Controlling Insulin-Like Growth Factor Activity and the Modulation of Insulin-Like Growth Factor Binding Protein and Receptor Binding

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ABSTRACT

The insulin-like growth factors (IGF) and insulin perform seemingly unique roles by causing the same metabolic effect: cellular hypertrophy. Although overlapping, there are different consequences to cellular hypertrophy induced by IGF and that induced by insulin. The IGF enhance the cell hypertrophy that is requisite for cell survival, hyperplasia, and differentiation, and insulin enhances cell hypertrophy primarily as a means to increase nutrient stores. The effects of IGF and insulin are controlled by the segregation of their receptors between different cell types. A model is discussed that describes the need for three hormones (IGF-I, IGF-II, and insulin) to control nutrient partitioning. Insulin receptor localization, as well as an episodic mode of secretion, evolved to perform the short-term action of clearing excess nutrients from the circulation. In contrast, a complex and interactive set of factors ensure that maximal IGF activity occurs only when conditions are optimal for growth. A relatively invariant rate of secretion and the IGF binding proteins serve to maintain a large mutable pool of IGF. This pool exists to ensure a constant supply of IGF to maintain the basal metabolic rate and to ensure that, once a cell begins to proliferate or differentiate, adequate exposure is available to complete the process even after severe short-term physiological insults. The IGF concentrations only change in response to prolonged differences in protein and energy availabilities, environmental and body temperatures, and external stress. Also, evidence is now emerging that describes a discrete role for trace nutrients in the regulation of IGF activity. In this latter regard, zinc has the notable role of targeting IGF binding proteins to the cell surface. New data are presented showing that zinc also changes the affinity of the type 1 IGF receptor and cell-associated IGF binding proteins to optimize IGF activity.

(Key words: insulin-like growth factor, binding protein, receptor, zinc)

Abbreviation key: BMR = basal metabolic rate, GH = growth hormone, IGFBP = IGF binding protein.

THE IGF SUPERFAMILY

Members of the IGF superfamily provide the ability to partition the use of nutrients. During the development of the IGF superfamily, the first duplication of an ancestral gene resulted in the divergence of relaxin from the proto-IGF gene. Prior to the evolution of vertebrates, a similar event separated insulin from the IGF (89), and then, prior to the appearance of mammals, a third branching separated the genes for IGF-I and IGF-II. The primitive chordate Amphioxus sp. makes a single peptide that has 50% homology to IGF-I, IGF-II, and insulin, but that peptide has the domain structure of the IGF, not insulin (15), indicating that an IGF-like peptide was the first to evolve.

Characteristics of the IGF superfamily are compared in Table 1. In addition to the lack of C and D domains in the mature insulin molecule, making the IGF approximately 50% larger, the three peptides are closely related. Three cystine bonds stabilize very similar tertiary structures (9). The histidines in insulin are required for the formation of a zinc-stabilized hexamer in secretory vesicles, which does not occur for the IGF. All three hormones cause similar intracellular effects because the type 1 IGF receptor (αβαω), the insulin receptor (α′β′β′αω), and the type 1 IGF-insulin hybrid receptor (αβωα) are similar in structure and share intracellular signaling cascades (87).

Such closely related hormones may exist as a means to enhance cellular responsiveness because both IGF and insulin could stimulate the metabolism of the same cell if both receptors were present (75). However, these hormones probably evolved to simulate the metabolism of different populations of cells. Because of segregation in the location of IGF and...
insulin receptors, the members of the IGF family took on distinct yet overlapping roles to partition nutrient utilization. Consumed nutrients can be used for basal metabolism, hyperplastic growth, differentiation, or storage. These four functions are listed by their probable evolutionary development (i.e., organisms had to survive (maintain metabolism) and multiply (hyperplasia) before specialized tissues evolved (differentiation) to store nutrients). The more primitive needs, such as basal metabolic rate (BMR), hyperplasia, and differentiation, require IGF-like actions and the latter (storage) an insulin effect. For optimal function, these processes should be controlled separately. Metabolic rate must be maintained for survival, and a continuous stimulus must be present to maintain metabolism. Hyperplastic growth and cell differentiation should not occur during periods of nutrient deficiency or environmental stress (physiological insults) as these processes would only intensify the predicament. Metabolism must also be controlled such that nutrients are stored only in times of plenty.

It has been long recognized that members of the IGF family stimulate cell hypertrophy (i.e., elevated nutrient transport, protein synthesis, and RNA synthesis) (90). The big difference among the three peptides is the consequence of their actions (Table 1): either size, hyperplasia, differentiation, or survival. Type 1 IGF receptors are generally found on cells that are capable of proliferation and differentiation. Insulin receptors are found on cells that are responsible for nutrient storage. The link between the four consequences is the requirement of hypertrophic events for all of these processes.

**Separate Hormones, Separate Functions**

**IGF driven hypertrophic growth, a prerequisite for hyperplasia and differentiation.** The physiological difference between insulin and the IGF results primarily from the localization of their respective receptors rather than from divergent signaling pathways. Among others, preadipocytes, myoblasts, chondrocytes, primitive mesenchymal cells, fibroblasts, endothelial cells, and activated or dividing hepatocytes all respond to IGF-I (31, 43). The cells respond because of the presence of type 1 IGF receptors. Many, if not most, proliferative competent cells have more type 1 IGF receptors than insulin receptors. Several of these cell types have the capacity to differentiate into functional specific cell types, and the IGF are required to drive differentiation (7, 30, 46, 51, 52, 83). Terminal differentiation and quiescence of cells result in a decreased number or loss of type 1 IGF receptors (50), an effect that can be reversed in quiescent but not terminally differentiated cells by competence factor stimulation (74). Insulin-like growth factors are required for proliferation and differentiation because of the need for cell hypertrophy prior to mitosis (synthesis of cell cycle specific mRNA and proteins to maintain cell size following mitosis) and during the development of tissue-specific functions (synthesis of differentiation-specific mRNA and proteins). However, the IGF pro-

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**TABLE 1. Characteristics of three members of the IGF superfamily.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IGF-I</th>
<th>IGF-II</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acids (mature)</td>
<td>70</td>
<td>67</td>
<td>51</td>
</tr>
<tr>
<td>Homology (A &amp; B domains)</td>
<td>43%</td>
<td>74%</td>
<td>41%</td>
</tr>
<tr>
<td>Cystine bonds</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>C Peptide</td>
<td>Intact</td>
<td>Intact</td>
<td>Removed</td>
</tr>
<tr>
<td>D Region</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Histidines (Zn binding)</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellular action</td>
<td>↑ Hypertrophy</td>
<td>↑ Hypertrophy</td>
<td>↑ Hypertrophy</td>
</tr>
<tr>
<td>Consequence</td>
<td>↑ Size</td>
<td>↑ Size</td>
<td>↑↑↑ Size</td>
</tr>
<tr>
<td></td>
<td>↑↑ Hyperplasia</td>
<td>↑↑ Hyperplasia</td>
<td>Hyperplasia</td>
</tr>
<tr>
<td></td>
<td>↑↑ Differentiation</td>
<td>↑↑ Differentiation</td>
<td>Differentiation</td>
</tr>
<tr>
<td></td>
<td>↑ Survival</td>
<td>↑ Survival</td>
<td>↑ Survival</td>
</tr>
</tbody>
</table>

**Receptors**

<table>
<thead>
<tr>
<th>Name</th>
<th>Type 1 IGF</th>
<th>IGF - Ins hybrid</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure, ~ size</td>
<td>αββα, 370 kDa</td>
<td>αββα', 370 kDa</td>
<td>αββα', 370 kDa</td>
</tr>
<tr>
<td>Ligand preference</td>
<td>I &gt; II &gt;&gt; Ins</td>
<td>I &gt; Ins ? II</td>
<td>Ins &gt;&gt; II &gt; I</td>
</tr>
</tbody>
</table>

*1The type 1 IGF-insulin hybrid receptor (αββα'), for lack of a better location in the table, is listed under IGF-II but is not the type 2 IGF receptor. An IGF-mediated function of the type 2 IGF receptor has not been clearly defined and is not included in the table. I = IGF-I, II = IGF-II, and Ins = Insulin.*
mote but do not control the proliferative or differentiation processes. Cell fate is determined by regulatory factors: competence factors, differentiation-inducing factors, and differentiation-inhibiting factors.

That the consequences of IGF action are controlled by regulatory factors is eloquently illustrated using skeletal myoblasts. Myoblasts either proliferate or differentiate in response to the IGF. The IGF determine the rate, not the fate. For example, L6A1 cells exposed to serum express intermediate levels of the members of the mitogen-activated protein (MAP) kinase intracellular signaling pathway (23). The addition of IGF-I increases signaling by elevating the expression of proteins involved in the MAP kinase pathway, and the cells proliferate. However, 24 h after serum withdrawal, the cells have a markedly diminished ability to proliferate in response to the IGF, and levels of the proteins involved in the MAP kinase signaling pathway have diminished. Proteins involved in the phosphatidylinositol-3-kinase signaling pathway are extremely low in cells exposed to serum but increase following serum withdrawal. After serum withdrawal, IGF-I increases the levels of the proteins involved in the phosphatidylinositol-3-kinase pathway inducing differentiation, and IGF-I accelerates differentiation rather than proliferation of these cells. These data indicate that the IGF enhance both cell proliferation and differentiation but do not control the fate of cells. In this case, cell fate is determined by the presence or absence of serum-derived factors; IGF-I only amplifies the rate at which the cells proceed toward that fate by stimulating nutrient transport, mRNA synthesis, and protein synthesis (i.e., hypertrophy) via the type 1 IGF receptor that is present on proliferating myoblasts and at the onset of myoblast differentiation (29). Type 1 IGF receptors are lost during myoblast differentiation with the concurrent accretion of insulin receptors as the cells terminally withdraw and take on new roles as contractile cells and protein depots (6, 48, 82).

**Insulin ability to drive hypertrophic growth for nutrient storage.** Through evolutionary pressure, it became advantageous to store nutrients during times of plenty for use in later times of scarcity. The major actions of insulin are to increase protein, lipid, glycogen, and mineral stores (i.e., cause cellular hypertrophy). Adipocytes, the major source of lipid synthesis in pigs and ruminants and the major site of lipid stores for all domestic species (= 85% body lipid), have abundant insulin receptors and few, if any, type 1 IGF receptors (26). Liver, the major site for gluconeogenesis and glycogen storage for most species and the major sources of lipid synthesis for humans and chickens again have abundant insulin receptors but few if any type 1 IGF receptors (35). Skeletal muscle myofibers, the major reserve of mobilizable protein, have more insulin receptors than do type 1 IGF receptors (42). Finally, osteoblasts and osteoclasts, which are responsible for mineral stores, have numerous insulin receptors (37). Insulin acts in a very acute and sensitive manner to increase the respective storage capacities of fat, liver, muscle, and bone. Insulin-induced storage can be reversed, and these stores are mobilized when insulin secretion is low. For example, fasting or uncontrolled insulin-dependent diabetes causes muscle wasting, osteoporosis, and loss of adipose stores, but elevated rates of insulin secretion cause obesity (36, 60).

Nutrient stores are not maintained by heightened signaling through two receptor types, but by the cell type-specific localization of the insulin receptor. In many cases, insulin receptors are abundant on cells that are incapable of division, and, thus, the receptor is not mitogenic (i.e., adipocytes, myofibers, and fully differentiated hepatocytes). If insulin receptors are present or are artificially expressed on proliferative competent cells (39, 62, 71, 78), then the receptor acts similar to the type 1 IGF receptor (inducing cell hypertrophy of competent cells with resultant hyperplasia). The functions of IGF and insulin are interactive, and several cell types contain both type 1 IGF and insulin receptors. A good example would be bone cells, which retain their ability to proliferate throughout life but are also responsible for maintaining and mobilizing mineral stores and, thus, are responsive to both IGF-I and insulin (81).

**Secretion to Meet Separate Functions**

If the concept of separate function for the IGF superfamily is correct, insulin secretion should be regulated to optimize nutrient storage. Indeed, the volume of literature in this area is enormous. Pancreatic β-cells monitor nutrient levels to increase storage as an adaptation to episodic feeding. Insulin secretion markedly increases following the consumption of a meal, primarily in response to elevated blood glucose concentrations (60). Insulin is rapidly cleared from the circulation because storage of excess nutrients occurs rapidly. Once excess ingested nutrients are removed from the circulation, basal concentrations of glucose and amino acids are available to fuel IGF action. The secretion of IGF does not increase with excess intake (i.e., no spike in IGF concentrations occur following a meal). There is no advantage from such an event because the cells that are responsible for storing excess energy are not very responsive to the IGF.
If the function of IGF-I is to promote hypertrophy of cells undergoing hyperplasia or differentiation, then IGF-I secretion must be controlled to optimize these functions. If IGF responsive cells began to use nutrients for hypertrophy in preparation for subsequent hyperplasia or differentiation during a physiological insult, this use would only exacerbate the problem. Thus, IGF-I secretion and action must be controlled by factors that monitor both nutrient availability and the environment. In addition, cell proliferation and differentiation are timely events. Nutrient transport into cells is increased in fewer than 15 min by IGF or insulin receptors; however, cells require continued stimulation (6 h to several days) to complete the cell cycle and the differentiation process (88). Thus, if IGF-I concentrations varied rapidly, as do insulin concentrations, cells would rarely complete either process. As discussed herein, IGF-I concentrations are controlled by four primary inputs, each of which monitor specific environmental or metabolic parameters (Figure 1) to ensure that cellular proliferation or differentiation does not begin unless conditions are optimal, and IGF-I levels change slowly to ensure adequate continued stimuli for cells already undergoing either of the events.

The growth hormone (GH)-dependent member of the IGF superfamily is IGF-I. Growth hormone secretion stimulates IGF-I secretion. Low GH concentration or low GH activity results in depressed IGF-I concentrations (86). Most important to this discussion therefore are the mechanisms that regulate GH activity. Growth hormone activity is primarily a function of protein intake as modified by concentrations of circulating free fatty acids and glucose (25, 36). The IGF-I secretory response to elevated GH is slow, taking several days to reach an apex. This slow response ensures that a continued source of protein and energy is present before IGF-I concentrations increase. Similarly, short-term diurnal changes in GH concentrations (e.g., nightly peaks) do not cause diurnal changes in IGF-I concentrations (4, 27, 34, 49). Thus, IGF-I secretion is highest only when the conditions that are favorable for growth are present for several days and when secretion remains high even if GH activity is rapidly depressed. Therefore, once GH-induced secretion begins, IGF-I is available long enough to ensure that cells that have begun to proliferate or differentiate have ample receptor stimuli to complete the process.

Concentrations of IGF-I are also conditionally insulin-dependent. Low insulin concentrations result in low IGF-I secretion, but elevated insulin does not enhance IGF-I secretion (84, 85). Because insulin monitors energy (glucose) availability, if energy intake is insufficient, IGF-I activity is suppressed, and hyperplastic growth and cell differentiation are depressed. Refeeding increases insulin secretion and, eventually, IGF-I secretion (18). A gradual (several days in humans) decrease or increase to changing insulin concentrations ensures that a short period of food deprivation (e.g., overnight fasting) or temporary realimentation (e.g., a meal) does not depress or prematurely enhance IGF activity, respectively.

Concentrations of IGF-I are also modified by the stress response largely in response to changes in glucocorticoid secretion. Normal glucocorticoid concentrations are required for optimal rates of IGF-I secretion, but elevated glucocorticoid concentrations depress IGF-I secretion and similarly depress body growth (66). This effect ensures that nutrients are not used for hyperplasia or differentiation unless a stress-free environment is maintained.

**Figure 1.** The regulation of IGF-I secretion. Many illustrations of this sort indicate that growth hormone (GH), insulin, thyroid hormone, and glucocorticoids act independently to regulate IGF-I secretion. However, when the actions of any one of these hormones is missing, IGF-I secretion is decreased independently of the status of the others. For example, GH does not stimulate IGF-I secretion in the absence of insulin (e.g., fasted animals). Although inhibitory at high concentrations, normal concentrations of glucocorticoids are required for optimal IGF-I secretion. All four hormones synergize to promote normal rates of IGF-I secretion. Alterations in any of the four hormones changes the responsiveness of the others. This phenomenon explains the inability of GH to stimulate IGF-I secretion during a number of physiological insults; IGF-I is only secreted at high levels when conditions favor hyperplastic growth. The secreted IGF-I can then either act locally as an autocrine or paracrine factor or be secreted and act in an endocrine fashion. The magnitude of each hormonal response to the monitored parameter changes under a variety of conditions such with age, pregnancy, puberty, and gender, resulting in altered IGF secretion during these physiological conditions. T3 = 3,5,3′-Triiodothyronine; T4 = thyroxine.
Concentrations of IGF-I are also dependent on temperature. The thyroid gland monitors body temperature and adjusts the BMR to heat or cool cells in response to changes in environmental temperature. Secretion of low amounts of thyroid hormone suppresses IGF-I secretion, and elevated thyroid hormone secretion increases IGF-I secretion (72). Synergistic with the direct effect of thyroid hormones, IGF-I concentrations enhance nutrient transport into cells to be used as fuel for heat production. This role is further evidenced by the finding that IGF-I expression by brown adipose tissue is enhanced by hypothermia (28). Because of the continued (albeit low) presence of type 1 IGF receptors on skeletal muscle, elevated IGF-I secretion would stimulate nutrient transport to support heat production by shivering. Thus, IGF-I secretion is regulated to maintain cell temperature, which is necessary for proliferation and differentiation.

All of these regulatory mechanisms clearly serve to control IGF-I secretion to ensure that rapid cellular hyperplasia and differentiation occur only in the absence of a physiological insult. Slow changes in IGF-I secretion ensure that once cells begin to proliferate or differentiate an IGF-I stimuli will be present long enough for cells to complete the process. Clearly, there is a need for multiple levels of regulation to control IGF-I secretion. However, IGF-II concentrations rarely change in parallel with changes in IGF-I concentrations. The reason for this difference has not been defined.

The secretion of IGF-II is usually high in the fetus (41). Concentrations of IGF-II decline to varying degrees after birth and vary little with environmental and nutritional insults. One of the more perplexing areas in the IGF field is a lack of a defined role for IGF-II after birth, although IGF-II is undeniably important in fetal development (43). The high concentrations of IGF-II in the fetal circulation are more than sufficient to promote IGF-I-like effects, enhancing cellular hyperplasia and differentiation, despite having half the affinity of IGF-I for the type 1 IGF receptor. However, there is evidence of a type 1 IGF receptor with high affinity for IGF-II (38) and evidence that IGF-II may act through the insulin receptor (62). Although little information exists, these receptors could have a role in mediating IGF-like effects of IGF-II during fetal development. The important point is that IGF-II levels in the circulation drive fetal development. There was no need for evolution to provide a detailed regulatory pattern for IGF-II secretion because the embryo and fetus attempt to grow, presuming that adequate nutrition (protein and energy) will be provided by the maternal circulation (or within the preformed yolk in egg-laying species) and that the mother will control the fetal environment (stress and temperature). The maternal ability to meet the nutrient needs of the embryo and to maintain environmental control depends on body condition and nutrient availability, although a signal to grow is always present in the fetal circulation in the form of IGF-II. Hence, fetal growth (hypertrophy for cell proliferation and differentiation) is propelled by IGF-II with little need for mechanisms to change the rate of IGF-II secretion.

What then is the role of invariant IGF-II secretion and basal IGF-I concentrations after birth? The IGF-I concentrations in postnatal blood decrease only by 50 to 75%, even with physiological insults such as severe nutrient deprivation, hypophysectomy, insulin-dependent diabetes, thyroidectomy, and high glucocorticoid concentrations. During these conditions, serum concentrations of IGF-I and IGF-II are still quite high (≈10⁻⁸ M) relative to those of most hormones (10⁻⁹ to 10⁻¹⁰ M), including insulin. I propose a role for this phenomenon. After birth, secretory rates of invariant IGF-II and basal IGF-I control nutrient utilization by cells to support nutrient transport, protein synthesis, and RNA synthesis to maintain the BMR of cells and, in doing so, to prevent apoptosis. It has become quite clear that several cell types die in vitro without IGF (3, 73), although no unique IGF signaling pathway exists that mediates cell survival (68). Type 1 IGF receptor signaling and possibly low insulin receptor signaling by the IGF probably maintain ongoing cellular hypertrophic events and maintain cell temperature for cell survival. This theory relates back to the ability of thyroid hormones and IGF-I to regulate BMR. Cell death because of inadequate IGF stimulation is probably a means to reverse the growth-promoting effects of the IGF just as nutrient mobilization is a means to reverse the growth effects of insulin.

Functional Regulation

The availability of insulin to cells is controlled by the rate of insulin secretion. This control does not exclude regulation of insulin action by changes in postreceptor signaling. Intracellular changes in sensitivity can result from the interaction of insulin-sensitive cells with a myriad of other hormones and regulatory factors. However, these changes are outside the scope of the current discussion. The action of IGF is also controlled by changes in receptor signaling and, as just discussed, altered secretion.

However, because of the importance of IGF effects for several critical functions that require a long-lived
stimulus for completion (cell survival, proliferation, and differentiation), it is not surprising that IGF concentrations are subjected to an additional level of regulation. A pool of IGF is maintained in the circulation. No such pool exists for insulin. At least seven IGF binding proteins (IGFBP) modulate IGF activity (64, 80). The endocrine role of the IGFBP is to ensure the presence of a labile pool of IGF. The function of the labile pool of IGF is to maintain temporarily the IGF delivery to target cells at a normal level when IGF-I secretion is compromised by a physiological insult (41). This mechanism, like the slow changes in IGF secretion, further ensures that transient insults do not drastically affect IGF actions in vivo and, further, ensures that cells requiring hyper trophy during the cell cycle or during differentiation have adequate metabolic stimulus to complete the process.

**Mobilizing the IGF pool.** The clearance of IGF from serum is controlled by the relative proportions of IGFBP. When animals are not stressed, most (>95%) of the IGF in the circulation is bound to IGFBP-3 as a trinary complex with an additional carrier, the acid-labile subunit. This trinary complex has 6 to 24 times longer (12 h) circulating half-lives than do binary complexes of IGF with either IGFBP-1 or IGFBP-2 (0.5 to 2 h) (43, 57). Following a physiological insult, the ratio of the IGFBP-3 acid labile subunit to IGFBP-1 plus IGFBP-2 slowly decreases in serum. This decrease occurs in response to either depressed GH activity (low protein intake), low insulin secretion (low energy intake), elevated glucocorticoids (stress) or hypothyroidism (altered temperature). Thus, the same factors that regulate IGF secretion are needed to keep this ratio high and to maintain the large pool of IGFBP-3 and acid labile subunit to form the trinary complex with the IGF.

An increase in the half-life of IGF in the circulation was originally postulated as being a means to inhibit IGF activity, but IGF have the greatest half-life when IGFBP-3 acid labile subunit levels are highest, and the trinary complex is most abundant when growth rate is highest. However, this ratio and the IGF half-life are the highest when growth rate is highest and the rate of IGF-I secretion (liver mRNA expression) is also at its peak. Thus, even though the pool is large, the flux of IGF through the circulating pool is greatest. Thus, the growth rate is proportional to the flux of IGF through the pool, not to the pool size (41).

Following a physiological insult, an increase in the ratio of IGFBP-1 plus IGFBP-2 to IGFBP-3 and a decrease in the acid labile subunit help to mobilize the circulating pool and thus temporarily maintain metabolism even while IGF-I secretion is decreasing. Proteolytic cleavage of the IGFBP may also act to increase further the IGF clearance from the circulating pool following a physiological insult (33). Proteolysis of IGFBP could act to free IGF. Free IGF are very rapidly cleared from the circulation to target cells (10-min half-life). Ironically, the IGFBP may be susceptible to extracellular proteolysis and not the IGF, as originally proposed; in addition, the IGF probably control IGFBP proteolysis (12). It should be realized, however, that there is considerable evidence for IGFBP proteolysis in serum but not plasma. The physiological relevance of serum proteolysis is still undefined although serum proteolysis is important in wound healing. That controversy aside, maintaining a mutable IGF pool is the only undisputed role of circulating IGFBP, and, apparently, only binary IGF-IGFBP complexes leave the circulation (8). After leaving the circulation, the IGFBP within the extracellular fluids still retain the IGF as binary complexes, as evidenced by the presence of such in lymphatic fluids. As discussed herein, the strength and location of this binary complex in extracellular fluids determine the activity of the associated IGF.

**IGFBP inhibitory mechanism.** The IGFBP, type 1 IGF receptor, and type 2 IGF receptor bind IGF-I at distinct sites. No evidence exists that either IGF-I or IGF-II can bind to two sites simultaneously. Hence, when bound to an IGFBP in extracellular fluids, IGF activity is delayed. In vitro, soluble IGFBP bind the IGF and decrease IGF binding to cell surfaces (55, 56). The in vitro inhibitory effect of the IGFBP is further supported by greater activity of IGF-I mutants that cannot bind to IGFBP compared with the activity of native IGF-I (14, 67). A great deal of in vitro evidence exists that IGFBP in extracellular fluids delay IGF activity. The term “delay” is used here because statements referring to inhibition of IGF activity are often incorrectly interpreted to imply that activity is prevented. In relatively short-term in vitro studies, IGFBP decrease the measured IGF response by delaying IGF activity. In vivo, the IGF are delayed but they will eventually reach and bind to cells within target tissues. Under nonstressed conditions, the rate at which IGF reach the cell surface is directly proportionate to the rate of IGF secretion (i.e., the rate of flux through the extracellular pool when pool size is not changing) (41). The forthcoming response then depends on cellular sensitivity to receptor activation. If the IGFBP prevented IGF access to cells and blocked IGF activity in vivo, IGF would continually accumulate in extracellular fluids because the IGF are apparently only inactivated by intracellular degradation (2, 32, 47). Continuous accumulation does not occur in vivo despite the presence of IGFBP.
IGFBP enhancing mechanism. In vitro, IGFBP can also expedite the activity of either IGF-I or IGF-II. The mechanism is not clearly defined, but appears to be a result of increased binding of the IGF-IGFBP binary complex to cell surfaces. The IGFBP-1, -2, -3, -5, and -6 have been shown to expedite the activity of either IGF-I or IGF-II (10, 13, 17, 54). In contrast, only the delaying effects of IGFBP-4 have been demonstrated. Our work was the first to show that the ability of the IGFBP to expedite IGF activity is related to their ability to adhere to cell surfaces (10, 55, 56); IGFBP binding to cell surfaces has been confirmed by several laboratories (20, 21, 24, 40, 53, 54, 65). The only IGFBP that has not been shown to bind to cell surfaces is IGFBP-4. Several studies (1, 16, 22, 61, 79) have now measured both IGFBP binding to cell surfaces and IGF activity. In all cases, IGFBP adherence to cells is proportionate to IGF-enhancing activity. The results indicate that, in a stationary system during a brief in vitro experiment, IGF have a greater access to the type 1 IGF receptor if IGFBP are present. Thus, IGFBP have the potential to expedite the IGF delivery process and possibly to target activity.

In addition to delivering IGF to the cell surface, the IGF-IGFBP binary complex must dissociate before the IGF can bind to receptors and activate target cells. The first report showing that cell-associated IGFBP-3 has a lower affinity (10-fold) for IGF-I than does soluble IGFBP-3 (56) ushered in a new area of research. Indeed, IGFBP with lower IGF binding affinity do a better job of expediting IGF activity (1, 44, 45, 79). Together with the IGF-enhancing effect of cell-associated IGFBP, this result indicates that, as IGFBP adhere to cell surfaces, their affinity drops, IGF is released, and the pericellular concentrations of free IGF increase. This cascade increases the potential for the activation of type 1 IGF receptors and thus expedites IGF activity. This issue is critical in understanding the role of IGFBP.

Adherence of IGFBP to cell surfaces through a receptor or anchor implies that IGF activity can be targeted to specific cells dependent on the type or amount of IGFBP receptor. Because all secreted IGF eventually gains access to a target cell, this type of mechanism is the only way to explain how long-term IGFBP administration can alter metabolism (5) or how IGFBP transgenes can differentially affect body growth (70) or the growth of specific tissues (63, 70). That is, IGFBP do not affect overall IGF activity per se, but instead target the activity to different cell types; thus, the functional consequence of IGF-induced hypertrophy may change.

Functional localization. Because IGFBP modulate IGF activity by cell association, specific mechanisms within tissues must exist that regulate IGFBP adherence to cells. An additional or allied mechanism must also exist to release IGF from the IGFBP at the cell surface to activate the type 1 IGF receptor. Such a regulatory mechanism apparently evolved to prevent IGF-stimulated hypertrophy in the event of another type of physiological insult, trace nutrient deficiency.

The ability of IGFBP-1 and IGFBP-2 to expedite IGF activity requires a serum-derived factor (10). At first it was presumed that the component was a growth factor, presumably peptide in nature. However, the ability to expedite activity was directly related to the ability of these IGFBP to adhere to cell surfaces (10, 19). Thus, this activity could result from any stimulus that could target IGF-IGFBP binary complexes to the cell surface. All media used in these in vitro experiments contained Ca2+ and Mg2+ but lacked other trace nutrients (multivalent cations), including Zn2+, Mn2+, Se3+, and Cr3+. These and other trace nutrients are necessary for cell survival. During subculturing prior to the growth assays, cells obtain trace nutrients from the serum added to the medium. When serum was absent during a growth assay, the IGFBP were unable to potentiate IGF activity. This result led to our hypothesis that trace nutrients may be involved in regulating IGF activity via the IGFBP.

Recently, multivalent cations were found (58) to control the adherence of IGFBP to cell surfaces. Both Zn2+ and La3+ (but not Mn2+) retain specific IGFBP on cell surfaces: IGFBP-3 on human GM-10 fibroblasts, IGFBP-3 on bovine MDBK kidney epithelial cells, and IGFBP-5 on human T98G glioblastoma cells (58). Retention of IGFBP-4 and IGFBP-2 is unaffected. Thus, there is IGFBP and cation specificity to the action. Soluble IGFBP-3 and IGFBP-5 both had a 10- to 15-fold higher affinity than did cell-associated IGFBP, even in the presence of La3+. Interestingly, when IGFBP are retained on the cell surface, La3+ increased IGF-I binding to the cell surface. However, Zn2+ retained IGFBP on the cell surface but did not increase cell surface [125I]-labeled IGF-I binding, which indicated a possible Zn2+-induced depression in the affinity of cell-associated IGFBP. Overall, the data indicated that a trace nutrient, Zn2+, specifically targets IGFBP-3 and IGFBP-5 to the cell surface, which confirms the earlier finding of a striking difference in affinity between cell-associated and soluble IGFBP.

Studies were then conducted to determine whether Zn2+ and possibly other cations affected the binding
activity of IGF. Human type 1 IGF receptor-transfected P2A2-LISN muscle cells (69) were used to quantify IGF binding to the type 1 IGF receptor, human T98G glioblastoma cells were used to study IGF binding to cell-associated IGFBP-5 (12, 58), and human GM-10 fibroblasts were used to study IGF binding to a combination of cell-associated IGFBP-3 and -5. The presence of Zn\textsuperscript{2+} and Cd\textsuperscript{2+} increased \( ^{[125]} \text{I}-\)labeled IGF-I binding to the type 1 IGF receptor, La\textsuperscript{3+} did not affect binding, and Au\textsuperscript{3+} decreased binding compared to control (59). In stark contrast, Zn\textsuperscript{2+}, Cd\textsuperscript{2+}, and Au\textsuperscript{3+} all decreased \( ^{[125]} \text{I}-\)labeled IGF-II binding to cell-associated IGFBP, but La\textsuperscript{3+} did not affect binding to cell-associated IGFBP (76, 77). Similar effects were found for IGFBP-5 on T98G cells and IGFBP-3/5 on GM-10 cells. Independent of the cell type, the affinity of the cell-associated IGFBP for IGF-II was twice that for IGF-I. In the absence of Zn\textsuperscript{2+}, the affinity of the cell-associated IGFBP (for IGF-I or IGF-II) was higher than that of the type 1 IGF receptor. However, in the presence of Zn\textsuperscript{2+}, the affinity of the type 1 IGF receptor for either IGF-I or IGF-II was equal to or slightly higher than the affinity of the cell-associated IGFBP for either ligand.

From these data, there appears to be a hierarchy in affinity: \( K_a \) rankings of soluble IGFBP >> cell-associated IGFBP > cell-associated IGFBP + Zn\textsuperscript{2+} = type 1 IGF receptor + Zn\textsuperscript{2+} > type 1 IGF receptor and affinity ratios of approximately 40:4:2:2:1, respectively. Binding favors the type 1 IGF receptor only in the presence of Zn\textsuperscript{2+} and when IGFBP are on the cell surface. Considering this information, it is easy to envision why IGFBP that remain in solution appear to delay IGF activity considering their affinity for IGF to be \( ≈ \) 40-fold that of the receptor in the absence of Zn\textsuperscript{2+}. A theoretical model for Zn\textsuperscript{2+} control of IGF access to cells is shown in Figure 2. Zinc can partition IGFBP and bound IGF to the cell surface and then, by altering ligand affinity, can partition the IGF to the receptor. These findings imply that low Zn\textsuperscript{2+} levels, such as the low serum concentrations that occur in Zn deficiency, would result in partitioning of IGF from the receptor to IGFBP and would therefore depress IGF activity. Also, during Zn\textsuperscript{2+} deficiency, IGFBP would come off cell surfaces, and the ability of IGFBP to target IGF activity could be altered. These changes could mediate the decrease in growth rate during Zn deficiency (11).

CONCLUSIONS

The IGF system is extremely complex. Now, with the possibility for discovering additional IGFBP, understanding the nuances of these important metabolic regulators is becoming even more difficult. It should be clear that the IGF and insulin have distinct roles in regulating nutrient utilization. Because of their overlapping actions but differing receptor locations, these hormones can differentially regulate metabolism. Insulin action is primarily a function of secretory rate and receptor localization. In contrast, IGF action has an additional regulatory mechanism provided by the IGFBP. The IGFBP maintain a pool
of IGF that serves as a reserve to maintain BMR during physiological insults. The IGF activity is controlled by several inputs to monitor energy intake, protein intake, body temperature, environmental temperature, environmental stress, and, as introduced here, micronutrient intake. These inputs regulate not only IGF secretion but localization and circulating half-life by regulating IGFBP levels. Their effects ensure that IGF concentrations and activity are highest only when conditions are optimal for hyperplastic growth and cell differentiation. In addition, the IGFBP deliver the IGF to the cell surface, endowing the capability to target IGF activity. As yet, the potential of this activity has not been investigated, although the role of trace nutrients to target IGF activity deserves considerable attention. It is to be hoped that this review and new information will stimulate new approaches for IGF studies.

REFERENCES


SYMPOSIUM: GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTORS


