INVITED COMMENTARY

GH, IGFs, IGF-binding protein-3 and acid-labile subunit: what is the pecking order?

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Is the title of this Commentary a question which is still relevant? The dependence of insulin-like growth factor-I (IGF-I) on growth hormone (GH), which formed the basis of the classical somatomedin hypothesis (1), is now almost old hat. After insulin-like growth factor-binding protein-3 (IGFBP-3) was identified in serum as the carrier protein for most circulating IGFs, its variations were shown also to be regulated by GH secretion (2, 3). Serum ALS (acid-labile subunit), which binds IGFBP-3 associated with IGF-I or -II to form 140 kDa complexes (4), is age- and GH-dependent in the same way as are IGF-I and IGFBP-3. However, over recent years, the use of recombinant IGF-I in both experimental investigation and treatment of dwarfism resulting from genetic disorders of the GH receptor (Laron syndrome) has raised controversy as to the respective physiological roles of GH and IGF-I. It was recently proclaimed that ‘long-term IGF-I therapy in patients with Laron syndrome consistently failed to demonstrate any increase in IGFBP-3 concentrations, indicating that IGFBP-3 production is under the direct control of GH’ (5). With equal conviction, another group has concluded in this issue of the European Journal of Endocrinology (6) that the rise seen in serum IGFBP-3 levels under IGF-I therapy provides confirmation of their earlier findings, which they considered as ‘the first proof that IGFBP-3 in humans is regulated directly by IGF-I and is GH-independent’ (7).

The first ambiguity arising out of these statements concerns the relationships made between ‘concentrations’ or ‘levels’ of IGFBP-3 and ‘production’ or ‘regulation’. It is well known that virtually all of the IGFs and IGFBPs found in the bloodstream arise in the liver (8) but, although their concentrations in the circulation reflect hepatic production, these also depend on their half-lives—approximately 12 h in the 140 kDa ternary complexes, 30 min as free IGFBPs or associated with IGFs in the form of binary complexes (9). It therefore seems useful to take a fresh look at some of the known data from in vitro and in vivo studies on animals and in man so as to clarify the apparent discrepancies or contradictory interpretations.

Let us consider just liver production and regulation of components of the 140 kDa complexes, which means that certain lines of research will deliberately be ignored. These include the numerous studies of (ubiquitous) IGFBP-3 expression and (more limited) ALS expression in other tissues which do not, or only moderately, contribute towards levels in the bloodstream and the various studies using cell lines with phenotypes different from those in physiological models.

Within the liver, different cell populations synthesize different proteins. IGFs and the ALS are produced by hepatocytes, and IGFBP-3 by Kupffer cells and sinusoidal endothelial cells. Analysis of their expression in the rat has shown that IGFBP-3 increases relatively little during post-natal development, whereas ALS increases dramatically in the first weeks after birth. With hypophysectomy, ALS mRNA levels are decreased by 90%, but IGFBP-3 mRNA by only 50% (10). In cultured endothelial cells from bovine periarteric tissue, IGFBP-3 mRNA is increased 2- to 10-fold by IGF-I, but reduced to 20% of control levels by transforming growth factor-β. The changes in mRNA match the levels of IGFBP-3 protein secreted into the media by the cells (11). In co-cultures of rat hepatocytes and Kupffer cells, IGFBP-3 synthesis is stimulated by insulin and IGF-I, but not by GH (12). In the same model, insulin stimulates IGFBP-3 gene transcription, whereas IGF-I prolongs IGFBP-3 mRNA stability (13). Recently, GH was shown to stimulate transcription of the ALS gene and ALS promoter activity in rat liver (14).

From all these findings, it can be assumed that ALS synthesis is the primary site for GH regulation of ternary complex formation and that both IGF-I and insulin are necessary for maintenance of normal IGFBP-3 production.

The next step is to consider in vivo studies, where most of the work has been done on hypophysectomized rats. IGF-I administration, independently of GH, normalizes IGFBP-3 mRNA levels in the liver (15) and more or less completely restores IGFBP-3 levels in the bloodstream (16–19), but only GH is capable of increasing serum ALS levels (19) so that 140 kDa complexes can be formed (17–19). In a particularly revealing study using two lines of transgenic mice, one GH deficient and the other over-expressing IGF-I and with doubled serum IGFBP-3 levels, when the two lines were crossed, offspring were obtained with serum levels of IGF-I and IGFBP-3 at 69 and 64% of those of controls, but no 140 kDa complexes (20). This suggested that IGF-I induction of IGFBP-3 may be a major mechanism by
which IGF-I directly regulates synthesis of its carrier. The sub-normal serum levels would be explicable in terms of the shorter half-lives of the binary IGFBP-3–IGF-I complexes, which, unlike the ternary 140 kDa complexes, are capable of crossing the capillary endothelium (21).

In man, the various studies of GH-deficient patients have all shown that GH therapy restores IGF-I and IGFBP-3 levels to normal (or close to it), although the rise in serum IGF-I precedes that in IGFBP-3 (22). In the course of treatment, there is a remarkably close correlation between IGFBP-3 and IGF-I levels (23), which as much reflects IGF-I regulation of IGFBP-3 as their common dependence on GH. It has also been shown that the dosages of GH required to normalize IGF-I levels are lower than those needed to restore IGFBP-3 and ALS levels (24). This means that GH could increase IGFBP-3 indirectly by stimulating IGF-I and ALS production. IGF-I then inducing IGFBP-3 synthesis.

Analysis of data for Laron syndrome patients may be tenuous in view of the often small numbers and the ages of the subjects, the differences in dosage administered and duration of treatment, and the different methodologies used to analyse IGFBP-3.

Input has been obtained from three major sources. The first concerned a group of Ecuadorian patients. In the initial, highly detailed study, six adult patients were treated for 7 days by subcutaneous injection of 80 μg recombinant IGF-I/kg per day. No significant changes were observed in serum IGFBP-3 levels as measured by RIA. Neither were there any noteworthy changes in half-life or metabolic clearance of IGF-I, but distribution volume increased, IGF-II levels dropping by almost 50% in the course of treatment (25). Despite the very low ALS levels, which did not vary during treatment, IGF-I was found to be equally distributed between the 40 kDa and 140 kDa complexes, whereas the relative proportions for IGF-II were 70 and 30% (26). This is an important point, because it demonstrates that in spite of the primary GH receptor deficiency, there was sufficient basal production of IGFBP-3 and ALS in the liver for 140 kDa complexes to be formed. Similarly, in GH-deficient children, as also in newborns in whom GH regulation of the IGF system is not yet functional, almost 50% of IGF-I and slightly less IGF-II elute from gel filtration in the 140 kDa material (27).

With IGF-I infusion in normal adults, there is a temporary rise in IGFBP-3 accompanied by increased IGFBP-2 as analysed by Western ligand blotting (WLB) and, as in the case of the Ecuadorian patients, a drop in serum IGFBP-3 as analysed by WLB rose significantly in four adults treated with the same dosages for 6 months, treatment (32). In a second study of two children and four adults treated with the same dosages for 6 months, serum IGFBP-3 as analysed by WLB an increase in the 42–39 kDa doublet characteristic of intact IGFBP-3 was observed within 4 h of IGF-I injection (35). Similarly, in two other patients, WLB in association with immunoblotting revealed an increase in the ratio of intact to proteolysed IGFBP-3 under IGF-I therapy, suggesting that an IGF-dependent mechanism regulates protease activity (36). We have confirmed the rapidity with which these changes occur in a case of LTD, where in WLB an increase in the 42–39 kDa doublet of intact IGFBP-3 was observed within 4 h of IGF-I injection (35).

More recent findings for 17 pre-pubertal Ecuadorian patients treated for 12 months with 120 μg IGF-I/kg per day have confirmed the lack of change in immunoreactive IGFBP-3 (30).

The second source of information was a study in international collaboration concerning 31 Laron syndrome patients who underwent 2 years of treatment at dosages of 80–240 μg IGF-I/kg per day. Again, RIA levels of IGFBP-3 were unchanged and IGF-II levels were depressed (31).

Thirdly, among the patients treated by Zvi Laron and his team, IGFBP-3 was measured by RIA in eight subjects receiving 120–150 μg IGF-I/kg per day over 7 days. The initially very low IGFBP-3 levels, as in all patients studied, on average dropped by 30% in response to treatment (32). In a second study of two children and four adults treated with the same dosages for 6 months, the RIAs currently available indiscriminately recognize both intact and proteolysed forms of IGFBP-3. It should be remembered that limited proteolysis of IGFBP-3 is a physiological phenomenon for which the regulatory pathways remain largely unknown. By reducing its affinity for the IGFs, and IGF-I in particular, IGFBP-3 proteolysis accelerates dissociation of the IGFs and enhances their bioavailability (33). Cotterill et al. were the first to note nycthemeral variations in IGFBP-3 levels as detected by WLB in two patients with Laron-type dwarfism (LTD) and attributed these to variations in protease activity (34). We have confirmed the rapidity with which these changes occur in a case of LTD, where in WLB an increase in the 42–39 kDa doublet characteristic of intact IGFBP-3 was observed within 4 h of IGF-I injection (35).

The second comment is that, in the single (and only reported) case where recombinant IGF-I administration provoked parallel increases in the concentrations of IGF-I, immunoreactive IGFBP-3 and ALS (6), all known data indicate that the rise in serum ALS could not result.
from increased synthesis which is controlled by GH. The elevated IGFBP-3 could be interpreted either in terms of IGF-I stimulation of its synthesis, or simply as a reflection of increased 140 kDa complex formation in the presence of additional IGF-I, which would prolong the half-lives of the three components in the serum. The same could apply to the other cases of LTD where IGFBP-3 levels were seen to rise in response to IGF-I treatment (7, 35, 36).

Thirdly, age of the subject and/or basal levels of IGFBP-3 also appear to play a role in the discrepancies observed during IGF-I therapy. In the single case above, the child presenting elevated immunoreactive IGFBP-3 was the one with the lowest basal levels before treatment (6). In the earlier report by the same team, where significant increases in IGFBP-3 were seen in the two children, but not in the four adults (7), IGFBP-3 was invisible in WLB before treatment in the children, but quite distinct in the adults. These aspects are to be borne in mind, especially when the phenotypic diversity of the Laron syndrome is becoming increasingly recognized and there is speculation that less severe forms of GH insensitivity may exist (37). In children with constitutionally short stature and GH levels that could be evocative of partial GH insensitivity, the increases in IGF-I and IGFBP-3 levels in response to GH are inversely correlated to corresponding basal levels (38).

Fourthly, the drop in immunoreactive IGFBP-3 early in IGF-I treatment in Laron’s eight patients (32) and in two of the three mentioned in this issue, in whom there was a concomitant drop in IGF-I and ALS (6), would initially appear more surprising. Baxter et al. (39) also reported an approximately 20% decrease in IGFBP-3 levels in healthy adults injected with 100 μg IGF-I/kg per day for 7 days, accompanied by a 30% drop in ALS levels. This could be attributable to negative feedback by IGF-I on GH secretion, which, however, could not apply to patients with GH receptor deficiency (who do nevertheless have low GH levels after IGF-I administration). Another possible cause of this short-term effect of IGF-I treatment may be its influence on glomerular filtration rate, which has been observed in normal subjects (40), whose urine contains the various IGFBP species (41). In LTD patients, creatinine clearance is reduced, but with IGF-I therapy it increases towards normal (42). It therefore seems possible that IGF-I treatment may stimulate clearance of GH and IGFBP-3, thus reducing their plasma levels.

In analysing the variations in plasma concentrations of the various components of the IGF system in response to long-term IGF-I therapy and the discrepancies between different teams’ observations for Laron syndrome patients, it becomes clear that in most cases IGFBP-3 levels remain unchanged. This does not necessarily mean that hepatic synthesis of IGFBP-3 is controlled by GH as opposed to IGF-I. Considering the short half-lives of IGFBP-3 unassociated with the ALS (43) and of the 40 kDa IGFBP–IGF complexes (9), IGF-I treatment of LTD patients may stimulate IGFBP-3 production sufficiently to maintain, but in most cases not to increase, serum concentrations. This hypothesis would be compatible with recent data for monkeys subjected to IGF-I administration by constant subcutaneous infusion (300 μg/day) between the ages of 13 and 32 months. Endogenous GH secretion was depressed and IGFBP-3 levels rose, but not proportionally with IGF-I levels. The authors justifiably concluded that IGF-I may regulate IGFBP-3 synthesis but that GH may be required to maintain equimolar proportions of IGF-I to IGFBP-3 (44). The insufficient levels of serum IGFBP-3 could in fact be attributable to the deficient ALS resulting from inhibition of GH secretion.

This brief review would not be complete without mention of intra-uterine growth retardation and post-natal growth failure associated with deletion of the IGF-I gene (45), which fans the controversy as to regulation of circulating IGFBP-3. Here, GH secretion was enhanced, plasma concentrations of IGF-I not detected and IGFBP-3 and ALS levels normal. The authors considered that their findings support the notion that ‘these peptides are controlled independently of IGF-I in humans’. Nevertheless, they did not comment on the high serum IGF-II levels (1430 ng/ml as opposed to 1010 ng/ml in pooled control serum), which is intriguing, given that in acromegalics IGF-II levels remain within the normal range (46). This was the basis for the hypothesis that IGF-II is less GH-dependent than IGF-I, which is more easily understandable in the light of the ALS having preferential affinity for IGFBP-3–IGF-I complexes (29). In this patient, the increased GH secretion and absence of competition from IGF-I would promote formation exclusively of ALS–IGFBP-3–IGF-II complexes, which would account for the elevated IGF-II. Nevertheless, returning to acromegalics (2), one would expect increased IGFBP-3 levels, but this was not the case. In addition, IGFBP-3 levels failed to respond to the test of 4 days of GH administration, possibly because of the patient’s high endogenous GH levels. Nonetheless, these data would not support the notion of hepatic IGFBP-3 synthesis under direct control of GH. However, if IGFBP-3 production is normally regulated by IGF-I, the question arises of whether, in this particular patient, IGF-II could replace IGF-I in this role, although it evidently cannot substitute for IGF-I in regulating somatic growth.

The findings of Bar et al. (47) indicate that type 1 and 2 IGF receptors are present in the endothelium of blood vessels and that IGF-I and IGF-II are potent stimulators of a variety of metabolic and anabolic processes in endothelial cells. As mentioned above, IGF-I is capable of stimulating IGFBP-3 synthesis in these cells (11, 12). It would be interesting to know if IGF-II has this capacity too. Finally, although experimental data have demonstrated the major role that GH plays in controlling ALS synthesis in hepatocytes (12, 14), further
studies are to be awaited to confirm or refute the lack of GH effect on Kupffer and endothelial cells (12). The question still remains.

References


