boutons leading to an imbalance in the glutamatergic/GABAergic synapse ratio in the hippocampus (269). The cognitive and synaptic deficits in mice with liver-specific IGF-I KO were ameliorated by prolonged systemic administration of IGF-I that normalized the density of glutamatergic boutons in the hippocampus (269), further demonstrating the importance of liver-derived circulating IGF-I for brain function.

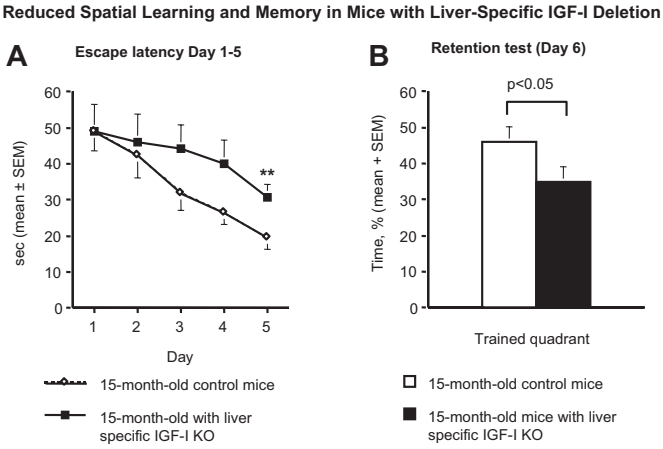


FIG. 7. Reduced spatial learning (A) and memory (B) in mice with liver-specific IGF-I inactivation. Reference memory was measured using the water maze test. During this test, the latency time for the mice to find a platform hidden in water is recorded. The release point, where the mice are let into the water, is randomly shifted. A, Latency time to find the escape platform in 15-month-old mice with liver-specific IGF-I KO (n = 9) and control mice (n = 9) during the first 5 d of the water maze. A two-way ANOVA for repeated measurements was performed, followed by Student Newman Keul's *post hoc* test. B, Results of the spatial reference memory (probe) test at day 6. The reference memory (probe) test was performed in the absence of the platform, and percentage of time spent in the trained quadrant was recorded. In B, the given *P* value is based on an unpaired *t* test. Values are given as means ± SEM. Adapted from Ref. 268.

7. Mechanisms showing how circulating IGF-I affects the function of the adult brain

As described above, liver-derived IGF-I exerts multiple effects on the brain, but it is yet not fully clear whether

these effects of IGF-I can be explained only by effects at the blood-brain barrier. IGF-I can pass the blood-brain barrier (270), and IGF-I has the direct capacity to modulate synaptic efficacy in several *in vitro* studies (271–273). Therefore, there are several possible explanations regarding how deficiency of liver-derived circulating IGF-I impairs spatial memory learning and synaptic function. At the current stage of knowledge, it is difficult to establish which mechanism is most important. Furthermore, deficiency of liver-derived circulating IGF-I results in unchanged circulating glucose but increased circulating insulin levels, indicating an adequately compensated insulin resistance (211, 214). Because systemic levels of insulin correlate with Alzheimer's disease (AD) neuropathology (274), the increased insulin levels observed in mice with liver-specific IGF-I KO might contribute to reduced spatial learning and memory in these mice.

8. Human studies

In humans, IGF-I gene mutations associate with sensorineural deafness, microcephaly, and mental retardation (25, 88, 89). In adult hypopituitary patients with severe GH deficiency and low circulating IGF-I levels, the impairment of cognitive function is less distinct, but a recent meta-analysis showed that in GH-deficient adult hypopituitary patients not receiving GH replacement, most cognitive domains display moderate to large impairments compared with matched controls (275). After GH replacement, cognitive performance improved moderately, particularly in terms of attention and memory (275). In older subjects (65 yr and over) lower circulating levels of IGF-I associated with lower Mini-Mental State Examination performance (276). Two studies showed that patients with AD or vascular dementia had lower circulating IGF-I concentrations than controls without dementia (277, 278). In contrast, total and free circulating IGF-I levels increased in patients with late-onset AD (279), consistent with the hypothesis that the early phase of AD may be a state of resistance to IGF-I signaling (279). There are few data regarding IGF-I levels in cerebrospinal fluid in subjects with AD. In one study, the cerebrospinal fluid level of immunoreactive IGF-I did not differ between subjects with dementia of the Alzheimer type and healthy subjects (280).

In conclusion, IGF-I is of major importance for brain development. In adult life, circulating IGF-I participates importantly in reactive vessel remodeling in the brain, the clearance of brain Aβ, cognitive function, and behavior. Reduced spatial learning and memory in mice with liver-specific IGF-I KO are not due to changes in GH secretion because these mice have increased circulating GH levels. The relative importance of liver-derived circulating IGF-I *vs.* brain IGF-I is presently unclear. Global deficiency of IGF-I produces a more severe central nervous system phenotype than deficiency of liver-derived IGF-I. However, the global IGF-I deficiency model includes developmental effects, whereas the liver-specific IGF-I KO model does not, rendering direct comparisons between the models difficult. In mice, systemic IGF-I treatment improves central nervous functions including spatial retention memory; it remains undetermined whether systemic IGF-I treatment will be a useful treatment option for diseases like AD in humans.

B. Cardiovascular system

Both the myocardium and blood vessels express IGF-I (35, 281), and IGF-I mRNA transcripts have been found in both aortic smooth muscle cells and endothelial cells (282). Moreover, functional receptors for GH (283–285) and IGF-I (286, 287) are present in the heart and vasculature. In addition, IGF-I production in the heart increases in response to GH (35, 281). Hence, there are possibilities of direct actions of GH as well as endocrine or autocrine/paracrine effects of IGF-I on the cardiovascular system.

Apart from stimulating cardiac growth and possibly also contractility, GH/IGF-I may also participate in the regulation of vascular tone and thereby peripheral resistance (288, 289). Studies indicate that IGF-I is a potent vasodilator and that this effect may be partly mediated by increased nitric oxide release from the endothelium (290–292). Accumulating evidence would also suggest that IGF-I plays a role in vascular diseases such as atherosclerosis and restenosis (293). IGF-I is a potent stimulator of vascular smooth muscle cell (VSMC) proliferation and migration, and this may play a role in the early development of atherosclerotic plaque formation (293). Providing evidence suggesting the involvement of locally produced IGF-I in this setting, Grant *et al.* (294) observed expression of IGF-I in VSMC derived from coronary atherectomy of both *de novo* and restenotic plaques, as opposed to VSMC from normal coronary arteries, where IGF-I expression could not be detected. In further support of a role of local IGF-I in the progression of atherosclerosis, genetic deletion of the IGFBP-specific protease PAPP-A, which results in reduced local IGF-I bioavailability, associates with resistance to atherosclerotic lesion development in apolipoprotein E-deficient mice challenged with a high-fat diet (295).

In addition, circulating IGF-I may participate importantly in the development of atherosclerosis because it has been reported that individuals with low serum IGF-I have significantly higher risk of developing atherosclerosis and coronary heart disease during a 15-yr follow-up period (296). Moreover, a polymorphism of the *IGF-I* gene linked to lower levels of serum IGF-I may associate with increased carotid intima-media thickness and aortic pulse wave velocity, which are considered to be early markers of atherosclerosis (297). In analogy, GH-deficient adult pa-

tients with low serum IGF-I values have been shown to have signs of early-onset atherosclerosis (298, 299), and this may be reversed by GH substitution (300, 301). Thus, it has been proposed that circulating IGF-I, largely regulated by GH, would have a protective role against atherosclerosis and cardiovascular disease, whereas IGF-I produced locally in blood vessels may actually promote atherogenesis by different mechanisms (302).

Several studies attempting to describe the consequences of cardiac overexpression of IGF-I have been published, although results are complex to interpret due to leakage of cardiac IGF-I to the circulation. Reiss *et al.* (303) reported a time-dependent increase in heart weight with a concomitant increase in cardiomyocyte cell number in mice with cardiac-specific IGF-I overexpression. However, it was also observed in this experimental model that body weight and levels of circulating IGF-I were clearly increased compared with wild-type mice, making additional endocrine effects of IGF-I probable. In more recent studies, cardiac overexpression of IGF-I was reported to protect against aging-related alterations in diastolic function (304), intracellular Ca^{2+} homeostasis, and apoptosis (305), although cardiac overexpression of IGF-I was again accompanied by a substantial increase in circulating IGF-I. Cardiac-specific overexpression of IGF-I also increased median survival (305). Somewhat conflicting results of cardiac IGF-I overexpression have also been reported, showing initially beneficial effects on systolic function followed by pathological cardiac hypertrophy and deterioration of systolic function in aging mice (306).

The KO models have also been used to clarify the importance of IGF-I for the regulation of cardiovascular function. Signaling through the IGF-I receptor in the heart has been believed to play a role during adaptive cardiac hypertrophy after hemodynamic load (307), and cardiomyocyte-specific KO of the IGF-I receptor with preserved serum IGF-I levels impairs physiological cardiac hypertrophy in response to exercise (308). Mice carrying a mutant IGF-I allele, with 30% of wild-type IGF-I levels present in all tissues and serum (309), showed a significant elevation of mean arterial pressure compared with wild-type controls. Somewhat surprisingly, these partially IGF-I-deficient mice had increased cardiac contractility, possibly due to a compensatory β-adrenergic coupling. When subjected to experimental myocardial infarction, the same mouse model of general partial IGF-I deficiency displayed altered cardiac remodeling, despite the preservation of cardiac function (310). However, due to the general lowering of IGF-I in both tissues and serum, as well as the fact that IGF-I deficiency was present since the beginning of lifespan in this experimental model, the role of endocrine *vs.* autocrine/paracrine IGF-I was not clarified.

A more recent study in mice with liver-specific IGF-I KO that resulted in an 80% decrease of circulating IGF-I showed an elevation of blood pressure, similar to that in the above described IGF-I mutant mice, as well as a comparable decrease in serum IGF-I, suggesting that decreased endocrine-acting IGF-I is responsible for this blood pressure-elevating effect (311). A decrease in circulating IGF-I also associated with endothelial dysfunction of mesenteric resistance vessels and increased expression of endothelin-1 in the aorta. In contrast to generally IGF-I-deficient mice, cardiac contractility remained unchanged in mice with liver-specific IGF-I KO compared with controls. Stroke volume and cardiac output decreased in mice with liver-specific IGF-I KO, although fractional shortening, velocity of circumferential shortening, and functional cardiac reserve, measured with stress echocardiography, remained unaffected, suggesting that reduced cardiac output was secondary to increased peripheral vascular resistance rather than decreased contractility *per se*. Although this experimental model associates with increased levels of circulating GH, it is less likely that increased GH activity is the explanation for increased vascular resistance because bovine GH transgenic mice have unchanged blood pressure compared with controls (312) and a number of both experimental studies and clinical studies show decreased peripheral resistance after GH administration (288, 289).

Mice with a specific KO of liver-produced IGF-I have been reported to have improved survival compared with wild-type controls when subjected to oxidative stress (313) and to have attenuated aging-related deterioration in contractile function and intracellular Ca^{2+} handling when isolated cardiomyocytes were studied *in vitro* (314).

In summary, different transgenic models with KO or overexpression of IGF-I, general or local, clearly demonstrate a wide range of cardiovascular effects that can be attributed to IGF-I signaling. However, the exact role of endocrine *vs.* locally acting IGF-I is difficult to elucidate in many models due to either spillover of IGF-I into the circulation from cardiac overexpression or IGF-I deficiency in both tissues and circulation due to a general KO. Nevertheless, results from mice with liver-specific IGF-I KO with decreased levels of circulating IGF-I and generally unchanged tissue IGF-I levels suggest a role of liver-derived IGF-I in the regulation of blood pressure and peripheral resistance.

C. Kidney

Both GH and IGF-I receptors are expressed in the kidney (315, 316). Rodent models illustrate the importance of the GH/IGF-I system for kidney size. Mice with global overexpression of GH and/or IGF-I have renal and glomerular hypertrophy (317–319). In IGF-I transgenic as well as IGF-I-treated rodents, IGF-I stimulates renal

growth to an approximately similar extent as it stimulates overall body growth (286, 317). In mice with global inactivation of the IGF-I gene that survive the postnatal period, the proportionally reduced kidney size associates with reduced glomerular size and decreased numbers of nephrons (320). GH receptor KO mice with very low IGF-I levels also display a proportional decrease in kidney weight (14, 15). The role of liver-derived circulating IGF-I for kidney size has been investigated in mice with liver-specific IGF-I KO (11, 321). Despite their secondary high circulating GH levels (57), mice with liver-specific IGF-I KO had an absolute and relative decrease in kidney weight, however, with no alterations in kidney morphology including the size, number, and distribution of renal structures. A microarray analysis of the kidneys of mice with liver-specific IGF-I KO revealed normal IGF-I mRNA levels, but a pronounced and tissue-specific decrease in renal IGF-II mRNA levels (321). Given the facts that mice overexpressing IGF-II have increased relative kidney weight (322–324), and that postnatally elevated IGF-II selectively increased the kidney weight of total IGF-I KO mice (324), it may be hypothesized that liver-derived circulating IGF-I increases renal IGF-II expression, resulting in symmetrical renal growth.

IGF-I has rapid effects on renal hemodynamics, including an increased renal blood flow and glomerular filtration rate (325–327). GH treatment has similar, but slower, effects on kidney function, and these are, therefore, considered to be mainly mediated by IGF-I (315). GH-deficient rats have a reduced glomerular filtration rate, which is restored by IGF-I treatment, supporting the notion that the low levels of local and/or circulating IGF-I in this model are responsible for this phenotype (328). However, the finding of unchanged creatinine clearance in mice with liver-specific IGF-I KO does not support a major role of liver-derived IGF-I for glomerular filtration (321). Thus, it may be hypothesized that local IGF-I, rather than liver-derived circulating IGF-I, participates in the maintenance of a normal glomerular filtration rate.

Administration of IGF-I causes dose-dependent retention of sodium and water (329), possibly through activation of distal tubular sodium channels (330). However, the role of IGF-I in the physiological regulation of water and sodium balance has been less clear (315). The role of liver-derived IGF-I for renal sodium handling was therefore investigated in mice with liver-specific IGF-I KO, which displayed increased urinary loss of sodium (321). In humans, GH treatment increases serum IGF-I levels, sodium reabsorption, and fluid retention. Therefore, it is likely that the increased urinary loss of sodium in mice with liver-specific IGF-I KO is caused by the deficiency of circulating IGF-I and that the sodium retention seen during GH treatment is mediated by the concomitant increase in circulating IGF-I.

Evidence suggests a role for the GH/IGF-I axis in the development of progressive glomerular sclerosis (315). Mice with overexpression of GH, but not mice with overexpression of IGF-I, develop progressive glomerulosclerosis (318, 319, 331), despite higher levels of circulating (and presumably renal) IGF-I in the IGF-I transgenic mice. Consequently, a role of GH, rather than IGF-I, has been emphasized for the development of glomerular sclerosis (315). This is in accord with results showing that mice with GH receptor gene disruption are protected against diabetes-induced renal damage (332). A direct effect of GH on the glomerular podocyte, with increased levels of reactive oxygen species and reorganization of the actin cytoskeleton in these cells in response to GH, has been proposed as one possible mechanism underlying the development of progressive glomerulosclerosis in transgenic mouse models with high GH levels (333). However, despite the fact that mice with liver-specific IGF-I KO are insulin resistant, although not diabetic (211), and have elevated levels of GH (57), there were no signs of glomerulopathy in older mice with liver-specific IGF-I KO (321). The absence of renal structural, vascular, and/or glomerular complications in the mice with liver-specific IGF-I KO (321) suggests that high circulating GH and low circulating IGF-I in itself does not cause glomerular sclerosis/renal failure. However, in diseases such as type 1 diabetes mellitus (202), where circulating GH is high and circulating IGF-I is low, the possibility cannot be fully excluded that high circulating GH levels combined with low circulating IGF-I levels can accelerate an already existing renal disease.

Taken together, available data suggest that liver-derived IGF-I is a regulator of kidney size and the renal excretion of sodium. In contrast, there is no support for a role of liver-derived circulating IGF-I in the regulation of glomerular filtration rate, where local renal IGF-I expression may be more important for the physiological effects. Although direct effects of GH may be less important in the regulation of kidney size and function, data suggest a role of GH, rather than IGF-I, in the development of progressive glomerular sclerosis in rodent models. Importantly, key pieces of knowledge are still lacking, and models with kidney-specific inactivation and overexpression of IGF-I would provide valuable new information.

D. Liver

1. IGF-I and the size of the intact liver

The liver is a major target organ for GH, and GH is an important regulator of hepatic IGF-I production. It is believed that IGF-I does not affect the function of hepatocytes directly because there are few IGF-I receptors on

these cells in the intact liver (196–198). This is in accord with results indicating that enhanced hepatic glucose production in mice with liver-specific IGF-I KO is not due to decreased direct effects of hepatic IGF-I on hepatocytes, but it might be secondary to enhanced GH secretion. This notion is supported by the results of crossbreeding liver-specific IGF-I KO mice with GH antagonist transgenic mice, showing that depletion of GH can reverse this effect of decreased liver IGF-I production (see *Section V.A*) (Fig. 6) (214). The lack of IGF-I receptors on hepatocytes would also mean that liver-derived IGF-I, as well as nonliver IGF-I, would be unable to stimulate liver growth during adulthood. Accordingly, mice with liver-specific IGF-I KO did not display decreased growth of the intact liver, but the liver instead became disproportionally large, likely due to direct stimulation by enhanced GH secretion (see *Section IV.A*) (Fig. 4) (11, 12). In line with this, GH receptor-deficient mice have reduced relative liver weight (14). Moreover, overexpression of GH in transgenic mice causes a disproportional growth of the liver, whereas this is less apparent in mice overexpressing IGF-I (245, 318).

2. IGF-I and liver regeneration

The comparatively slow liver growth in normal healthy mice differs in many ways from the very rapid liver growth occurring after hepatectomy. In rodents, this is an established model, where almost normal liver size is achieved again about 7–10 d after two thirds partial hepatectomy. The regulation of liver regeneration has been studied extensively, and it has been shown to be dependent on the cytokines IL-6 and TNF-α (334, 335), which also seem to stimulate the growth of the intact liver (336). In addition, growth factors, such as hepatocyte growth factor and TGF-α/EGF, have been implicated in liver growth (334). It has been shown in various models that GH is important for liver regeneration in both rats and mice (198, 337–339). IGF-I treatment, like GH treatment, to hypophysectomized rats accelerates DNA synthesis during liver regeneration. Later, it was reported that depletion of liver IGF-I and ALS, which reduces serum IGF-I levels to about 10% of normal intact mice, delays hepatocyte proliferation with about 12 h (198). Moreover, depletion of IGF-I receptors normally present in hepatocytes and epithelial cholangial cells of regenerating liver reduces hepatocyte proliferation in regenerating liver by about half in male but not female mice (340). It remains unknown why IGF-I stimulates liver regeneration more effectively than the growth of intact liver, but there are some possible mechanisms. First, IGF-I effects may be potentiated by EGF/TGF-α (341), and this growth factor is more active during liver regeneration (334). Second, IGF-I has been shown to increase the production of the potent liver growth factor hepatocyte growth factor from hepatic stellate cells, one of the cell types besides hepatocytes in the liver, providing a possible alternative route for how IGF-I indirectly stimulates hepatocyte proliferation and thereby liver growth (342). Third, Alternatively, the stimulatory effect by IGF-I on liver regeneration may be exerted directly on hepatocytes because the expression of IGF-I receptors as well as IGF-I binding increases in the remaining liver after partial hepatectomy (196, 197, 340), although this effect has not been seen in all studies (198). The levels of the intracellular signaling molecule insulin receptor substrate-1 also increase in regenerating liver, providing another possible mechanism for enhanced IGF-I sensitivity (340). And finally, IGFBPs may play a role for IGF-I effects on liver regeneration. IGFBP-1, one of the IGFBPs that also may exert IGF-I independent effects (343), increases rapidly after partial hepatectomy (344). Targeted disruption of the IGFBP-1 gene causes delayed and decreased DNA synthesis in hepatocytes of regenerating liver (345).

In conclusion, endogenous GH is needed for the growth of both intact and regenerating liver in rodents. Liver-derived IGF-I also stimulates liver regeneration, but it does not seem to stimulate growth of the intact liver directly. Liver-derived IGF-I may instead decrease relative liver size indirectly by suppressing GH secretion (Fig. 4).

E. Prostate

In this section, only the effect of IGF-I on prostate size will be reviewed. The role of IGF-I in relation to prostate cancer will not be described because this has recently been reviewed in *Endocrine Reviews* (5).

The prostate is composed of two compartments, glandular and fibromuscular. In primary culture, prostatic epithelial cells express the IGF-I receptor, and IGF-I stimulates them to proliferate and produce IGFBPs (346, 347). Prostatic stromal cells also express the IGF-I receptor (348–350). There is local production of IGF-I in the prostatic stromal cells (351, 352), and locally produced IGF-I can act in a paracrine fashion on prostatic epithelial cells (352). Thus, both endocrine and locally derived IGF-I may have the capacity to regulate the growth of prostate-derived cells.

Mice with overexpression of bovine GH display increased ventral prostate weight (353). GH treatment of hypophysectomized rats increased the expression of the androgen receptor (AR) as well as the expression of IGF-I and the IGF-I receptor in prostate (354).Transgenic mice with global inactivation of the IGF-I gene, as well as mice overexpressing a GH antagonist, have decreased prostate size (355). Administration of bovine GH had no effect on restoring prostate development in transgenic mice with global inactivation of the IGF-I gene (355), suggesting that reduced prostate size in these mice was due mainly to low IGF-I activity. Transgenic mice expressing human IGF-I in basal epithelial cells of prostate had leakage of human IGF-I into the circulation and

prostate hyperplasia in early life (and later neoplastic changes in prostate) (356). Another study, which focused mainly on prostate cancer development, examined transgenic (PB-Des) mice with specific overexpression in prostate epithelial cells of human IGF-Ides, which encodes a mature isoform of IGF-I with decreased affinity for IGFBPs due to a 3-amino acid deletion in the N terminus (357). IGF-Ides was not detected in the serum of PB-Des mice, confirming that the expression of the exogenous IGF-I was confined to the prostate, and no difference *vs.* controls was seen in terms of genitourinary tract weight normalized to body weight (357). Finally, conditional deletion of the IGF-I receptor in prostate epithelium did not affect total body or prostate weight (358). Therefore, taken together, overexpression or inactivation of IGF-I or its receptor specifically in prostate epithelium does not appear to result in any major change of total prostate weight.

Mice with adult liver-specific inactivation of IGF-I displayed clearly reduced prostate weight, with a more marked effect on the ventral prostate (VP) lobe than that on the dorsolateral prostate (DLP) and anterior prostate lobes (359). Reduced AR mRNA and AR protein levels were observed in the VP lobe of mice with liver-specific IGF-I KO, and immunohistochemistry revealed reduced intracellular AR immunoreactivity in the VP and DLP lobes (359). The nonaromatizable androgen dihydrotestosterone increased VP weight to a lesser extent in orchidectomized mice with liver-specific IGF-I KO compared with orchidectomized controls, indicating that the down-regulation of AR expression in the prostate of mice with liver-specific IGF-I KO was functionally important (Fig. 8).

Mice with global inactivation of the IGF-I gene displayed impaired development of the glandular prostate compartment (355). It was later shown that IGF-I can affect the development of both prostate compartments (360). In mice with liver-specific IGF-I KO, analysis of prostate morphology showed reductions of both the glandular and the fibromuscular compartments of the VP and DLP lobes that were proportional to the reductions of the weights of these lobes (359). It is unlikely that the reduction of the fibromuscular prostate compartment of the mice with liver-specific IGF-I KO were due to reduced AR expression in these mice because previous studies show that androgen inhibition or treatment primarily acts on the prostate glandular epithelium, whereas the effect on the fibromuscular tissue is relatively small (360–363). It could, therefore, be hypothesized that liver-derived IGF-I regulates the glandular prostate compartment by affecting AR mRNA and protein levels in the prostate, whereas the effects on the fibromuscular compartment are due to other androgen-independent mechanisms.

In humans, GH excess (acromegaly) results in high serum IGF-I values and increased prostate size (364). Prostate size decreases in GH-deficient adults with low IGF-I values (365), and GH replacement therapy increases both serum IGF-I levels and prostate size in such patients (365). Furthermore, combined GH and testosterone treatments have additive affects on prostate size in adults with GH deficiency and low serum IGF-I values (365). Thus, in accord with findings in liver-specific IGF-I KO mice, human data provide support for the notion that IGF-I and androgens interact in the regulation of prostate size (Fig. 8).

In conclusion, IGF-I regulates prostate size and probably mediates the effect of GH on prostate size. Although the relative importance of circulating IGF-I *vs.* that of locally produced IGF-I is not fully clear, the fact that mice with liver-specific IGF-I KO display clearly reduced prostate weight demonstrates that liver-derived circulating IGF-I is important for the regulation of prostate weight. We propose that liver-derived IGF-I increases prostate size, at least partly, by increasing the number of ARs and thereby androgen responsiveness (Fig. 8). This could, hence, be a mechanism for circulating IGF-I to converge with the androgenic pathway in the regulation of prostate size.

VII. Clinical Implications

A. Indications for the use of IGF-I and adverse effects

IGF-I treatment increases linear growth in patients with severe IGF-I deficiency resulting from IGF-I gene mutations or GH insensitivity (see *Section II.D*) (48, 49, 85, 90, 366). However, treatment of GH-deficient patients with GH results in a more pronounced postnatal growth response than treatment of GH-insensitive patients with IGF-I, supporting the notion that GH exerts IGF-I-independent effects on body growth (48, 49, 366). In 2005, the United States Food and Drug Administration approved the use of rhIGF-I for long-term treatment of children with short stature resulting from severe primary IGF-I deficiency, defined as height and IGF-I SD scores below/equal to −3 and normal or elevated GH levels. In 2007, similar approval was granted in Europe by the European Medicines Agencies. The cause of IGF-I deficiency was defined as various mutations in the GH receptor, defects in the post-GH receptor signaling pathway, and/or IGF-I gene defects. Treatment was also approved for children with GH deficiency who had developed neutralizing antibodies to GH.

Information about the long-term efficacy and dose effect of IGF-I treatment in children with various severities and forms of IGF-I deficiency is still lacking. However, results from the Increlex (Tercica) Growth Forum Database show that treatment with rhIGF-I for a period of 1 yr stimulates height velocity in prepubertal children with moderate IGF-I deficiency (height SD score, −2.5), in a dose-dependent manner (367). Chernausek *et al.* (366)

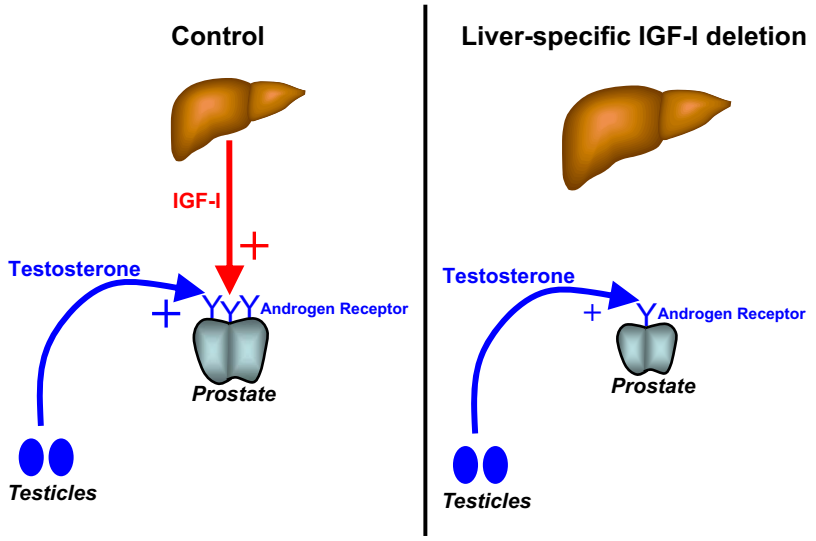


FIG. 8. Mice with liver-specific inactivation of IGF-I displayed reduced prostate weight. The clearly decreased prostate weight in mice with liver-specific IGF-I KO associated with decreased mRNA and protein levels of the AR in the ventral prostate. Thus, liver-specific IGF-I deletion reduces the number of ARs expressed in the prostate (*right panel*). Testosterone from the testicles is then less effective in increasing prostate size. This could, hence, be a mechanism for circulating IGF-I to converge with the androgenic pathway in the regulation of prostate size.

previously reported a similar dose-dependent effect in children with severe primary IGF-I deficiency. Chernausek *et al.* (366) reported the adverse events in 76 children with severe IGF-I deficiency due to GH insensitivity treated with rhIGF-I for up to 12 yr. The most common adverse events were hypoglycemia (49%), injection site lipohypertrophy (32%), and tonsillar/adenoidal hypertrophy (22%). Other adverse events included benign increase in intracranial pressure and coarsening of facial features (366). The authors concluded that adverse events are common but rarely of sufficient severity to interrupt or modify treatment. Other side effects, *e.g.*, myalgia and arthralgia, may be transient, and the frequency and severity of side effects of IGF-I treatment have been suggested to be less severe in patients with mild IGF-I deficiency compared with those with severe IGF-I deficiency (368).

B. Potential indications for combined GH and IGF-I treatment

It is quite clear that both GH and IGF-I are critical components for stimulation of statural growth and that GH stimulates the production of IGF-I in various organs including liver and bone (see *Section II*). As summarized in Fig. 1C, GH stimulates longitudinal bone growth by both IGF-I-dependent and IGF-I-independent mechanisms. It seems that endocrine IGF-I and local bone-derived IGF-I have to some extent overlapping growth-promoting effects and can partly (= redundancy) replace each other in the maintenance of normal longitudinal bone growth (see *Section II.B*). Therefore, it is logical that pharmacological administration of IGF-I can replace not only liver-derived IGF-I but also the effect of locally produced IGF-I on bone growth. A major finding supporting the hypothesis that both normal IGF-I and GH actions are required for normal bone growth is the finding by Lupu *et al.* (19) that GHR-IGF-I double KO mice had a more severe reduction in bone length than mice with IGF-I KO or GHR KO. These experimental findings suggest that some patients with both affected GH and IGF-I action might benefit from combined GH and IGF-I treatment. In the clinical setting, it seems quite logical that patients with growth disorders that have pronounced GH deficiency will benefit from GH replacement. Likewise, it appears clear that patients with pronounced primary IGF-I deficiency will benefit from IGF-I treatment/replacement therapy. In clinical practice, however, patients with growth disorders often do not exhibit complete deficiencies of GH and/or IGF-I, respectively, but rather partial deficiencies. The potential efficacy of IGF-I treatment in large groups of children with growth disorders that exhibit partial hormone deficiencies, currently labeled as GH deficiency, intrauterine growth retardation, idiopathic short stature, GH insensitivity, and IGF-I deficiency is presently unclear. Because the majority of the patients in these quite large and heterogeneous groups of patients do not have a defined geno- and phenotype, it appears that patient groups with these labels overlap to some extent and that the most obvious common denominator is a poor growth velocity. Therefore, clinical studies are needed in children with growth disorders exhibiting partial hormone deficiencies. Such studies should aim to explore whether the combined effect of GH and IGF-I stimulates statural growth more effectively than monotherapy of IGF-I and GH, respectively, and whether side effects during combined GH and IGF-I treatment will be acceptable compared with those seen during monotherapy with IGF-I or GH.

One further potential indication for combined GH and IGF-I treatment is growth retardation in chronic renal failure (CRF). CRF in children is associated with growth retardation despite normal or elevated GH levels, consistent with a state of GH resistance (369, 370). The IGF-binding capacity of CRF serum is increased by 7- to 10-fold due to alterations in IGFBP concentrations, resulting in de-

creased IGF bioactivity of CRF serum despite normal total IGF-I levels (370–372). Thus, CRF in children is associated with a functional IGF-I deficiency (370). GH treatment improves catch-up growth and allows most children with CRF to achieve normal adult height (373), but final adult height is still below the genetic target (373). In uremic rats, GH or IGF-I treatment alone induced an approximately similar increase in body growth, and an additive effect on longitudinal growth was demonstrated when both agents were administered together (374, 375). In addition, concomitant GH treatment prevented the hypoglycemia that was noted with IGF-I treatment alone (375). It remains, however, to be determined whether combined GH and IGF-I treatment will be more effective than GH alone in children with CRF and whether side effects will be acceptable for the combined treatment.

C. Diabetes mellitus

Available clinical data show elevated 24-h GH release in both untreated and treated type 1 diabetic patients; exaggerated GH release in response to GHRH occurs consistently (202). Main regulators of GH secretion in patients with type 1 diabetes are nutritional intake, metabolic factors, and serum IGF-I concentration (202, 217). Several lines of evidence suggest that insulin deficiency in the portal vein causes, at least partly, the GH hypersecretion observed in type 1 diabetic patients by regulating hepatic IGF-I regeneration (217). In short, in type 1 diabetic patients, the lack of insulin in the portal vein, combined with deteriorated nutritional status, results in reduced liver production of IGF-I, and as a consequence, low serum IGF-I levels (217). The low serum IGF-I levels, by lack of negative feedback action (see *Section IV.B*), result in compensatory high GH secretion. The GH hypersecretion then results in reduced insulin sensitivity due to the well-known diabetogenic effects of GH (see *Section V.A*). Furthermore, GH hypersecretion in type 1 diabetic patients may accelerate diabetic microvascular complications because there is an association between GH secretion and diabetic retinopathy (376–378). Treatment with sc injected insulin does not fully normalize the aberrations in the GH/IGF-I axis in type 1 diabetic patients, at least partly because sc injected insulin reaches the portal vein only to a small extent (217). It could, therefore, be hypothesized that addition of IGF-I treatment to insulin treatment in type 1 diabetic patients may reduce GH hypersecretion, thereby improving insulin sensitivity, retinopathy, and possibly also other diabetic microvascular complications in these patients (217, 379). Because KO of IGF-I receptors in vascular endothelial cells protects against retinal neovascularization, this hypothesis is not without controversy (380).

Treatment trials performed thus far indicate that administration of a single dose of IGF-I or IGF-I treatment can reduce GH hypersecretion, improve insulin sensitivity, and decrease the requirement of insulin in adolescents and adults with type 1 diabetes mellitus for up to 3 months (218, 381–385). Moreover, combined administration of IGF-I and IGFBP-3 for 2 wk induced similar types of improvements (235). However, beneficial effects did not last beyond 6 months of IGF-I treatment, likely due to poor compliance with the multi-injection regime required rather than a reduced effect of IGF-I (217, 382).

In patients with type 2 diabetes mellitus, GH secretion is dependent on age and body composition, but clearly elevated GH levels are usually not seen (202). This gives further support for the notion that deficiency of insulin in the portal vein, via a reduction of circulating IGF-I, at least partly causes GH hypersecretion in type 1 diabetic patients. However, also in type 2 diabetic patients, short-term IGF-I treatment (234, 386) or combined IGF-I and IGFBP-3 treatment (387, 388) can improve glycemic control and reduce the requirement of insulin.

In conclusion, GH hypersecretion in type 1 diabetes mellitus associates with insulin resistance and retinopathy progression. Treatment studies performed thus far indicate that IGF-I administration can reduce GH hypersecretion, improve insulin sensitivity, and decrease the dose of insulin required. However, additional long-term IGF-I treatment studies are needed to evaluate whether addition of IGF-I treatment to insulin treatment can affect diabetic microvascular complications. Furthermore, it needs to be thoroughly evaluated whether possible beneficial effects of IGF-I treatment to patients with diabetes mellitus type 1 or 2 will be meaningful when taking into account side effects of IGF-I.

D. Other potential future indications

Based on experimental animal studies, blockade of endocrine IGF-I might speculatively be beneficial for treatments of some types of tumors (see recent review in Ref. 5) and for the reduction of prostate size in patients with benign prostate hyperplasia, but might result in detrimental effects on brain function as well as insulin resistance, hypertension, and cortical bone osteoporosis. Although IGF-I treatment could be hypothesized to be beneficial for cortical bone mass and memory function, it might also result in benign prostate hyperplasia, increased risk of progression of some tumors, hypoglycemia, and other well-known side effects of IGF-I. Therefore, an overall evaluation of positive and negative effects of IGF-I on various organ systems must be performed in these patient groups before determining the usefulness of IGF-I treatment.

VIII. Summary: The Role of Liver-Derived IGF-I

Studies using mice with liver-specific IGF-I inactivation have clearly established that the major part of circulating IGF-I is liver-derived. This indicates that liver-derived IGF-I might exert important endocrine effects. The experiments using different mouse models with tissue-specific IGF-I inactivation indicate that endocrine IGF-I and local bone-derived IGF-I to some extent have overlapping growth-promoting effects and might partly but not completely have the capacity to replace each other (= redundancy) in the maintenance of normal longitudinal bone growth. In contrast to the moderate importance of liver-derived IGF-I for the maintenance of normal longitudinal bone growth, it is now well established that liver-derived IGF-I is a major regulator of several other phenotypes. Importantly, and in contrast to the regulation of bone growth, locally derived IGF-I cannot replace liver-derived IGF-I for the regulation of these phenotypes (= lack of redundancy).

Loss of liver-derived IGF-I feedback on the hypothalamic pituitary system increases GH levels, an effect that seems to be exerted at the pituitary rather than the hypothalamic level. It is clear that some of the phenotypes in the liver-specific IGF-I KO mice are indirect, mediated via the elevated GH levels (Fig. 9). The phenotypes mediated indirectly via elevated GH levels include insulin resistance, increased liver size, and affected sexually dimorphic liver functions (Fig. 9, *right column*), whereas others including reduced cortical bone mass, reduced kidney size, reduced prostate size, increased peripheral vascular resistance, reduced sodium retention, reduced tumor progression (of some but not all tumors evaluated; see recent review in Ref. 5), and several of the affected brain functions are probably direct IGF-I effects (Fig. 9, *left column*) that are not mediated via the elevated GH levels.

Some of the physiological effects of liver-derived IGF-I are summarized below. However, it should be emphasized that it is only possible to directly evaluate the relative importance of liver-derived IGF-I *vs.* local IGF-I for bone- and pancreas-related phenotypes because bone and pancreas are the only nonhepatic tissue in which IGF-I, thus far, has been successfully inactivated (52, 53, 224). Nevertheless, the role of liver-derived IGF-I can be determined for all evaluated phenotypes. Liver-derived IGF-I is a major stimulator of cortical bone size. Importantly, the serum IGF-I threshold below which IGF-I influences cortical bone mass is higher than that for body length. In contrast, local bone-derived IGF-I seems equally important for cortical bone and longitudinal bone growth. Specific elimination of liver-derived IGF-I results in increased insulin levels as well as decreased insulin sensitivity in muscle, liver, and fat tissues. It is now well established that liver-derived IGF-I regulates specific brain functions in adult mice. Circulating IGF-I is required to mediate the effect of exercise on anxiety and spatial learning, for reactive vessel modeling, and for clearance of brain amyloid β. Mice with liver-specific IGF-I KO demonstrate reduced exploratory activity as well as impaired spatial learning and memory.

Mice with liver-specific IGF-I inactivation display elevated blood pressure, endothelial dysfunction in resistance vessels, and increased peripheral vascular resistance. These data suggest that liver-derived IGF-I regulates blood pressure by modulating peripheral vascular resistance. Analyses of the kidneys in mice with liver-specific IGF-I inactivation demonstrated that liver-derived IGF-I is a specific stimulator of kidney size associated with major alterations in kidney IGF-II expression. In addition, liver-derived IGF-I reduces renal excretion of sodium.

Liver-specific IGF-I KO results in larger livers, probably due to decreased feedback inhibition by IGF-I on GH secretion and thereby resulting in GH-stimulated liver

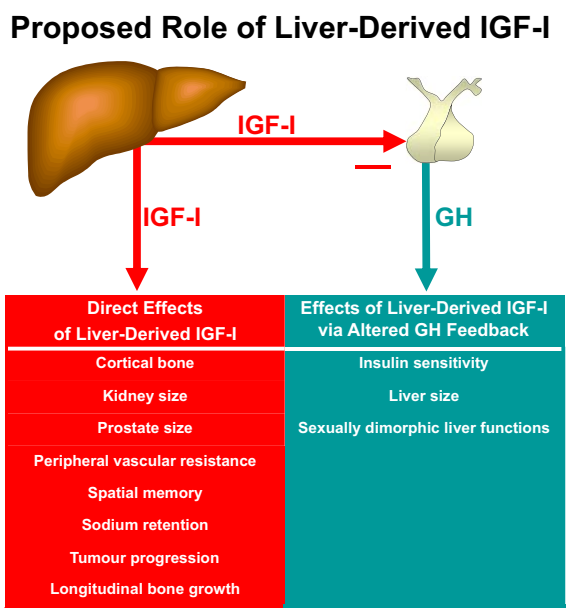


FIG. 9. Proposed role of liver-derived IGF-I. This figure shows the authors' proposed endocrine effects of liver-derived IGF-I discussed in this review. It is clear that a major role of liver-derived IGF-I is to regulate GH secretion and that some of the phenotypes in the liver-specific IGF-I KO mice are indirect via reduced GH feedback, resulting in elevated pituitary GH secretion. The phenotypes proposed to be mediated via the altered GH feedback (*right column*) include insulin resistance, increased liver size, and affected sexually dimorphic liver functions, whereas other effects such as reduced cortical bone mass, reduced kidney size, reduced prostate size, increased peripheral vascular resistance, reduced spatial memory, reduced sodium retention, and reduced tumor progression (of some but not all tumors evaluated, see Ref. 5 for recent review; not discussed in this review) are proposed to be direct IGF-I effects (*left column*). In addition, liver-derived IGF-I has the capacity to stimulate longitudinal bone growth directly (*left column*), but it is not required for essentially normal bone growth in the presence of normal IGF-I expression in bone (= redundancy). Importantly, and in contrast to the regulation of longitudinal bone growth, locally derived-IGF-I cannot replace liver-derived IGF-I for the regulation of the other described phenotypes in this figure (= lack of redundancy).

growth. Finally, adult mice with liver-specific inactivation of IGF-I display reduced prostate weight, prostate AR expression, and androgen-induced weight gain. We propose that liver-derived IGF-I, at least partly, increases prostate size by increasing the number of ARs and thereby androgen responsiveness. These experimental findings clearly establish liver-derived IGF-I as an endocrine factor with a variety of important physiological functions.

IX. General Conclusions

Studies comparing the impact of liver-derived IGF-I and local bone-derived IGF-I demonstrate that both sources of IGF-I have the capacity to stimulate longitudinal bone growth, although liver-derived IGF-I is not required for an essentially normal longitudinal bone growth. We propose that liver-derived circulating IGF-I and local bone-derived IGF-I have some overlapping growth-promoting effects and might have the capacity to replace each other (= redundancy) in the maintenance of normal longitudinal bone growth. Importantly, and in contrast to the regulation of longitudinal bone growth, locally derived IGF-I cannot replace (= lack of redundancy) liver-derived IGF-I for the regulation of a large number of other physiological functions including cortical bone mass, kidney size, prostate size, peripheral vascular resistance, spatial memory, sodium retention, insulin sensitivity, liver size, progression of some tumors, and sexually dimorphic liver functions. It is clear that a major role of liver-derived IGF-I is to regulate GH secretion and that some, but not all, of the phenotypes in liver-derived IGF-I KO mice are indirect, mediated via the elevated GH levels.

Future studies developing and evaluating several mouse models with local disruption of IGF-I in different IGF-I-dependent tissues are required for direct evaluation of the role of local IGF-I. However, these studies might be difficult to perform and/or evaluate because: 1) there are some limitations in the tissue specificity and efficiency for the different available tissue-specific promoters driving Cre expression in mice; 2) most tissues are heterogeneous, including several different cell types that might contribute to the local pool of IGF-I by paracrine mechanisms; and 3) liver-derived IGF and local IGF-I might exert redundant effects in some tissues.

We believe that all of the described multiple endocrine effects of liver-derived IGF-I should be considered in the development of possible novel treatment strategies aimed at increasing or reducing endocrine IGF-I activity. The substantially increased knowledge about the endocrine role of liver-derived IGF-I might result in improved/novel treatment strategies of IGF-I-dependent diseases in the future, but it is clear that further research in this area is required.

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