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IGFBP-3

Functional and Structural Implications in Aging and Wasting Syndromes

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Over the last several years, the authors have studied the relationship of insulin-like growth factors (IGFs) and the insulin-like growth factor binding proteins (IGFBPs) in the circulation in a number of clinical settings. Patterns have emerged that seem to be characteristic of various conditions. In aging, there are marked decreases in IGF-I and -II, normal levels of IGFBP-3, and marked increases in IGFBP-1 in serum. Using ligand blotting and an IGFBP-3 proteolysis assay, BP-3 is intact. Based on native gel electrophoresis, IGFBP-1 is in its most highly phosphorylated state in those elders who have high IGFBP-1 levels. This pattern is slightly different in catabolic conditions such as AIDS (wasting in adults; failure to thrive in children), uncontrolled diabetes mellitus, trauma, and severe burns. In these conditions, serum levels of IGF-I and -II are markedly diminished, IGFBP-3 levels are also decreased, and IGFBP-1 levels are markedly increased. In addition, there is increased proteolysis of IGFBP-3 (AIDS failure to thrive, uncontrolled diabetes mellitus) and disruption of the ternary complex with decreased levels of ALS (AIDS wasting and burns). IGFBP-1 is in its most highly phosphorylated state in all catabolic conditions studied. Thus, the alterations in the circulating levels of IGFs and the changes in the physical state of the IGFBPs may lead to decreased anabolic activity and be a part of the mechanism of increased catabolism and wasting.

Key Words: IGFBP-3; aging; wasting.

Introduction

The majority of the insulin-like growth factors (IGFs -I and -II) in circulation are carried by a large ternary complex, 150 kDa *(1)*. This ternary complex consists of insulin-like growth factor binding protein (IGFBP)-3, an acid-labile subunit (ALS) and the IGFs. When the IGFs are bound to this complex they do not leave the vascular compartment and their half-lives are prolonged to 12–15 h *(2)*. Other IGFBPs found in appreciable quantities in circulation are IGFBP-1, -2, and -4 *(1)*. These smaller IGFBPs are largely unoccupied or not completely saturated *(2)* and are capable of crossing intact endothelial barriers.

The IGFBPs have several major functions that impact on the biological activities of the IGFs. They act as transport proteins in plasma and control the efflux of IGFs from the vascular space; they prolong the half-lives of IGFs and regulate their metabolic clearance; they provide a means of tissue-specific localization, and are able to directly modulate interaction of the IGFs with their receptors *(1)*. The exact mechanism of how these functions are carried out is not entirely clear. In the case of the large store of IGFs in the ternary complex, it is thought that this is a functional pool that is readily accessible to the organism during periods of increased need such as stress and growth. The identification of several serum proteases for IGFBP-3 *(3–5)*, some which are active during stress *(6)*, would be consistent with this concept. These proteolytic fragments have much less affinity for IGF-I, thus theoretically making more peptide available to interact with target tissues.

Post-translational modification of the IGFBPs, such as phosphorylation, can increase the binding affinity of the IGFBPs. This has been demonstrated for IGFBP-1 *(7)*. When IGFBP-1 is phosphorylated it has a sevenfold higher affinity for IGF-I, which is greater than the affinity of IGF-I for the IGF-I receptor *(8)*. This could potentially limit the access of IGF-I to the target tissues, thus inhibiting IGF-I action *(9)*.

In light of this work, the authors' laboratory has assessed IGFs, IGFBPs, and the physical state of the IGFBPs in a number of wasting syndromes. They have reported marked alterations in the IGF system that may be a part of the etiology of the metabolic derangements that occur. These data will be reviewed in this article and contrasted to the changes seen in aging.

Aging

It has been well-documented that GH secretion declines with age *(10)*. It begins in the fourth decade of life and shows

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an incremental decrease with each decade thereafter *(11)*. At age 70–80 yr, approx 50% of all subjects have no significant serum immunoreactive GH around the clock. It is well accepted that the decline in GH secretion is part of the etiology of the alterations in the IGFs and IGFBPs that are associated with aging *(12)*.

Changes in IGF Levels

The authors have studied two groups of elderly adults. One group ($n = 115$) was fully ambulatory, living at home with no disabilities *(13)*. The age range was 60–88 yr, mean age 74 yr. Thirty-three of these elders were on antihypertensive therapy, the remainder of the group were on no medications. In this population, IGF-I (130 ± 6 ng/mL, mean ± SE) and IGF-II (301 ± 12 ng/mL) serum levels were significantly ($P < 0.01$) lower when compared to young adults ($n = 20$), aged 20–40 yr (IGF-I 254 ± 17 ng/mL; IGF-II 432 ± 11 ng/mL). Forty percent of these individuals had IGF-I levels less than 100 ng/mL.

The second group of elderly adults ($n = 105$), ranged in age from 65–94 yr, with a mean age of 78 yr *(14)*. This group was considered frail based on decreased muscle strength as measured by isokinetic dynamometry and significant musculoskeletal impairment as measured by Guralnik short physical performance battery *(15,16)*. IGF-I levels were lower in the frail group (93 ± 4 ng/mL) as compared to the healthy elderly (130 ± 6 ng/mL).

The differences in IGF-I between the two groups is striking. The cause for this is unclear. None of the women in either group were on estrogen replacement therapy. All subjects had normal thyroid function and there was no evidence of obvious malnutrition. The sex distribution in both groups was similar, with greater than 50% women. There is evidence in young adults that IGF-I levels may be correlated with physical activity, independent of age *(17,18)*. However, this association appears to be less convincing in the elderly. In a study of elderly men (70–94 yr), there was no association of IGF-I levels to functional status independent of age *(19)*. It is not clear if this is the same in women. Thus, other factors may be involved in the observed differences in the two groups.

Changes in IGFBP Levels

In the elderly adults studied, IGFBP-3 serum levels were not diminished when compared to control populations. In the healthy elderly, serum levels were 3.7 ± 0.25 mg/L vs 4 ± 0.34 mg/L in young adults. There was, however, a significant ($P = 0.05$) positive correlation between IGF-I and IGFBP-3 levels in the elderly ($r = 0.227$).

Ligand blotting of sera from the elderly adults demonstrated an intact doublet at 39–41 kDa representing IGFBP-3, which was no different in intensity from young adults. In addition, there was no evidence of IGFBP-3 proteolysis using a IGFBP-3 protease assay (Fig. 1).

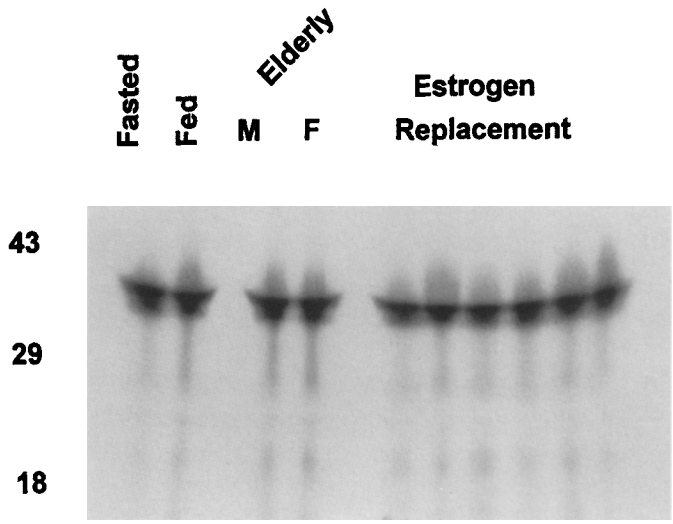

Fig. 1. IGFBP-3 proteolysis was measured by incubating [^{125}I] IGFBP-3 (30,000 cpm) with 6 µL serum in TRIS buffer for 6 h at 37°C. The reaction was stopped by addition of sample buffer. The reaction products were separated by SDS-PAGE. [^{125}I] IGFBP-3 proteolysis in control sera (lanes 1 and 2) were compared to elderly adults (lanes 3 + 4) and elderly women on estrogen replacement therapy (lanes 5–9). There was no evidence of IGFBP-3 proteolysis in any of the samples. The positions of the molecular mass markers in kilodaltons are noted in left margin and correspond to: 44, ovalalbumin; 29, carbonic anhydrase.

These data are different from several other studies, reviewed by Corpas et al. *(12)*, showing decreases in IGFBP-3 serum levels with advancing age. The decreases in IGFBP-3 are not very great, varying 20–30% lower than young adults, and are certainly not as dramatic as the changes seen in IGF levels. The discrepancies in the data may reflect differences in assays techniques and /or the population of adults studied.

In contrast to IGFBP-3, IGFBP-1 levels were markedly elevated in the healthy elderly (48 ± 6 ng/mL) as compared to young adults (2.6 ± 0.8 ng/mL). Based on native gel electrophoresis in the elderly with elevated IGFBP-1 levels, IGFBP-1 was in its most highly phosphorylated form. A similar elevation in IGFBP-1 in the elderly was also reported by Rutanen and colleagues *(20)*. They showed that this increase was related to the insulin resistance in this age group.

The diminished levels of IGFs in the elderly coupled with normal circulating levels of IGFBP-3 would result in a decrease in the molar ratio of IGF-I/IGFBP-3, suggesting less free IGF-I in circulation *(21)*. In addition, the increased levels of phosphorylated IGFBP-1, a potent inhibitor of IGF action *(8,9)*, would further limit access of IGFs to the IGF receptor in target tissues. These changes may in part be responsible for the decreases in lean body mass, increased adipostiy, and impaired immune function that occur with aging *(12,13)*.

Catabolic (Wasting) Clinical Syndromes

The authors have studied a variety of clinical conditions (AIDS wasting, trauma, severe burns, and uncontrolled diabetes mellitus), which are characterized by alterations in muscle protein synthesis, loss of lean body mass, weight loss, and, in children, failure to grow. All these conditions have significant alterations in the IGF system that may contribute to the catabolic state.

Changes in IGF Levels

In all conditions studied, IGF-I levels were markedly diminished ranging from a 55–70% reduction from control values *(22–26)*. The reduction in IGF-II was much less dramatic averaging 30–50% below healthy adult levels. In the trauma patients, IGF-I levels remained decreased by 30–40% throughout their hospital stay, despite normal levels of stress hormones and adequate nutritional intake *(24)*. This was similar to two of three patients with AIDS wasting who were followed longitudinally over 25 mo *(22)*. These two patients showed IGF-I levels that were 50% of healthy controls. Therefore, the GH/IGF axis may take a prolonged period of time to recover in these patients.

Changes in IGFBP Levels

Circulating levels of IGFBP-3 either by ligand blotting (AIDS wasting, burns, trauma) or direct measurement of serum levels (uncontrolled diabetes mellitus) were diminished *(22–26)*. In adult patients with AIDS wasting *(22)* and children with growth failure secondary to either AIDS *(23)* or uncontrolled diabetes mellitus *(26)* there was significant proteolysis of IGFBP-3 based on Western immunoblotting and an IGFBP-3 protease assay. In the adult patients with AIDS wasting, the level of proteolysis was not correlated with the loss of body weight, unlike what was observed in the children.

There was also decreased formation of the ternary complex in these patients. In the patients with AIDS wasting, burns, and uncontrolled diabetes mellitus, the levels of ALS were also markedly diminished *(22–24)*. In the patients with AIDS wasting the ability to form the ternary complex was correlated with the levels of IGF-I, i.e., those patients with higher IGF-I levels had normal complex formation. In this population, the patients who had wasting had the poorest ability to form the ternary complex based on nondenaturing gel electrophoresis (Fig. 2) *(22)*.

Insulin therapy in the children with uncontrolled diabetes mellitus was able to decrease IGFBP-3 proteolysis *(27)* and restore ALS levels to normal *(28)*. It also increased IGF-I levels to that of control subjects *(25)*. In the burn patients, insulin alone was not able to increase ALS or IGF-I levels in the circulation *(26)*. However, insulin plus growth hormone therapy restored both ALS and IGF-I

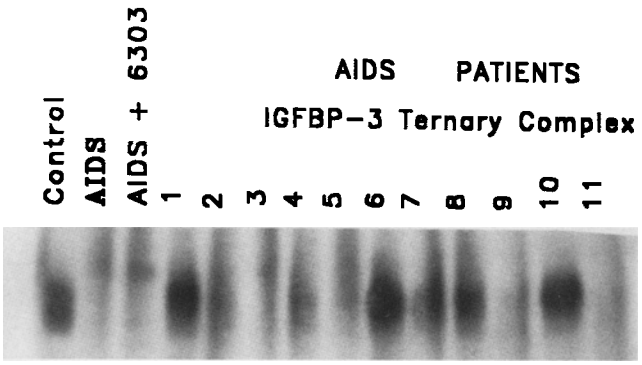

Fig. 2. IGFBP-3 ternary complex formation was compared in 11 AIDS patients by incubation of serum with ^{125}I-IGF-II followed by nondenaturing PAGE. Lane 1 (control sera), lane 2 (AIDS patient sera), lane 3 (AIDS patient sera + anti-BP-1), lanes 4–13 (AIDS patient sera). IGFBP-3 complex formation was reduced in patients 2–5, 7, 9, and 11 with the lowest IGF levels.

levels to values observed in healthy controls *(25)*, suggesting that IGF-I may be an important regulator of ALS (Fig. 3).

Another common feature to all these conditions, is a marked increase in IGFBP-1 levels *(22–26)*. Based on native gel electrophoresis, IGFBP-1 is also in its most highly phosphorylated form. Again, insulin therapy in uncontrolled diabetes mellitus decreased IGFBP-1 levels to basal values *(24)*. Insulin also decreased IGFBP-1 levels in the burn patients by 50%, but this was still fourfold higher than in control subjects *(26)*. This observation is consistent with the known severe insulin resistance that occurs in burn patients *(27)*.

The alterations in the IGF system seen in these catabolic conditions may indeed be part of the etiology of the abnormal metabolism and loss of lean body mass that is observed. The diminished levels of IGFs plus decreased formation of the ternary complex and markedly elevated IGFBP-1 levels would result in increased clearance of IGFs from circulation and decreased action of IGFs at the target tissue level.

Summary

In both aging and the wasting syndromes studied, IGF-I is markedly diminished and IGFBP-1 is elevated. Unlike aging, in wasting conditions, the ternary complex is abnormal with increased IGFBP-3 proteolysis and decreased levels of ALS. IGF-II levels are also decreased to a greater extent in wasting than is observed in aging; this probably relates to the mechanisms operating to bring about these changes, which may be secondary to the severity of the insult or disease process.

In the elderly, GH secretion is low *(8–12)*; there is mild insulin resistance *(20)* and perhaps abnormal cortisol metabolism *(29)*. In the patients with wasting and trauma, there is a GH resistant state *(24,26,30)* with increased GH

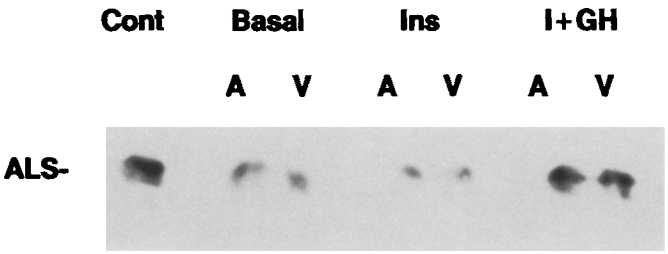

Fig. 3. A representative Western immunoblot of ALS in serum from control subjects and burn patients under basal conditions, after infusion of insulin, and after the administration of insulin plus GH. Note the diminished ALS in burn patients, which remains low despite the administration of insulin but is restored with insulin and GH.

levels, severe insulin resistance *(22,31)* and hypercortisolism *(22)*. These hormonal alterations may be a part of the same process, which becomes more abnormal as the individual becomes more debilitated. Thus, in these wasting syndromes, the ternary complex may be the index for the severity of the illness, i.e., as the individual becomes more compromised, IGF levels continue to diminish, and then there is disruption of the ternary complex with IGFBP-3 proteolysis and decreased levels of ALS. This would imply that the "functional" pool of IGFs carried in the ternary complex is an important protective mechanism for the individual.

The changes in the hormonal environmental and the IGF system, seen in the wasting syndromes may be a result of proinflammatory cytokines. The authors have reported that in rats treated with proinflammatory cytokines such as tumor necrosis factor alpha (TNF α) and interleukin-1β, there are marked decreases in IGF-I and increased levels of IGFBP-1 *(32,33)*. In addition, TNF α can stimulate IGFBP-1 production directly in a human hepatoma cell line and make these cells resistant to the dominant negative effect that insulin has on IGFBP-1 expression *(34)*. In the rats treated with these proinflammatory cytokines, IGFBP-1 levels are also increased in peripheral tissues such as skeletal muscle *(35)*. This suggests that IGFBP-1 can exit the vasculature rapidly and localize to specific tissues. In this model of sepsis, there was a positive linear correlation between IGF-I content and the rate of protein synthesis in skeletal muscle *(35)*. This suggests that in conditions where IGF-I levels are diminished, there are reduced amounts of the peptide to interact with target tissues in addition to dramatic changes in IGFBPs, which together may result in disruption of anabolic processes that conserve muscle protein in healthy individuals.

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