Insulin-Like Growth Factor Binding Proteins as Glucoregulators

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Circulating insulin-like growth factors (IGFs) represent an important pool of potential hypoglycemic activity, which is largely inhibited by their sequestration in a heterotrimeric complex comprising growth factor, IGF-binding protein-3 (IGFBP-3), and acid-labile subunit (ALS). Less than 1% of total IGFs circulate in the free form, yet even this amount might contribute significantly to circulating insulin-like activity. The ternary binding protein complex appears to inhibit insulin-like activity of bound IGFs by preventing their egress from the circulation. Although the integrity of this complex might be affected by limited proteolysis of IGFBP-3 in pregnancy and catabolic conditions, the evidence that this increases IGF bioavailability, and thus hypoglycemic potential, is as yet unclear. However, in patients with IGF-II–secreting tumors, hypoglycemia may result from a failure of the ternary complex to adequately sequester the IGFs. Improvement in complex formation, by treatment with corticosteroids or growth hormone, alleviates the hypoglycemia, even if (as seen with growth hormone treatment) IGF-II hypersecretion persists. In these patients, blood glucose levels are inversely correlated with IGFBP-2 levels, suggesting that this protein might play a part in transporting IGFs to their target tissues. Conversely, ALS levels correlate positively with blood glucose, emphasizing the importance of the ternary complex in preventing hypoglycemia. Unlike the other IGF-binding proteins, IGFBP-1 is acutely regulated in the circulation, in a manner consistent with its acting as a glucose counterregulator. It might act in this way by inhibiting the activity of free IGFs in the circulation. Its acute rise following hypoglycemia, and suppression after glucose ingestion, are consistent with such a role. Following IGF-I administration in humans, adaptive changes in IGFPB-3, ALS, and IGFBP-1 are seen, which might affect subsequent responses to IGFs. Understanding the mechanisms and consequences of these changes will be important in optimizing the therapeutic applications of IGF-I.

THE INSULIN-LIKE growth factors (IGF-I and IGF-II) are responsible for a range of biological actions, including the stimulation of growth and differentiation in a variety of cell types.[1] They also exert many actions similar to those of insulin, which may be mediated by either the heterotetrameric type I IGF receptor or the structurally related insulin receptor. For example, at the cellular level, stimulation of tyrosine aminotransferase, amino acid transport, and glycogen synthase by IGF-II in H-35 rat hepatoma cells[2] and of glycogenesis by IGF-I and IGF-II in fetal rat hepatocytes[3] are believed to be insulin receptor–mediated. However, the metabolic effects of IGF-I in muscle cells and the majority of its metabolic effects in other tissues appear to require the IGF-I receptor, as recently reviewed by Ballard et al.[4]

Studies in vivo indicate that IGF-I can act as a potent glucoregulatory agent. In adult rats, IGF-I has been reported to have approximately 2% of the potency of insulin in inducing hypoglycemia.[5] However, the reported relative potency of IGF-I in humans is higher. A seminal study by Guler et al[6] in 1987 showed that in healthy adults, a single intravenous injection of insulin or IGF-I caused almost identical hypoglycemic responses, with the potency of IGF-I being approximately 6% that of insulin. Although responses to the two agents of the counterregulatory hormones glucagon, epinephrine, norepinephrine, and cortisol were described in that report as being indistinguishable,[6] a more recent study in which insulin and IGF-I were infused over a 2-hour period showed slightly greater epinephrine and norepinephrine responses to IGF-I than to insulin, but no glucagon response and a blunted recovery of serum glucose levels following IGF-I infusion as compared with insulin.[7] In that study, the potency of IGF-I in eliciting the initial hypoglycemic response was 4.6% that of insulin.

Due to its potential to increase glucose disposal, suppress growth hormone (GH) secretion, and overcome insulin resistance, IGF-I has been tested in a number of clinical trials in subjects with both insulin-dependent and non–insulin-dependent diabetes, including those with extreme insulin resistance syndromes.[8-10] However, to date, these studies have paid relatively minor attention to the effects of IGF-I administration on the IGF-binding proteins (IGFBPs), a family of circulating proteins known to be central to the regulation of endogenous IGF bioavailability and likely to play an equally vital role in regulating the activity of administered IGFs.

IGFBPs

IGF-I and IGF-II are found in all biological fluids and in culture media conditioned by IGF-secreting cells, in association with one or more IGFBPs. The six IGFBPs (IGFBP-1 to IGFBP-6) are proteins of core molecular weight of approximately 22 to 30 kd, which may be modified posttranslationally by glycosylation and/or phosphorylation.[11] These proteins show a high degree of structural similarity, with nine disulfide bonds conserved in IGFBP-1 to IGFBP-5, and eight of these present in IGFBP-6. All of these proteins can form simple binary complexes with IGF-I or IGF-II, but only IGFBP-3 has the additional property of combining with a liver-derived glycoprotein, the acid-labile subunit (ALS), to form a ternary complex of approximately 140 kd.[12] Circulating concentrations of ALS generally exceed those of IGFBP-3 by severalfold, so that even when—as is generally the case—most of the IGFBP-3 is in the 140 kd form, there is always substantial free ALS in the circulation.[13]

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The ternary complex, consisting of IGF-I or IGF-II, IGFBP-3, and ALS, is believed to carry most of the circulating IGFs in healthy adults. Because free IGFs disappear from the circulation within minutes, whereas IGFs in the ternary complex have a half-life of 15 hours,[14] concentrations of free IGFs in the circulation are extremely low. Measured by a direct immunoradiometric assay, a free IGF-I level of 0.7% of total IGF-I has been reported,[15] whereas an ultrafiltration method yielded a value for free IGF-I of 0.4% of total, and for IGF-II, 0.2% of total.[16] An earlier gel filtration study yielded values as high as 10% of total for free IGF-I and 1.3% of total for free IGF-II,[14] although this technique might lead to some dissociation of IGFs from IGFBP complexes.

Even low levels of free IGFs have the potential to exert considerable insulin-like activity. If only 0.4% of a total IGF concentration of 100 nmol/L, or 400 pmol/L, is in the free form, with a potency of 5% that of insulin, insulin-like activity equivalent to 20 pmol/L insulin would be exerted, contributing significantly to the total hypoglycemic potential. In fact, steady-state free IGF measurements may seriously underestimate IGF availability. A recent study in which rats received an intravenous bolus of human IGFBP-3 found rapid complexing of the IGFBP-3 into the 140-kd form.[17] This implies that both IGFs and ALS were readily available, since ALS does not bind to IGFBP-3 unless the binding protein is occupied by IGF-I or IGF-II.[18] Whereas ALS is known by direct measurement to circulate in excess of the ternary complex, the ready availability of IGF-I, which did not dissociate from the endogenous rat ternary complex, suggests a rapid flux of IGFs through the circulation at a concentration far in excess of that measurable as free peptide.[17]

GLUCOREGULATORY ROLE OF IGFBP-3 AND ALS

Since most of the circulating IGFs are carried in the ternary complex together with IGFBP-3 and ALS, the factors regulating this complex therefore have the potential to modulate insulin-like activity of IGFs. Indeed, the ternary complex can be seen as a major glucoregulatory system, since it controls the bioavailability of a circulating pool of IGFs, which, if fully active, would exert vastly more hypoglycemic activity than that due to insulin itself. The blocking of IGF egress from the circulation by the ternary complex may simply be a result of the large size ($\sim$140 kd) of the complex, preventing its passage through the capillary endothelium. This conclusion was drawn from a study that found extremely low levels of high–molecular-weight IGFBPs in human lymph as compared with serum.[19] In contrast, IGF-I and IGF-II were shown to form a ternary complex in ovine mammary lymph, indicating the presence of both IGFBP-3 and ALS and raising the question whether in this species, the ternary complex might be able to leave the circulation.[20]

There is little doubt that IGFs in the ternary complex do not exert insulin-like activity. Thus, despite their insulin-like potential, an increase in total circulating IGFs, as seen, for example, in acromegaly, does not lead to hypoglycemia, because IGFBP-3 and ALS also increase in this condition.[21,22] This may be compared with a similar increase in circulating IGFs seen after IGF-I infusion, in which a profound hypoglycemia may be seen despite the initiation of normal counterregulatory responses.[7] In this situation, there is no acute compensatory increase in the ternary complex to block the insulin-like activity—indeed, a more prolonged administration of IGF-I causes a significant decrease in both ALS and IGFBP-3 levels.[23]

In pregnancy and some catabolic states, the appearance of serum proteolytic activity, resulting in limited IGFBP-3 proteolysis, has been described.[24-26] It has been widely speculated that this might increase the bioavailability of IGFs to the tissues. However, given the clear-cut hypoglycemic activity of exogenous "free" IGF-I, a significant increase in IGF bioavailability resulting from IGFBP-3 degradation, and a subsequent impairment of IGF binding, might be expected to affect glucose homeostasis. The failure to see hypoglycemia in these conditions implies that despite the possibility of limited IGFBP-3 proteolysis, the ternary complex remains functional in its ability to sequester IGFs into an inactive form.

Although the possibility remains that counterregulatory responses in pregnancy and catabolic states mask a potential IGF-induced hypoglycemia, the best evidence that the ternary complex does not easily release IGFs in these conditions due to IGFBP-3 proteolysis is the observation that the complex circulates at normal to increased concentration and carries the same proportion of IGFBP-3 and IGFs as in nonpregnancy serum.[27,28]

GLUCOREGULATION IN NON–ISLET CELL TUMOR HYPOGLYCEMIA

A well-characterized illustration of the importance of the ternary complex in glucoregulation comes from the condition known as non–islet cell tumor hypoglycemia (NICTH), in which the presence of an IGF-II–secreting tumor leads to recurrent hypoglycemia.[29] Most of the IGF-II in the circulation of these patients is in incompletely processed forms of 10 to 15 kd[30,31] and is found, together with IGFBP-3, in a binary complex of approximately 40 kd rather than the usual ternary complex of approximately 140 kd. Surgical removal of the tumor leads to a normalization of glucoregulation, concomitant with a return of the IGFs and IGFBP-3 to the high–molecular-weight form.[32]

The impairment of ternary complex formation appears to be a major factor in the occurrence of hypoglycemia in these patients.[29] As discussed earlier, an increase in circulating total IGFs per se does not lead to hypoglycemia, since the IGFs would normally (eg, in puberty or acromegaly) be sequestered in the high–molecular-weight complex. Thus, if complex formation can be achieved in patients with NICTH, normoglycemia may be attained even in the presence of continuing oversecretion of IGF-II. This is seen in the case of treatment of tumor hypoglycemia with GH. Teale et al[33] observed that alleviation of hypoglycemia by GH was associated with an increase in IGFBP-3. More recently, a shift in IGFBP-3 from binary to ternary complexes has been

reported following GH treatment,[34] despite the fact that GH treatment does not decrease IGF-II hypersecretion.[35]

Table 1 illustrates serum analyte concentrations in an 87-year-old woman with NICTH, before and during 5 weeks of treatment with GH. As previously described in a preliminary report, the patient had initially been treated with prednisolone.[36] Although IGF-II levels showed a slight increase during the period of GH treatment, normoglycemia was achieved in parallel with a twofold increase in IGFBP-3 and an almost fourfold increase in ALS. Gel chromatographic analysis of her serum IGFBP-3 indicated a marked redistribution into the 140-kd complex during GH treatment, although a normal IGFBP-3 profile (80% to 90% in the ternary complex) was not achieved.[36]

Before treatment, the patient's serum IGFBP-3 concentration was less than one third of her total IGF concentration, in contrast to the usual approximately equimolar relationship between IGFs and IGFBP-3.[21] ALS, which normally circulates in excess of the complex,[22] was also in severe molar deficiency, but increased into the midnormal range on treatment. The abnormally high IGFBP-2 concentration—some 10-fold above the normal mean value—suggests that this protein might have carried the considerable excess IGF-II in this unusual situation. GH treatment approximately reversed the IGFBP-2 and IGFBP-3 concentrations, with IGFBP-2 remaining above the normal range. Figure 1a shows that over the entire period of treatment of this patient with prednisolone and GH, IGFBP-2 showed a significant inverse relationship with the blood glucose level. IGF-I, IGFBP-3, and ALS all showed a positive relationship with blood glucose, which was strongest in the case of ALS (Fig 1b). These observations suggest that formation of the ternary complex is a significant factor in the maintenance of normoglycemia, but also may indicate that when the ternary complex is insufficient to carry IGFs, IGFBP-2 can act to transport IGFs to the tissues.

GLUCOREGULATORY ROLE OF IGFBP-1

While the ternary complex plays a major role in regulating the insulin-like activity of IGFs over the long term, it

Table 1. Serum Analyte Concentrations in a Patient With NICTH Before and During Treatment With Recombinant Human GH

Analyte	Pretreatment (n = 3)	During GH Treatment (n = 3)*	Normal Range†
Glucose (mmol/L)	2.5 ± 0.3	5.5 ± 0.8	
IGF-I (nmol/L)	8.3 ± 0.4	14.9 ± 4.1	16-45
IGF-II (nmol/L)‡	122 ± 9	149 ± 16	47-94
IGFBP-3 (nmol/L)	39.3 ± 4.6	79.5 ± 11.0	51-107
ALS (nmol/L)	75.7 ± 9.8	285.9 ± 74.4	176-400
IGFBP-2 (nmol/L)	81.5 ± 13.5	38.1 ± 14.7	2.2-14.4

NOTE. Values are the mean ± SD. This case has previously been reported in abstract form.[36]

*Three samples taken while the patient, an 87-year-old woman with a pleural fibroma, received recombinant human GH 4 U/d for 1 week, followed by 8 U/d for 4 weeks. She had previously received prednisolone for 3 weeks.

†Determined in the author's laboratory.

‡Mostly present as high–molecular-weight precursor forms.

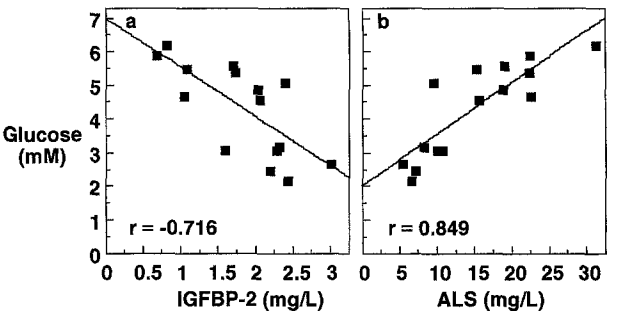

Fig 1. Association between (a) serum glucose and IGFBP-2 levels and (b) serum glucose and ALS levels in 15 blood samples taken from an 87-year-old woman with a pleural fibroma associated with NICTH. Samples were taken before treatment (n = 3), over 3 weeks of treatment with prednisolone (n = 5), and over 11 weeks of treatment with GH (n = 7). This case has previously been reported in abstract form.[36]

appears that short-term regulation of IGFs not sequestered in the ternary complex may be provided by IGFBP-1. This protein differs from the other IGFBPs in that it shows acute regulation by metabolic status,[37] being powerfully suppressed by insulin[38,39] and independently by carbohydrate.[40,41] The effect of carbohydrate is apparently mediated by inhibition of a stimulatory cyclic adenosine monophosphate–dependent mechanism.[40] Although, as reviewed recently,[42] insulin suppresses IGFBP-1 production in the presence of adequate substrate (for example, in subjects undergoing a euglycemic clamp), insulin administration under conditions in which hypoglycemia ensues leads to an increase in serum IGFBP-1 levels,[37] just as the blocking of substrate entry into liver cells stimulates IGFBP-1 secretion in vitro.[40]

It has been suggested that the stimulatory effect of hypoglycemia may merely reflect decreased insulin secretion into the hepatic portal circulation.[42] However, the effect, also seen in rats, is significantly attenuated following adrenalectomy, suggesting the involvement of an adrenal factor, probably not corticosteroid.[43] In type I diabetic subjects, the IGFBP-1 increase is not significant and is preceded by a significant decrease in response to insulin injection,[44] possibly reflecting increased hepatic glucose production or altered adrenal function in these patients.

The pattern of IGFBP-1 suppression by carbohydrate and insulin and stimulation by hypoglycemia is characteristic of the regulation of counterregulatory hormones, leading to the suggestion in 1988 that IGFBP-1 might play a counterregulatory role in glucose homeostasis.[37,45] This view now appears to be well established.[42] For IGFBP-1 to exert a counterregulatory action, its increased serum concentration in response to hypoglycemia would have to be inhibitory to the insulin-like activity of the fraction of total IGFs not sequestered in the ternary complex. The IGFBP-1 increase following hypoglycemia has been reported to be approximately 100 μg/L (~4 nmol/L),[37] or in a more recent study, 35 μg/L (~1.4 nmol/L).[44] As discussed earlier, estimates of circulating free IGFs vary, but it is clear that a free concentration of 1 nmol/L could be significantly neutralized by an IGFBP-1 increment of up to 4 nmol/L in response to hypoglycemia.

This hypothesis implies that IGFBP-1 can block the hypoglycemic activity of IGF-I, and that there exists in the circulation a tonic glucoregulation by IGFs free from the ternary complex, which is amenable to regulation by IGFBP-1 fluctuations. Studies involving injection of human IGFBP-1 into rats support this idea.[46] Figure 2a shows that the hypoglycemic activity of IGF-I in overnight-fasted rats is totally inhibited by co-injection of equimolar human IGFBP-1. This is apparently achieved by retarding the egress of IGF-I from the circulation and inhibiting its effect on glucose uptake by heart and skeletal muscle.[47] Furthermore, IGFBP-1 administered alone exerts a significant hyperglycemic effect, implying inhibition of the tonic hypoglycemic activity due to IGFs (Fig 2b). The transient nature of this effect may be explained by the rapid removal of IGFBP-1 from the circulation, with a half-life of approximately 12 minutes.[46]

Possible evidence of the glucoregulatory role of IGFBP-1 is seen in fasted healthy subjects following a single injection of recombinant human IGF-I (0.1 to 0.125 mg/kg), which elicits a profound acute increase in serum IGFBP-1 levels, accompanied by a marked suppression of insulin levels and a 20% decline in blood glucose.[23] Food intake 6 hours later rapidly reverses the IGFBP-1 increase and leads to a marked glucose elevation to a mean peak value of approximately 9 mmol/L. The peak IGFBP-1 response at 6 hours is highly associated with the peak glucose response at 8 hours (R^2 = .89), suggesting that the insulin-like activity of IGF-I (under conditions in which insulin secretion is greatly suppressed) may be closely regulated by IGFBP-1.[23]

In fed subjects, IGF-I administration elicits an IGFBP-1 response of less than 10% of that seen in fasted subjects,[23] presumably because the presence of adequate carbohydrate would obviate the requirement for a strong counterregulatory response. However, interestingly, after daily IGF-I injections for 7 days, IGF-I is able to stimulate a strong IGFBP-1 response even in fed subjects. Associated with this increased responsiveness to IGF-I is a significant decline in levels of IGFBP-3 and ALS, the proteins that sequester IGFs in the inactive high–molecular-weight complex.[23] It may be speculated that the decreased ternary complex would allow more of the insulin-like activity of administered IGF-I to be expressed, prompting a greater compensatory IGFBP-1 response. These studies highlight the intimate link between IGFs, IGFBP-1, and blood glucose levels, and suggest that the adaptive response of IGFBPs to extended IGF-I administration have the potential to modulate IGF-I biopotency.

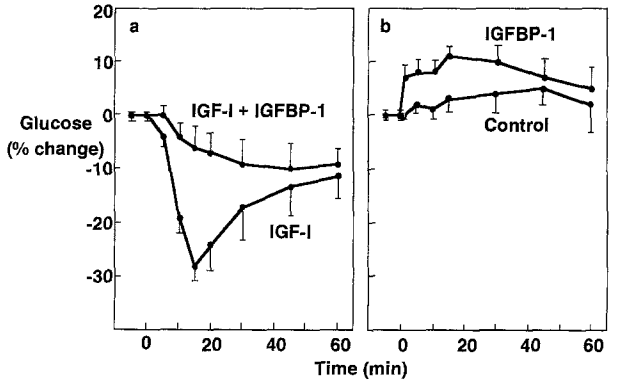

Fig 2. Effect of IGFBP-1 on glucose levels in (a) IGF-I–treated or (b) saline-treated rats. Plasma glucose levels are expressed as the mean percent change (±SEM) from baseline levels. IGF-I (25 μg) and human IGFBP-1 (100 μg) were administered intravenously, separately or premixed (30 minutes previously), to chronically cannulated male rats of 210 to 300 g (5 to 7 per group). Effect of IGFBP-1 is significant by ANOVA, $P < .001$ (a) or $P < .002$ (b). Reprinted with permission from *Endocrinology* 129:2254-2256, 1991.[46] ©The Endocrine Society.

SUMMARY AND CONCLUSION

Although the insulin-like activity of the IGFs was established more than two decades ago by the pioneering study reported by Oelz et al,[48] the precise role of the growth factors as endocrine regulators of blood glucose levels has been difficult to demonstrate. In recent years, administration of recombinant human IGF-I to humans and animals has shown clearly that this growth factor can elicit a hypoglycemic response similar to that of insulin, adding weight to the earlier conclusion that endogenous IGFs must also have a glucoregulatory action. Although it was recognized that IGF carrier proteins must somehow be involved in regulating this activity, it required the purification, assay development, and administration of IGFBPs to demonstrate the mechanisms involved.

At our present state of knowledge, it appears that the IGF–IGFBP-3–ALS ternary complex is the principal regulator of the overall bioavailability of serum IGFs. Important lessons about the role of this complex have been provided by patients with IGF-II–secreting tumors causing hypoglycemia, in whom the complex fails to form normally. Because of this central role of the ternary complex, measurement of serum concentrations and distribution of IGFBP-3 and ALS, possibly in a variety of posttranslationally modified forms, therefore appears to be essential to a complete understanding of the stability, distribution, and activity of the IGFs.

A strong case has also been established for an acute glucoregulatory role for IGFBP-1, with its fluctuations in concentration in response to metabolic stimuli reflecting acute regulation of free IGFs, ie, IGFs not inactivated by sequestration in the ternary complex. Whether these free IGFs are truly in an entirely unbound form or associate, at least transiently, with the other IGFBPs is not yet clearly established. IGFBP-1 itself appears to be under dual regulation by insulin (an inhibitor) and cyclic adenosine monophosphate (a stimulator) responding to intracellular substrate availability. IGFBP-2 and possibly other IGFBPs may also be shown to be important in glucose regulation, but this has not yet been clearly proven.

The recent evidence that IGF administration to humans can lead to adaptive changes in glucoregulatory IGFBPs emphasizes the importance of understanding the various IGFBP systems as IGF therapy becomes a reality. A greater knowledge of these systems is likely to yield important therapeutic dividends by allowing development of optimal preparations and schedules for IGF administration.

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