Regulation of Insulin-Like Growth Factor-I by Nutrition

Jean-Paul Thissen, Véronique Beauloye, Jean-Marie Ketelslegers, and Louis E. Underwood

KEY POINTS

• Nutrition is one of the principal regulators of circulating IGF-I. Many mechanisms are involved in the nutritional regulation of IGF-I.
• Both energy and protein are critical to the regulation of serum IGF-I concentrations. After fasting, adequate energy and protein are necessary for restoration of serum IGF-I, but energy may be somewhat more important than protein. While a low intake of protein is able to increase IGF-I in the presence of adequate energy, there is a threshold requirement of energy below which optimal protein intake fails to raise IGF-I after fasting.
• The decline of serum IGF-I during dietary restriction is independent of diet-induced alterations in pituitary GH secretion. In severe dietary restriction (fasting), a marked decrease in the number of liver somatogenic receptors suggests that a GH receptor defect is involved in the decline of serum IGF-I. In protein restriction, the decline of serum IGF-I results from a postreceptor defect in GH action at the hepatic level.
• Nutritional deprivation decreases hepatic IGF-I production by diminishing IGF-I gene expression. Decline in IGF-I gene expression results from both transcriptional and posttranscriptional mechanisms.
• Diet restriction also increases the clearance and degradation of serum IGF-I through changes in the levels of circulating IGFBPs.
• The molecular mechanisms leading to the decline of IGF-I in catabolic stress seem to be similar to those operational in food deprivation.
• Nutrients may also control the biological action of IGF-I, either directly or indirectly, through changes in IGFBPs.

1. INTRODUCTION

Starvation causes growth arrest and decreases body cell mass. Although secondary changes in the hormonal milieu play a major role, the mechanisms by which insufficient nutrition cause growth retardation are not well elucidated. Given its stimulatory...
effect on cell proliferation and differentiation and its anabolic effect on protein metabolism (1), the decline in insulin-like growth factor (IGF)-I in states of undernutrition likely contributes to the observed growth arrest and loss of cell mass observed. In this chapter, we will review the mechanisms responsible for the decrease of IGF-I in response to fasting and food deprivation and compare the responses to insufficient nutrition to those that occur during critical illness (2).

2. EVIDENCE FOR THE NUTRITIONAL REGULATION OF IGF-I IN HUMANS

2.1. Fasting and Malnutrition

Fasting among normal volunteers causes serum IGF-I to begin to decline within 24 h and to reach 20% of prefast values by 10 d (3). Changes in serum IGF-I concentrations parallel changes in nitrogen balance, suggesting that decreased IGF-I might mediate a decline in protein synthesis or an increase in protein breakdown. Decreased serum IGF-I is not restricted to fasting but also is observed in protein calorie malnutrition (marasmus, kwashiorkor, anorexia nervosa, celiac disease, AIDS, inflammatory bowel diseases) (4). In general, the magnitude of IGF-I reduction relates to the severity of the nutritional insult as reflected by serum albumin concentrations, weight deficit, or loss of body cell mass. The sensitivity of serum IGF-I to nutrient deprivation and repletion makes it a useful marker of nutritional status (reviewed in Chapter 4). Growth hormone (GH) secretion often is increased in malnourished patients, suggesting that they have resistance to the action of GH (5). The injection of pharmacological doses of GH, however, may still increase IGF-I and cause nitrogen retention in some malnourished patients (6).

Serious illness is also associated with low circulating concentrations of IGF-I despite augmented GH concentrations (7). This rise in GH secretion persists despite the calorie and protein supply afforded by artificial feeding, indicating that nutrient deprivation is not always responsible for the GH resistance observed in such patients.

2.2. Respective Roles of Energy and Protein Intake

Both energy and protein are important in regulating IGF-I because each is essential for restoration of serum IGF-I concentrations after fasting. Refeeding a diet sufficient in calories and protein raises IGF-I to nearly 70% of the basal prefast values within 5 d, whereas refeeding a protein-deficient isocaloric diet results in a 2-d delay in the upward inflection of IGF-I and increases IGF-I to only 50% of control prefast values. In contrast, refeeding a diet deficient in both protein and energy results in a further decrease of IGF-I in serum (8). The importance of energy intake in regulating IGF-I is supported by the observation that there is a threshold energy requirement (similar or equal to 11 kcal/kg/d) below which optimal protein intake fails to raise IGF-I during fasting (9). The source of energy also might be critical for the regulation of serum IGF-I because the carbohydrate content of the diet appears to be a major determinant of the response of IGF-I to GH when energy intake is severely restricted (10). The role of protein intake in the regulation of IGF-I is illustrated by the observation that the increase in IGF-I after fasting is proportional to the protein content of the refeeding diet. The quality of dietary protein is also important because IGF-I concentrations after fasting
are restored more readily by a protein-restricted diet rich in essential amino acids than by one that is rich in nonessential amino acids (11).

Energy and amino acid deprivation do not appear to play a major role in the decline of IGF-I observed in critically ill patients. Evidence obtained mainly from animal models suggests that factors, such as cytokines, glucocorticoids, and acidosis, are important in this regard.

3. MECHANISMS INVOLVED IN THE NUTRITIONAL REGULATION OF IGF-I PRODUCTION

3.1. Role of GH Secretion

Because GH is the principal hormonal stimulus of IGF-I production, impaired GH secretion may cause decreased IGF-I concentrations when food intake decreases (Fig. 1). In rats, the pulsatile secretion of GH is markedly attenuated by decreased availability of nutrients (12), and this could result in the decline of serum IGF-I. Unlike rats, however, humans (13) and other species have increased GH secretion when food intake is decreased. Fasting for 5 d increases the 24-h integrated GH concentration and the maximum amplitude and pulse frequency of GH (13). These observations suggest that impaired GH secretion is not responsible for decreased serum IGF-I concentrations in food-restricted humans.

Decreased GH secretion in starved rats could result from increased somatostatin, somatotropin-releasing inhibitory hormone (SRIH), tone or from reduced growth hormone-releasing hormone (GHRH) stimulation of somatotroph cells (14). The involvement of SRIH is suggested by in vivo immunoneutralization studies in which GH secretion is restored in fasted rats after intravenous injection of antiserum to SRIH (15). Increased SRIH observed in the peripheral circulation of fasted rats likely originates more probably from peripheral sources (gastrointestinal tract and pancreas) and not the hypothalamus (12,16,17). These observations suggest that a relative or absolute excess of SRIH is involved in fasting-induced inhibition of GH secretion. The decline in GH secretion during fasting could also result from diminished GHRH secretion because levels of GHRH mRNA in the hypothalamus are decreased markedly after 72 h of fasting (16). This decrease, however, is not associated with a reduction in hypothalamic GHRH peptide content, suggesting that release of peptide may be decreased in parallel with the reduction in mRNA. Given the evidence for an inhibitory effect of SRIH on GHRH release (18), the imbalance between GHRH mRNA and peptide in the hypothalamus might be secondary to high SRIH tone. Lack of dietary protein blunts spontaneous GH secretion (19). The protein content of the diet seems to be critical for the regulation of hypothalamic GHRH gene expression (20). Furthermore, dietary protein restriction attenuates GH responsiveness to GHRH and reduces pituitary size and GH content.

Several extrahypothalamic hormones are also implicated in the regulation of GH secretion and might be involved in nutrition-induced alterations in GH secretion. Leptin, a product of adipose tissue, is capable of entering the brain to inhibit food intake and increase energy expenditure. Because administration of leptin antiserum to rats causes a marked decrease in GH secretion (21) and leptin levels are reduced by fasting in rats as in humans (22–24), it has been suggested that leptin may be an integrative
signal for coordinating growth with nutritional status. This is supported by the observation that exogenous leptin prevents the fasting-induced fall of serum GH in rats (25–27). Despite its stimulatory effect on GH secretion, exogenous leptin does not restore serum IGF-I in fasted animals to normal. The effect of leptin on GH secretion in rats appears to be exerted at the hypothalamic level by regulating neurons that produce GHRH, SRIH, and neuropeptide Y (NPY). The rescue of GH secretion by leptin may be mediated by preventing decline of GHRH mRNA, and induction of NPY mRNA in fasted rats (26). Alternatively, the GH-releasing activity of leptin may be mediated in part by inhibition of SRIH (28), as suggested by the ability of leptin to decrease SRIH mRNA and secretion in vitro (29). Finally, leptin may stimulate GH secretion directly,
as illustrated by in vitro experiments (30). The effects of leptin on GH secretion in humans remain to be clarified.

Ghrelin, an important regulator of GH secretion and energy homeostasis (31) produced mainly by the stomach, is regulated by nutrient intake (32) and promotes GH release at both the hypothalamic and pituitary levels (33). Although both GH and ghrelin increase during fasting in humans, no correlations seem to exist between plasma ghrelin and GH or IGF-I concentrations. Further investigation is needed to delineate the role of ghrelin in enhancing GH secretion in fasting humans. Thyroid hormones stimulate the production of growth hormone in cultures of GH1 pituitary cells (34), and decreased plasma triiodothyronine (T3) in fasted rats has been implicated in the decrease of GH mRNA in the pituitary during fasting (35). Moreover, retinoic acid, acting in synergy with thyroid hormones, stimulates production of growth hormone in cultured pituitary cells (36,37). We speculate that the stunted growth accompanying vitamin A deficiency might be mediated by decreased GH production.

GH secretion of rats is impaired in several other models of dietary manipulation that are accompanied by growth retardation and low serum IGF-I concentrations (lysine deficiency, potassium deficiency, zinc deficiency and selenium excess) as well as in response to stressful stimuli such as sepsis (38).

3.2. Role of GH Receptor and Postreceptor Defects

It is unlikely that impaired GH secretion alone causes the decrease in IGF-I observed in nutritionally restricted rats because the administration of GH to fasted or protein-restricted rats does not produce a normal increase of IGF-I in blood (39–43). Also, protein energy malnutrition in humans is accompanied by high circulating concentrations of GH that fail to maintain IGF-I in the normal range (44,45). These observations suggest that GH resistance accompanies nutritional deprivation. Because the liver is a principal site for production of IGF-I (46,47), reduction of GH binding by the liver could impair production of IGF-I. Rats that are fasted exhibit parallel decline in serum IGF-I concentrations and in somatogenic (GH) binding capacity in liver (48).

Changes in hepatic GH receptors (GHR) caused by dietary restriction of rats are paralleled by changes in concentrations of GH binding protein (GHBP) in serum (49). In humans, GHBP decreases in parallel with serum IGF-I concentrations during severe dietary restriction (50). However, the consequences of changes in circulating GHBP in the control of GH action are disputed.

Regulation of the GHR by fasting occurs at the level of gene expression (51). GH receptor mRNA is reduced by fasting (52,53), and the magnitude and time-course of this decline is similar to the decline in liver IGF-I mRNA, suggesting that there may be a causal relationship between the two. This decline in GHR mRNA may be driven in part by nutritionally induced changes in hormones, such as glucocorticoids, thyroid hormones, and insulin, or in metabolites such as glucose. Glucocorticoids in excess (54,55), insufficient thyroid hormones (56–58) or insulin (59–61), and reduced supply of glucose (62,63) that occur in response to food deprivation are associated with low liver GHR binding and mRNA. Decreased liver GHR mRNA occurs in humans who have GH resistance caused by malnutrition (64) or postoperative catabolism (65). GH resistance associated with decreased liver GHR mRNA and binding is also present in
other dietary models of malnutrition, such as zinc deficiency (66) and in catabolic conditions, such as chronic renal failure (67) and sepsis (68).

In contrast to fasting, the role of the liver GH receptors in the decline of serum IGF-I in protein restriction is more questionable. Although protein restriction in young rats results in a dramatic decline of serum IGF-I, liver GH binding sites decrease only modestly. In older animals, protein restriction also causes a decline in serum IGF-I but does not reduce GH binding to liver membranes (69) or to freshly isolated hepatocytes (70). Also, no changes in serum GHBP are observed (71). Serum IGF-I responses to a single injection of GH in hypophysectomized protein-restricted animals are severely blunted (42). Despite the fact that continuous infusion of GH into protein-restricted rats increases liver GH binding to the level of control-fed rats, serum IGF-I is not increased (43). Taken together, these studies suggest that a postreceptor defect in GH action may participate in the GH resistance observed in protein restriction.

Intracellular defects in GH action could be a direct effect of limited nutrients or could result from secondary hormonal changes. Although it is not clear whether this postreceptor defect is specific to GH or is one that limits the synthesis of IGF-I regardless of the stimulus, the latter alternative seems the more likely. In hepatocyte primary cultures, the stimulation of IGF-I gene expression by insulin (72) as well as by GH (73) is blunted by a reduction in the concentration of amino acids in the medium.

### 3.3. Role of GH Transduction Pathways

Although the decrease in liver GH binding associated with fasting is well established, the consequences of this decline on the GH transduction pathway are less clear (Table 1). To investigate the effect of fasting on the liver GH transduction pathway, Beauloye et al. assessed the activation of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway in response to GH injected in the portal vein of fasted and fed rats (74). Although GH stimulated the phosphorylation of JAK-2, GHR, and STAT-5 in fed animals, the phosphorylation of these molecules was blunted markedly in the fasted animals. The inhibitory effect of fasting on these GH signaling molecules occurred without any changes in their protein content. The effect of fasting on GH-induced GHR and STAT-5 phosphorylation was detectable as early as 3 min after GH treatment and persisted at least until 30 min after GH injection. These findings suggest that the effects of fasting on the GHR signaling pathway are not caused by

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P, phosphorylated; JAK, Janus kinase; STAT, signal transducers and activators of transcription; SOCS, suppressors of cytokine signaling; GHR, growth hormone receptor; ND, not determined.
delayed GH activation. The observation that the phosphorylation of STAT-5 by JAK-2 is required for GH to stimulate IGF-I expression (75) supports the role of the alterations caused by fasting in the decrease of liver IGF-I production.

Although the mechanisms by which fasting alters the JAK-STAT pathway remains to be determined, it is likely that this defect results mainly from decreased liver GH binding sites. Another mechanism might be the superinduction of an intracellular negative feedback loop mediated by suppressors of cytokine signaling (SOCS) proteins. The ability of overexpressed SOCS-3 to blunt JAK-STAT activation in transfected cells (76,77) and the increased SOCS-3 expression by fasting (74) suggest a role for SOCS-3 in the fasting-induced JAK-STAT alterations. Impaired JAK-STAT signal transduction, possibly mediated through decreased GH receptors and SOCS proteins overexpression, may also contribute to the GH resistance observed in chronic renal failure (78) and in sepsis (79).

3.4. Role of Hormones in the Nutritional Regulation of IGF-I

3.4.1. INSULIN

Dietary restriction causes serum insulin concentrations to decline. Insulin-deficient diabetic rats have low serum IGF-I and decreased liver GH binding (60,80,81). Replacement of insulin restores both to normal. Insulin, therefore, might regulate serum IGF-I concentrations through changes in liver GH binding (82). The addition of insulin to hepatocytes in culture increases GHR mRNA and GH binding (61,83). Recent onset or mild streptozotocin-induced diabetes in rats, however, can be accompanied by low serum IGF-I without change in hepatic GH binding (84). In this situation, the decline of IGF-I is attributed to a postreceptor defect, likely related to the insulin deficiency directly, or less likely to metabolic abnormalities caused by insulin deficiency. In primary cultures of hepatocytes, insulin stimulates the accumulation of IGF-I mRNA in the absence of GH (85,86). Insulin also potentiates the stimulatory effect of GH and amino acids on IGF-I production (72,85,87). Although its exact mechanism of action is not known, the stimulatory effect of insulin seems to result from an enhanced rate of IGF-I gene transcription (Fig. 2) in adult hepatocytes (88) and from enhanced IGF-I mRNA stability in fetal hepatocytes (89). Despite insulin’s stimulatory role on IGF-I production, protein restriction (and perhaps decreased availability of specific amino acids) may be more important mechanistically than decline in serum insulin concentrations for the decreased circulating IGF-I in protein-restricted rats (90). In rats made diabetic with streptozotocin who are treated with insulin and then submitted to a low- or normal protein diet, the diet decline in IGF-I persists despite high circulating insulin (two to three times normal values). This suggests that dietary protein restriction decreases serum IGF-I independent of insulin and that protein restriction by itself is the major cause of reduced serum IGF-I in this model.

3.4.2. THYROID HORMONES

The involvement of the thyroid hormones in the nutritional regulation of IGF-I is suggested by the close relationship between the decline of circulating IGF-I and of thyroxine (T3) in fasted humans (91) and rats (92). Low serum IGF-I in hypothyroid animals reinforces the possibility of thyroid hormone regulation of IGF-I (93). It is possible, however, that the major effect of thyroid hormones on IGF-I synthesis is
exerted on the pituitary (94), where thyroid hormones regulate GH gene expression (95). Hypothyroidism is not only accompanied by a decline in GH secretion but is also associated with blunted IGF-I production in response to GH and resistance to the growth-promoting effect of GH (93). This suggests that thyroid hormones participate in IGF-I production by the liver. Studies show that thyroid hormones potentiate hepatic IGF-I synthesis in response to GH both in vivo and in vitro (83,96). Hepatic GH binding is upregulated by thyroid hormones. This supports the role of thyroid hormones in the potentiation of GH induction of IGF-I. Low serum T3 concentrations might play a role in the decline in GH and serum IGF-I concentrations in fasted rats because treatment of fasted rats with T3 seems to attenuate the decline in serum IGF-I (97).

### 3.4.3. Glucocorticoids and Proinflammatory Cytokines

A role for glucocorticoids in the GH resistance caused by food deprivation is suggested by their inhibitory effect on GH-induced IGF-I production in vivo (98) and in

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**Fig. 2.** Role of hormonal changes and nutrient availability in the regulation of IGF-I production by liver in response to food deprivation. GH, growth hormone; GHR, growth hormone receptor.
vitro (55,99) concurrent with their increased circulating concentrations during fasting. Part of the GH resistance caused by excess glucocorticoids might be caused by their capacity to inhibit GHR mRNA and GH binding to hepatocytes (54,55) and to other cell types (99,100). In diabetic rats, which have increased glucocorticoids and GH resistance, adrenalectomy restores the GH-induced IGF-I response (101,102). In contrast with diabetic rats, however, adrenalectomy of fasted animals failed to reverse the fasting-induced inhibition of the early steps of the liver GH receptor-signaling pathway (74). In addition to inhibit IGF-I production, glucocorticoids may also impair growth by induction of IGFBP-1 and reduction of free IGF-I (103) in the circulation.

In severe catabolic states such as sepsis, proinflammatory cytokines, namely tumor necrosis factor-α, interleukin-1β, and interleukin-6 are implicated in the development of GH resistance and the decrease in circulating IGF-I (refs. 104–106; see also chapter 16). As reviewed in Chapter 3, metabolic acidosis, as in renal failure, also has been shown to cause resistance to GH (107) and to decrease circulating IGF-I (108).

3.5. The Role of Nutrient Availability in the Nutritional Regulation of IGF-I

Evidence for a direct role of amino acid availability in regulating IGF-I comes from experiments using primary cultures of rat hepatocytes (109). In this model, amino acid deprivation causes a rapid and progressive decline in IGF-I mRNA and IGF-I peptide production, whereas amino acid excess causes an increase (73,88). Among the amino acids, tryptophan seems to be the most critical in regulating IGF-I, as its removal from the medium for 48h causes a dramatic decline in IGF-I mRNA and in IGF-I release. Across a broad range of amino acid concentrations, GH and insulin raised IGF-I mRNA levels in proportion to the amino acid concentration (110). Taken together, these observations suggest that GH, insulin and amino acids can regulate hepatic production of IGF-I independently.

It is not known, however, whether amino acids, insulin and GH control IGF-I synthesis at the same point. Whereas insulin and GH are believed to act at the transcriptional level, decreased IGF-I mRNA in amino acid-deprived cultured hepatocytes results from decreased transcription rate (88) and enhanced degradation (111). An amino-responsive element has been described in the IGF-I gene (112). It does not seem, however, to share common features with the one characterized in the CHOP and asparagine synthase genes, two genes induced by amino acid deprivation (113). Several transcription factors appear to be affected by dietary protein/amino acid availability, but it remains to be determined whether these are involved in the observed effects of amino acids on IGF-I transcription. Restriction of amino acids leads to accumulation of uncharged transfer RNA, which could impair gene expression by altering the rate of transcription (114).

In parallel with inhibition of IGF-I, amino acid deficiency stimulates the in vitro expression and release of IGFBP-1, a binding protein considered to inhibit the actions of IGF-I. Limitation in any one of the essential amino acids causes strong induction of IGFBP-1 (73,115). However, restriction in vivo of single essential amino acids is not sufficient to induce IGFBP-1, and general amino acid depletion is necessary (116). The direct effect of amino acid deprivation on IGFBP-1 explains why higher levels of IGFBP-1 are observed in protein-restricted rats than in starved rats.

IGF-I gene expression also may be regulated by glucose in hepatocytes, particularly those of fetal origin (63,117). In this model, glucose stimulates IGF-I mRNA and pep-
tide secretion. Experiments with analogs suggest that glucose-6 phosphate is necessary for the induction of IGF-I mRNA. A similar observation was made for IGF-II. The glucose-induced rise in IGF-II mRNA was mediated by stimulation of gene transcription and increased transcript stability. This effect was observed in the absence of GH, indicating that it did not result from the potentiation of the GH action on IGF-I (63).

Although reduced amino acid availability is thought to be responsible for the decrease of liver IGF-I mRNA in response to protein restriction, such a direct mechanism does not seem to be the rule for all nutrients. For example, chelation-induced depletion of zinc in rat hepatocytes does not cause IGF-I gene expression to decline, despite the clear inhibition of the metallothionein mRNA (118).

3.6. Role of Changes in IGF-I Gene Expression

Decreased serum IGF-I in dietary energy or protein restriction correlates with reduced steady-state levels of hepatic IGF-I mRNA (119–122), suggesting that nutritional regulation of IGF-I gene expression takes place at a pretranslational level. In fasted animals, the levels of IGF-I mRNAs with different 5′ untranslated regions (class 1 and class 2 transcripts) appear to be coordinately decreased (123,124). However, although the levels of IGF-1B mRNA declined markedly, IGF-1A mRNA was not altered significantly (125). Despite this preferential decrease in IGF-1B mRNA, fasting caused both propeptides IGF-1A and IGF-1B to decrease (123,125). All size-class transcripts (0.8–1.2, 1.7, 4.7, and 7.5 kb) are proportionately reduced by fasting (53). Dietary protein restriction, however, caused a greater decrease of the 7.5 kb IGF-I mRNA than the other size-classes (122,126).

Based on nuclear run-off studies (53,111,125) and analysis of nuclear transcripts (127,128), both transcriptional and posttranscriptional mechanisms may mediate the decrease in IGF-I expression observed in fasted as well as protein-restricted animals.

The weight of evidence suggests that transcription is the major locus for nutritional regulation of IGF-I. The co-ordinate decrease in transcription of nutritionally sensitive genes, such as albumin, transthyretin, and IGF-I in protein-restricted rats might result from altered activity of transcription factors. The DNA binding activity of factors involved in the expression of the IGF-I gene in liver (hepatocyte nuclear factor [HNF]-1α, HNF-3, HNF-4, CCAAT/enhancer binding protein [C/EBP], Sp1) is altered in response to protein restriction (129). The transcriptional activity of HNF-1α is reduced in hepatocytes exposed to medium deficient in amino acids (130). In contrast, C/EBP homologous protein (the CHOP transcription factor) is stimulated in these conditions (131,132). The induction of CHOP is of interest as increased levels of CHOP could interfere with C/EBP binding (133). Although the role of HNF-1α and C/EBPα in the stimulation of basal expression of IGF-I in liver is well established (134,135), their contribution to reduced IGF-I gene expression in the liver of protein-restricted rats is unsettled. Other transcription factors involved in the transcriptional regulation of IGF-I, such as Sp1 (136), might also direct the response to nutrient availability (137).

Nutritional regulation also seems to take place at the nuclear RNA splicing step. The observation that IGF-1A and IGF-1B mRNAs result from alternative splicing after transcription of a single gene, yet only IGF-1B mRNA levels are decreased by fasting suggests that the decrease in liver total IGF-I mRNA might also involve regulation of the pre-mRNA processing (111).
Protein restriction may result in decreased stability of the IGF-I mRNA, in particular for the 7.5-kb transcript (122,126). Reduction of the 7.5-kb transcript in protein restricted rats is confirmed by the observation that IGF-I mRNA stability in vitro is decreased in hepatocytes exposed to amino acid deprivation (111). In this model, the 7.5-kb mRNA is degraded faster than the two smaller IGF-I mRNA species. Differential regulation of the 7.5-kb IGF-I mRNA might be related to its 3′-untranslated region, which contains several AU-rich sequences (138). Such sequences in other genes are known to interact with cytosolic RNA-binding proteins and to be involved in the regulation of the stability and/or translation of the mRNA (139).

Regulation of IGF-I synthesis by nutrients also may be under translational control because discrepancies have been observed between serum IGF-I peptide concentrations and liver IGF-I mRNA levels (122,140–142). In protein-restricted rats, injections of high doses of GH for 1 wk restores liver IGF-I mRNA abundance to normal without normalization of liver or serum IGF-I concentrations (122). This divergent response to GH is not the result of a secretory defect of IGF-I because there was no accumulation of IGF-I in liver. It also seems unlikely that tissues other than liver might make significant contributions to the IGF-I released into the serum, given the observation that 80–90% of circulating IGF-I is produced by the liver (47). The mechanisms responsible for this presumed impairment of the translation of IGF-I mRNA are not known. It appears, however, that all IGF-I mRNA size-classes are associated with polysomes, even in the liver of protein-restricted rats (143), suggesting that they have the capacity to engage in IGF-I synthesis.

In addition to liver, reduced IGF-I mRNA during diet restriction is observed in most other organs (52,123). The decrement in IGF-I gene expression, however, is most dramatic in the liver. Fasting of young rats for 48 h decreases IGF-I mRNA levels by 80% in liver and lung, by 60% in kidney and muscle, and by only 30% in stomach, brain, and testes. No changes are observed in the heart. In a different study, prolongation of fasting for 3 d caused IGF-I mRNA in heart muscle to decrease but caused no change in the brain (52). Protein restriction for 1 wk, however, does not seem to reduce IGF-I mRNA in tissues (kidney, heart, diaphragm, brain, and aorta) other than liver (52) and muscle (144). The decline of circulating IGF-I in mice in whom the ability of liver to produce IGF-I has been knocked out indicates that the decline of circulating IGF-I during protein restriction originates from nonhepatic as well (145).

4. MECHANISMS INVOLVED IN THE NUTRITIONAL REGULATION OF IGF-I ACTION

4.1. Role of IGF Binding Proteins

Among the proposed functions of the IGF binding proteins (IGFBPs) are the prolongation of the plasma half-life of IGF-I and IGF-II, control of the rate of IGF transport from the vascular compartment, and regulation of the interaction between IGF-I and the type 1 IGF receptor on the cell surface (146–148). Because they control the bioavailability of IGF-I to tissues (149), the IGFBPs are believed to exert both stimulatory (150–152) and inhibitory (153–155) effects on IGF-I actions (Table 2). IGF-I bioavailability may be controlled by posttranslational modifications of IGFBPs (partial proteolytic degradation by specific IGFBP proteases, selective dephosphorylation)
resulting in IGFBPs with reduced affinity for IGF-I. Less than 1% of IGF-I circulates as free peptide (156), and most (>90%) is bound to the 150-kDa complex, which consists of IGF-I, IGFBP-3, or IGFBP-5 and an acid labile subunit (ALS). This complex is believed not to cross the capillary endothelium (157) and is credited with prolonging the half-life of IGF-I in the circulation. The complex has a relatively long half-life of 3–6 h in rats (158) and 12–15 h in humans (159), whereas free IGF-I disappears with an apparent half-life of 14 min (160). IGFBP-3 probably serves as a storage pool for IGF-I. IGFBP-1, IGFBP-2, and IGFBP-4 are associated with IGF-I in smaller complexes (30–40 kDa) that can cross the capillary endothelium (161). IGFBP-1 and IGFBP-2 contain RGD sequences (Arg-Gly-Asp) (149) that may allow attachment to cell surface integrin receptors. They may be involved in the delivery of IGF-I to tissues. Although serum concentrations of IGFBP-3 correlate positively with ALS and total IGF-I, IGFBP-1 and free IGF-I correlate inversely. It is hypothesized that IGFBP-1 is an important determinant of free IGF-I in vivo (162,163). IGF-independent actions have been shown for IGFBP-1, -3, and -5 (149). In particular, IGFBP-3 binds specifically and with high affinity to the cell surface of various cell types and inhibits monolayer cell growth, presumably by specific interaction with cell membrane proteins that function as IGFBP-3 receptors (164). The functions and nutrition-related regulatory mechanisms of IGFBP-5, IGFBP-6, and IGFBP-related proteins (Mac25, CTGF, NOV, CYR61) (165) are largely unknown.

Nutrient intake is a major regulator of the plasma concentrations of the IGFBPs. Serum IGFBP-3 concentrations in humans are relatively constant throughout the day, and this is the likely mechanism for the stability of serum IGF-I concentrations (166). In contrast, serum IGFBP-1 is markedly and rapidly suppressed by nutrient intake (167,168). An effect mediated primarily by increased insulin (169,170), glucose (171,172), and probably by amino acid concentrations (173,116). Insulin also may selectively stimulate the transport of IGFBP-1 to the extravascular space (173), which might explain the rapidity of the insulin-induced fall in IGFBP-1. IGFBP-2 levels are more stable than IGFBP-1 and are not subject to postprandial changes (174).

Dietary manipulations change the abundance of serum IGFBPs in humans and animals. In general, dietary restriction decreases serum IGFBP-3 and ALS concentrations

Table 2
Effect of Food Deprivation on Circulating IGF-I and IGFBP Levels and Their Hepatic Gene Expression

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<th>Circulating levels</th>
<th>Hepatic gene expression</th>
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<td>IGF-I</td>
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<td>Free ✓</td>
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<td>IGFBP-1</td>
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IGFBP, insulin-like growth factor binding protein; ALS, acid-labile subunit.
while it increases serum IGFBP-1 and IGFBP-2 (5). Fasting of rats for 24 h increases serum IGFBP-1 and -2 (175,176), and produces parallel increases of liver IGFBP-1 and IGFBP-2 mRNA (177). Likewise, 24-h fasting in humans causes a 4-fold increase of IGFBP-1 (162,168) whereas prolonged fasting for 9 d or protein restriction for 6 d is needed to cause an increase in plasma IGFBP-2 (174,178). Maternal fasting also induces an increase of IGFBP-1 and IGFBP-2 in fetal serum (179), thereby increasing the IGF binding capacity of serum. Free IGF-I changes in parallel with total IGF-I in fasted rats (180) and children (162). Unlike IGFBP-1, fasting for 24 h does not affect serum IGFBP-3 (181) and ALS (180) in rats and humans. A decline of serum IGFBP-3 and ALS, however, occurs after fasting for 48–72 h (179,180,182).

During more chronic dietary restriction, serum IGFBP-3 declines and a parallel change in the liver IGFBP-3 mRNA is observed (180,183). Unlike other situations in which IGFBP-3 concentrations are low, that is, pregnancy (184), after surgery (185), and catabolic states (186), the decline of IGFBP-3 in food-deprived rats and humans is not associated with proteolytic activity in serum (187). In response to reduced nutrition, serum ALS levels also decline, but steady-state hepatic ALS mRNA levels are not changed, indicating that ALS synthesis in fasting is regulated primarily at the posttranscriptional level (180,188–190). Because both IGFBP-3 and ALS production is stimulated by GH and insulin (188,191,192), it is not clear whether their decrease in response to food deprivation is due to decreased GH secretion, to defects in the GH action pathway and/or to the lack of the stimulatory effect of insulin. IGFBP-3 and ALS genes are stimulated directly by insulin, even in the absence of GH (192). The decline of IGFBP-3 may be related to decreased serum IGF-I itself, because IGF-I infusion in protein-restricted-rats normalizes serum IGFBP-3 (193). The induction of IGFBP-3 by IGF-I provides a mechanism by which the concentrations of these two peptides could be regulated coordinately. As with fasting, an increase in liver IGFBP-1 and -2 mRNA and increased serum concentrations of IGFBP-1 and -2 occurs with chronic energy or protein restriction (71,183,189,190,194).

Changes in IGFBPs similar to those produced by food deprivation have been observed in response to catabolic states. The most dramatic changes are an increase in circulating IGFBP-1 levels and the presence of an IGFBP-3 proteolytic activity. Proteolytic activity that degrades IGFBP-3 specifically appears in the circulation after major surgery (185) and in severe catabolic states (186). The decreased affinity for IGF-I caused by the enzymatic alteration of IGFBP-3 is associated with increased free IGF-I (195). This might increase the bioavailability of IGF-I for the tissues. Increases in IGFBP-2 and IGFBP-4 and decline in ALS levels are also observed (186,196,197).

As a consequence of these changes, when the IGF binding by serum is evaluated by chromatography after the serum sample has been incubated with radiolabeled IGF-I, dietary restriction consistently causes a decrease in the 150-kDa complex and an increase in the small binding protein complex (198,199). These changes might alter the transport of IGF-I across the endothelium and the bioavailability of IGF-I to the tissues.

4.2. Role of the Clearance of Circulating IGF-I

Because IGFBPs are responsible for transport of IGF-I in the circulation, nutrient-induced changes in the concentrations of the IGFBPs could alter the clearance of circulating IGF-I. Plasma IGF-I clearance is accelerated in situations where serum IGFBP-3
is decreased, for example, hypophysectomized (200) or pregnant rats (184). By monitoring the decline of radioactivity in the circulation of rats injected with $[^{125}I]$-IGF-I, it has been shown that the clearance and the volume of distribution of $[^{125}I]$-IGF-I were increased in protein-restricted rats by 50% and 75%, respectively (199). Pharmacokinetic analysis indicates that the accelerated clearance of IGF-I in protein-restricted rats is the result of more rapid distribution of IGF-I into tissues (shorter $t_{1/2}$) rather than a change in the elimination half-life ($t_{1/2}$). In fed rats, IGF-I is almost equally distributed between the 150-kDa and the 30-kDa binding protein complexes, whereas in protein-restricted rats, IGF-I is bound preferentially to IGFBPs in the 30-kDa complex (199). Because the small IGFBP complexes are believed to facilitate the transport of IGF-I from serum to tissues, the preferential association of IGF-I with these IGFBPs in protein-restricted animals might allow faster transcapillary passage and distribution to tissues. Unlike the rat, clearance of radiolabeled IGF-I in fasted sheep is not enhanced. This could result from species differences, or it could indicate that only chronic or a more specific dietary restriction affect clearance and distribution of IGF-I. Despite the rapid decline in circulating IGF-I after LPS injection, pharmacokinetic analysis of blood $[^{125}I]$-IGF-I decay curves indicates that the half-time for whole blood clearance is not altered by LPS (201).

4.3. Role of the Sensitivity to IGF-I

In addition to decreasing IGF-I production, dietary restriction impairs the anabolic actions of IGF-I. When protein restricted or zinc-deficient rats were infused with IGF-I by osmotic minipump, carcass growth (body weight and tibial epiphyseal plate) was not stimulated, despite the normalization of serum IGF-I (193,202). In contrast, growth of the spleen and kidney was enhanced. Similarly, IGF-I failed to increase cancellous and periosteal bone formation in protein-restricted rats, while exogenous IGF-I increased the bone formation rate in well-fed rats (203). In rats fed parenterally with a limited supply of amino acids, anabolic effects of IGF-I on carcass lean mass were not observed, in contrast to visceral tissues (204). Treatment of neonatal protein energy-deprived rats with IGF-I does not stimulate somatic growth. In contrast, erythroid precursors in bone marrow are increased, suggesting that the actions of IGF-I on growth and erythropoiesis are unrelated (205). These results suggest that, in addition to its effects on IGF-I gene expression and IGF binding proteins, food deprivation causes organ-specific resistance to the anabolic properties of exogenous IGF-I. These observations support the concept that nutrient insufficiency can block the anabolic properties of IGF-I, but do not block other properties (206–208).

Resistance to IGF-I during food deprivation occurs despite increased binding of IGF-I by tissues. Rats that are fasted for 48 h have increased IGF-I binding to stomach, lung, testes, and kidney, as reflected by alterations in the IGF-I receptor number and/or affinity (123). Change in the abundance of the type 1 IGF receptor mRNA parallels the change in binding. Protein restriction also is associated with an increase of IGF-I binding to muscle (209).

That nutrients can control the response to IGF-I directly has been observed in fibroblast cultures where zinc depletion inhibits the mitogenic action of IGF-I (210). Although the interaction between anabolic action of IGF-I and amino acid availability has not been studied at the cellular level, several pieces of evidence indicate that cross-
talk between the pathways activated by these stimuli are likely. For example, induction of the transcription factor CHOP by amino acid depletion requires the presence of IGF-I (132). In muscle cells, a target for IGF-I action, amino acids activate p70 S6 kinase, an intermediate in the initiation of protein synthesis, in synergy with insulin (211). Furthermore, both insulin and amino acids are required to stimulate protein synthesis and to inhibit protein degradation in muscle tissue (212). If these observations can be extended to IGF-I, they suggest that amino acids might modulate the anabolic action of IGF-I, at least on protein metabolism.

The possibility that nutrients and hormones modulate the anabolic action of IGF-I has been investigated in humans. In energy restricted obese subjects, exogenous GH produced a twofold increase in serum IGF-I, accompanied by attenuation of nitrogen loss (213). After a few weeks, however, resistance to the anabolic properties of IGF-I occurred. In similar experiments, infusion of IGF-I for 1 wk increased serum IGF-I concentrations fourfold and produced marked attenuation of the nitrogen wasting (214). In volunteers made catabolic by glucocorticoid treatment, IGF-I infusion did not produce any significant decrease of proteolysis, suggesting that the anabolic action of IGF-I in humans may depend on the hormonal and nutritional environment (215). GH administered together with IGF-I appears to function in a complementary fashion to promote protein anabolism (216). There are several ways by which GH could produce nitrogen retention that are not fully replicated by infusion of IGF-I. For example, GH may alter concentrations of IGFBPs and modify the metabolism of carbohydrate or fat to facilitate IGF-I action.

The loss of anabolic response to IGF-I has been described in catabolic states, such as AIDS (217), sepsis (218), and uremia (219). In uremia, resistance is caused by a post-receptor defect characterized by inhibition of the autophosphorylation of the type 1 IGF receptor (IGF-1R) β-subunit and IRS-1 (220). Although perturbed IGFBPs in chronic renal failure may play an important role in altering IGF-I storage and delivery to tissues, IGF-I analogs with low affinity to IGFBPs fail to obliterate this IGF-I resistance (220). In addition, malnutrition and acidosis have been excluded as mediators of the IGF-I resistance of uremic rats (220). In more severe catabolic states, such as sepsis, the anticalcatabolic action of IGF-I can also be blunted. In rats made septic by cecal ligation and puncture, IGF-I failed to inhibit muscle proteolysis, despite the suppression of the gene expression of several components of the ubiquitin-proteasome proteolytic pathway (221). However, IGF-I retained its antiproteolytic action in burned rats (222). Careful dissection of the factors controlling the anabolic actions of IGF-I will need to be performed.

5. SUMMARY AND CONCLUSIONS

Nutrition is one of the principal regulators of circulating IGF-I.

• In humans, serum IGF-I concentrations are markedly reduced by energy and/or protein deprivation.
• Both energy and proteins are critical in the regulation of serum IGF-I concentrations. After fasting, optimal intake of both energy and protein is necessary for the restoration of circulating IGF-I, but energy may be somewhat more important than protein in this regard. While a low intake of protein is able to increase IGF-I in presence of adequate
Fig. 3. Changes in IGF-I system induced by food deprivation. GH, growth hormone; GHBP, growth hormone binding protein; GHR, growth hormone receptor; ALS, acid-labile subunit; IGF-R, insulin-like growth factor receptors; IGFBP, IGF-binding proteins; BP-3, IGF-binding protein-3.
energy, there is a threshold requirement of energy below which optimal protein intake fails to raise IGF-I after fasting.

- When energy intake is severely reduced, the carbohydrate content of the diet is a major determinant of IGF-I responsiveness to GH.
- The content of essential amino acids in the diet is also critical for the optimal restoration of IGF-I after fasting.

Many mechanisms are involved in the nutritional regulation of IGF-I (Fig. 3).

- Decline of serum IGF-I during dietary restriction is independent of the diet-induced alterations in pituitary GH secretion.
- The role of liver GH receptors is dependent of the severity of the nutritional insult. In severe dietary restriction (fasting), a marked decrease of the number of somatogenic receptors suggests that a receptor defect is involved in the decline of circulating IGF-I. In contrast, in less severe forms of dietary restriction (protein restriction), the decline of IGF-I results from a postreceptor defect in GH action at the hepatic level.
- Nutritional deprivation decreases hepatic IGF-I production by diminishing IGF-I gene expression.
- Decline in IGF-I gene expression results from both transcriptional and post-transcriptional mechanisms.
- Decline in IGF-I gene expression is mainly caused by nutrient deficiency, and less importantly by nutritionally induced changes in hormones (insulin, T3).
- Diet restriction also increases the clearance and degradation of serum IGF-I through changes in the levels of circulating insulin-like growth factor-binding proteins (IGFBPs).
- Finally, nutrients may also control the biological action of IGF-I, either directly or indirectly through changes in IGFBPs.

GH resistance with decreased IGF-I production is also present in situations characterized by catabolic stress.

- Proinflammatory cytokines and glucocorticoids probably mediate the decreased IGF-I production in these situations.
- The molecular mechanisms leading to the decline of IGF-I seem to be similar to those operational in food deprivation.
- As with nutrients, these hormonal factors can control the anabolic action of IGF-I, in particular on skeletal muscle.

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