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BIOLOGICAL EFFECTS OF THE INSULIN-LIKE GROWTH FACTORS IN VITRO: RELEVANCE TO THEIR ACTIONS IN VIVO

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Whenever growth and its regulation are discussed today the term "somatomedin" comes into play. The terms "somatomedin" and "insulin-like growth factor" are synonyms for one and the same class of peptides. The definition of a somatomedin, however, is somewhat more restrictive in that it postulates a growth hormone-dependent regulation of a factor in addition to its non-suppressible insulin-like and growth promoting effects in vitro.
According to the somatomedin hypothesis put forward years ago by Daughaday on the basis of his pioneering work on the serum sulfation factor (see ref. 1 for a review) growth hormone secreted by the pituitary gland stimulates the production of somatomedin in the liver. On the basis of several pieces of indirect evidence it has been assumed and postulated that the somatomedins in turn mediate a variety of peripheral actions of the somatotrophic hormone. Furthermore, there is additional evidence, recently supported by the work of Berelowitz and Hintz (2), that a negative feedback exists between peripheral somatomedin levels and growth hormone secretion.
The question which shall be discussed here is: how do the insulin-like growth factors (IGF) fit into this concept and how relevant are their well-defined biological actions in vitro (see ref. 3 for a review) to their actions in vivo?
Through the pioneering work of Rinderknecht and Humbel (4, 5), two insulin-like growth factors have been identified in human serum. They have been termed IGF I and IGF II. Rinderknecht and Humbel have established the partial structural homology of these two factors to the insulin molecule: In fig. 1 the areas encircled by the closed lines represent amino acid sequences within IGF I and II which are identical, whereas the areas encircled by the broken lines represent amino acid sequences which are identical in human proinsulin and IGF. From this comparison it becomes apparent that nearly 50 % of the sequence of the

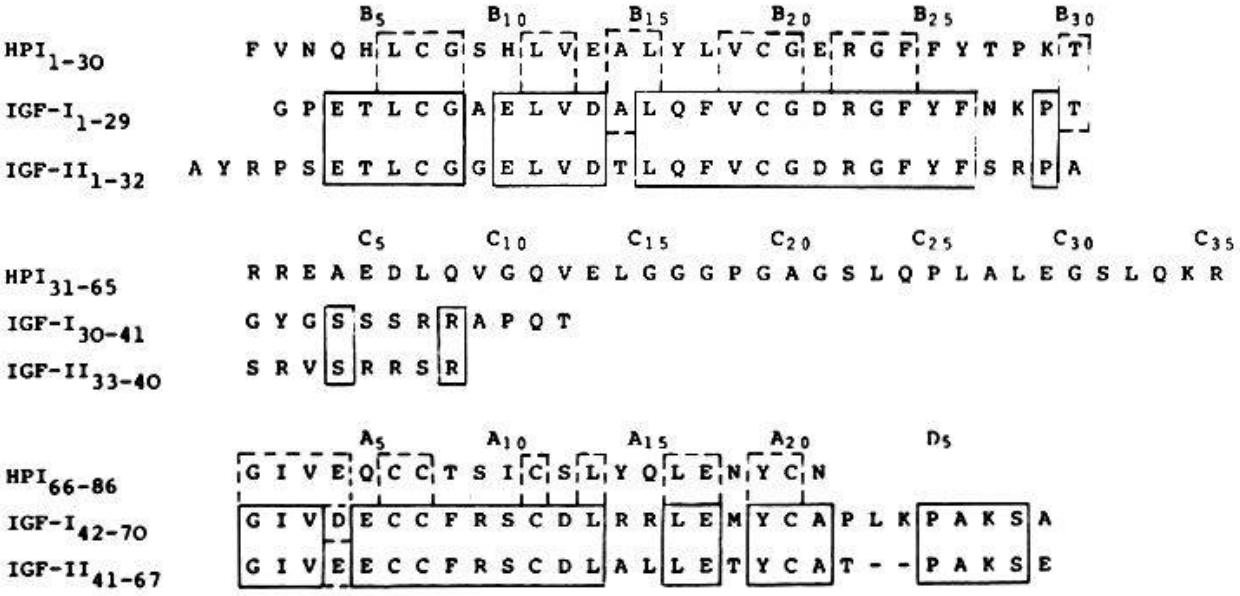

Fig. 1. Primary structure of human proinsulin (HPI), IGF I and IGF II. From ref. 5.

A- and B-chains in proinsulin is identical to the corresponding A- and B-domains in the IGF molecules. The differences between the structures of proinsulin and IGFs lie mainly in the amino terminal, the connecting peptide and the carboxyl terminal extension (D-domain), which is lacking in proinsulin.

Given this striking structural homology, it is not surprising that the insulin-like growth factors mimic all the biological effects of insulin in all tissues hitherto tested. In contrast to insulin, however, these effects are also observed in the presence of an excess of insulin antibodies. Furthermore, the potency ratio between insulin and IGF varies from one tissue to the other and can therefore serve as a characteristic index of the tissue specificity for IGF. The biological potency ratio and tissue specificity may, in first approximation, be taken as a clue to the physiological role of IGF relative to that of insulin.

Fig. 2 summarizes the biological effects of IGF on various tissues in vitro. According to the time of onset the anabolic effects can be ranged in two different categories which, taken together, can be classified as pleiotypic responses: a) acute metabolic effects which are observed within a few seconds and b) long-term effects which are observed only after several minutes to hours.

The acute effects of IGF are found in the typical insulin target tissues, adipose tissue and muscle. In the adipocyte the IGFs stimulate glucose transport, lipogenesis, glycogen synthesis, and they inhibit lipolysis and glycogenolysis. There are good reasons to assume that all of

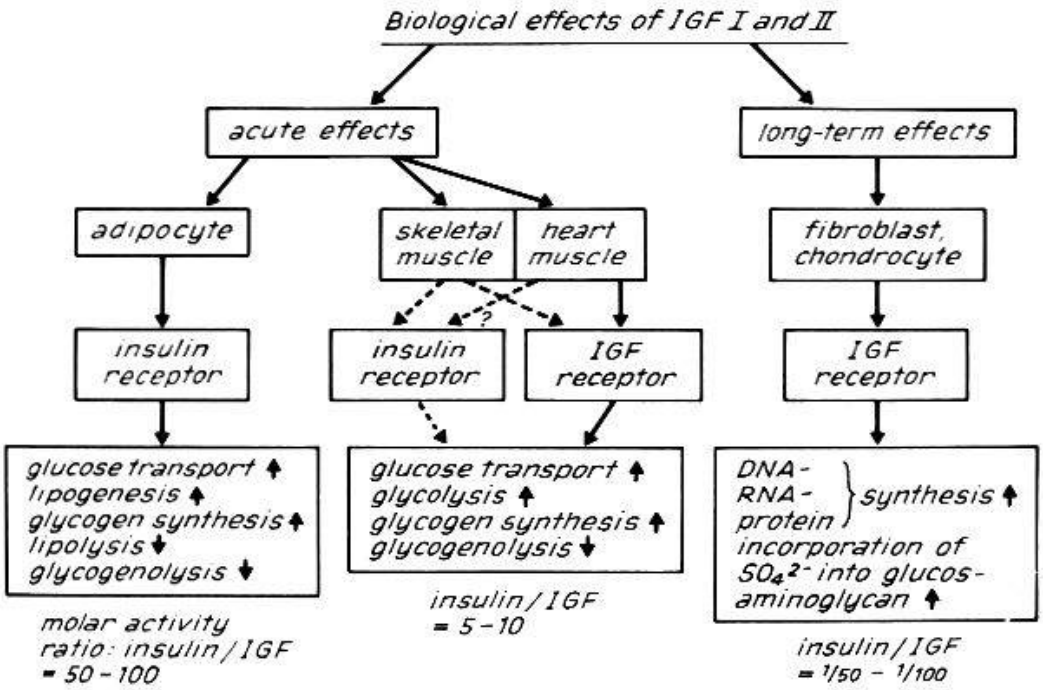

Fig. 2. Biological effects of IGF I and II.

these metabolic effects are mediated via the insulin receptor of the adipocyte (3, 6). In heart and skeletal muscle IGFs elicit metabolic responses similar to those observed in the adipocyte. These effects appear to be mainly mediated by a specific IGF receptor (3). However, from the data available it cannot be excluded that in skeletal muscle IGFs may also act via the insulin receptor and insulin via the IGF receptor (7).

A more clear-cut situation is obtained in tissues concerned with growth, like fibroblasts or chondrocytes, where IGFs stimulate DNA-, RNA- and protein synthesis as well as the incorporation of sulfate into glycosaminoglycans. These latter actions are clearly mediated through a specific IGF receptor. The comparison between the tissue specificities as represented by the molar activity ratios between insulin and IGFs indicates that, at least in vitro, the more potent anabolic hormone responsible for acute effects is insulin, whereas IGFs seem to represent the more potent anabolic principle for the stimulation of long-term growth effects.

The question which arises from these observations is whether or not these biological effects of IGF are relevant in vivo. There are two main ways that can be followed to reach an answer: 1. The measurement of IGFs in various physiological and pathophysiological conditions might allow to compare and possibly correlate IGF levels with clinical symptoms related to effects or lack of effects of IGF known from in vitro studies. 2. The second possibility would be to administer pure IGF in vivo, to quantitate the observed biological effects and to compare them with the in vitro effects.

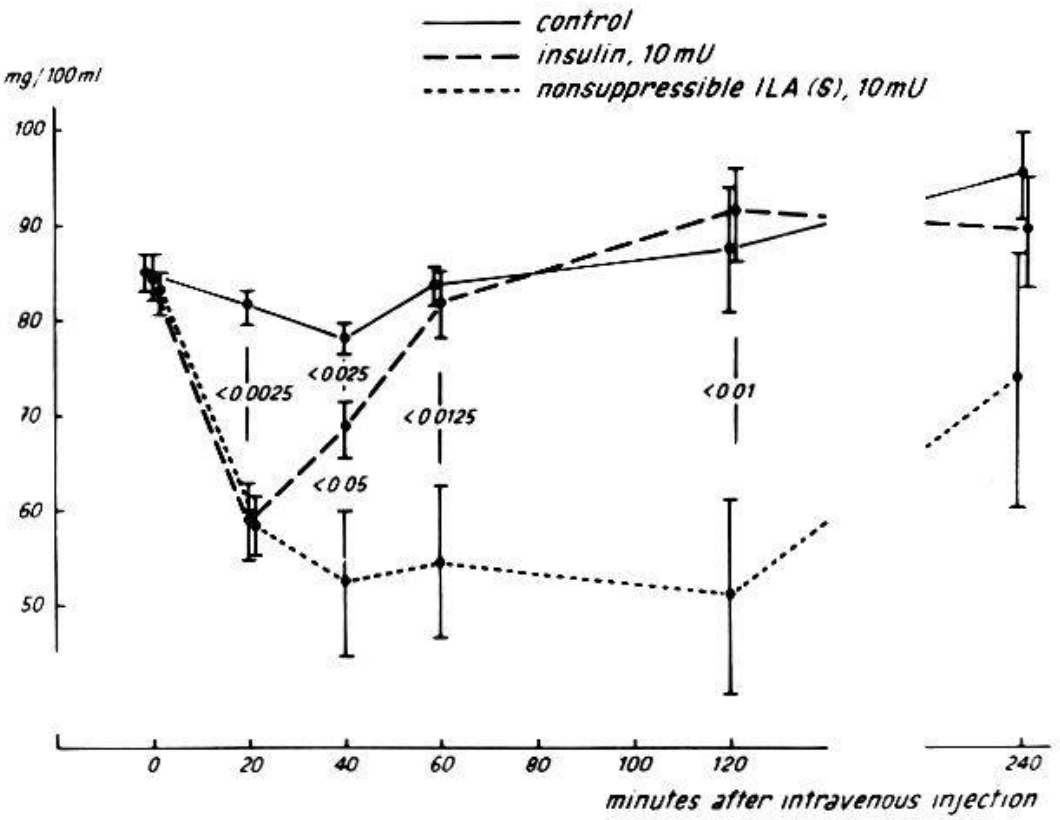

Fig. 3. Effect of intravenous administration of IGF (NSILA-S; 10 mU of a partially purified preparation) on the blood sugar level of adrenalectomized rats as compared with the effect of insulin (10 mU). The IGF preparation was standardized in mU of insulin equivalents in the fat pad assay. From ref. 8.

The second possibility has been checked very thoroughly early in IGF research by Froesch and his collaborators (see 8 for a review). They clearly demonstrated that IGF administered intravenously or intraperitoneally to rats or mice elicited the same acute biological effects as insulin. One of these examples is shown in fig. 3: the intravenous administration of 10 mU of IGF to adrenalectomized rats causes a pronounced fall of the blood sugar level similar to that caused by insulin, and even of a much longer duration. The latter observation is best explained by the longer half-life of IGF which, in contrast to insulin, is largely resistant to degradation by the liver and at the receptor sites. In parallel, Froesch and coworkers could show that intravenous or intraperitoneal administration of IGF leads to an increase of protein and glycogen synthesis in muscle, liver and adipose tissue and to increased lipogenesis in adipose tissue, effects that are very much the same as those observed after the administration of insulin.
At the time when these experiments were carried out it had not yet been known that IGFs carry intrinsic growth promoting activity. However, even then it would not have been possible to demonstrate any growth promoting effects in these short-time experiments.

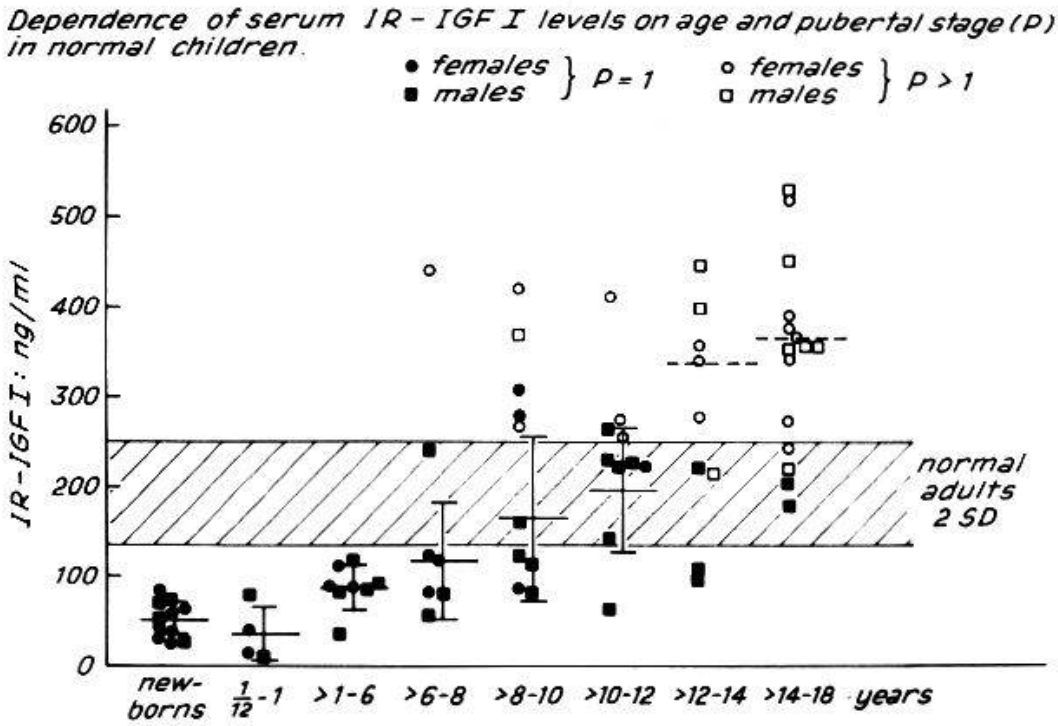

Fig. 4. Dependence of serum IR-IGF I levels on age and pubertal stage in normal children. From ref. 9.

Before going into the most important and intriguing question of whether or not IGFs are able to produce growth in long-term experiments - experiments which require large amounts of these growth factors - let us deal with the first-mentioned possibility of testing the relevance of IGFs in vivo, that is the correlation between clinical symptoms and IGF levels in various physiological and clinical situations. In this context, it may be useful to approach this issue by asking a couple of questions:

1. How high are normal IGF levels and do they correlate with biological actions of IGF known from in vitro studies?
2. Is there any clinical situation in which symptoms indicative of an acute insulin-like action might be explained by an elevation of IGF I or II?
3. Do we know clinical situations in which high IGF levels correlate with excessive growth, and others in which low IGF levels correlate with diminished growth?

The first half of question number 1 is answered by fig. 4 and fig. 5. We have developed two specific RIAs for IGF I and II (9). The crossreactivity of IGF II in the IGF I RIA is approximately 1 %, that of somatomedin A is ~10 %, and that of somatomedin C is more or less identical to that of IGF I. Thus, the IGF I RIA measures predominantly IGF I or somatomedin C depending on one's preferred terminology. The crossreactivity of IGF I, somatomedin A and somatomedin C in the IGF II RIA is approximately 10 %. All IGF II values measured in this assay are therefore corrected for crossreactivity with IGF I.

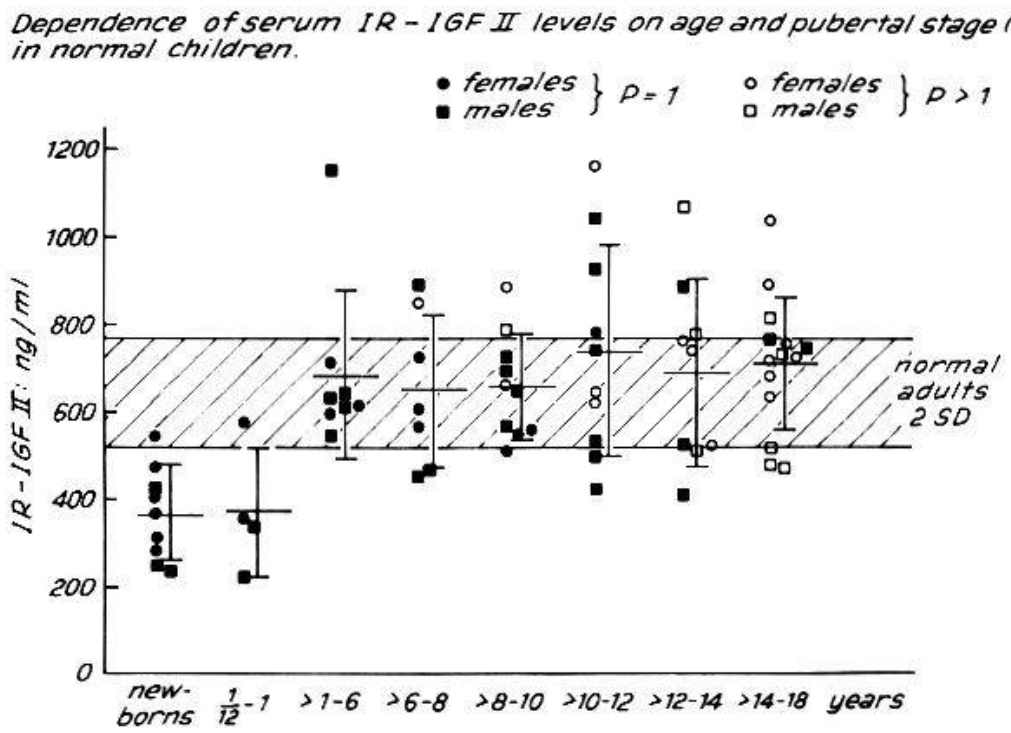

Fig. 5. Dependence of serum IR-IGF II levels on age and pubertal stage in normal children. From ref. 9.

Fig. 4 shows IGF I levels in normal children of different ages. The hatched area represents the mean IGF I level ± one standard deviation in a group of 32 normal adults between 21 and 54 years of age. In this latter group the mean IGF I level lies around 200 ng/ml serum. There is no correlation with age or sex in this group. In contrast, a clear-cut age-dependence can be seen in children of different ages. IGF I levels lie significantly below the normal adult level in newborn cord sera and during the first year of life with mean levels between 30 and 50 ng/ml. After the first year of life IGF I gradually rises and approaches the normal adult level. As indicated by the open symbols, which represent a pubertal stage of greater than I according to the definition of Tanner (10), there is a significant increase of IGF I coinciding with the onset of puberty. Fig. 5 shows the corresponding IGF II levels. As indicated by the hatched area, IGF II levels in normal adults lie between 400 and 800 ng/ml. That means that IGF II levels in serum are 3 - 4 times higher than IGF I levels. In contrast to IGF I, IGF II appears to be independent of age and of the pubertal stage. Only in newborn cord sera and during the first year of life do we observe slightly decreased IGF II levels. The question now is: how do these data fit into our general physiological concepts and, above all, how can we reconcile them with our present understanding of growth? If we add serum IGF I and II values in normal adults we come up with a total IGF level of 800 - 1000 ng/ml. When calculated in insulin equivalents the amount of total IGF present in 1 ml of

serum corresponds to 250 - 300 μU (specific biological activity of pure IGF I or II in the fat pad assay using insulin as a standard: ~300 mU/mg protein). This is the amount of insulin-like activity with which we constantly live without showing any symptoms of hypoglycemia! This puzzling phenomenon can be explained by the presence of a highly specific carrier protein in serum to which IGF is tightly bound (3). There is no conclusive evidence as yet for the presence of significant amounts of free IGF in native serum. Restricted capillary diffusion of the IGF-carrier complex (11) and the observation that the insulin-like actions of IGF are inhibited or even abolished by the carrier protein (12) could explain the absence of acute insulin-like effects in vivo despite the high IGF levels. On the other hand, the acute insulin-like actions observed after a bolus injection if IGF (8) can be explained as follows: The binding capacity of the carrier protein is overridden by an intravenous shot of IGF, and most of the administered dose reaches the receptors of the insulin target organs and can thus elicit acute insulin-like effects and hypoglycemia. Under normal physiological conditions, however, IGF does not appear to be involved in glucose homeostasis.

How then can IGF be involved in the regulation of growth in vivo? It has recently been shown by the group of Rechler and Nissley that endothelial cells of capillaries contain specific receptors for IGF-like peptides (13). It is attractive to speculate that these receptors would detach IGF from its carrier protein as the complex passes through the capillary bed and that IGF would then be conveyed via the intracellular space to the specific growth receptors of the tissues. This process would be too slow and result in too low concentrations of free IGF in the interstitial spaces to elicit acute insulin-like effects on insulin target organs, which have a low affinity for IGF (3). On the other hand, it is conceivable that the amounts of free IGF released by the afore-mentioned process would be high enough to stimulate tissues concerned with growth, in which the affinity for IGF is particularly pronounced (3). If we accept this hypothesis for the moment, how do IGF levels then correlate with the growth rate of children?

Fig. 6 compares IGF I levels with the growth rate curve during development taken from the work of Tanner (10). It is apparent at first sight that there is a huge discrepancy and even an inverse relationship between IGF I levels and the growth rate during the first years of life. There is the same discrepancy between the reportedly elevated growth hormone levels in newborns and the low IGF I levels. At present we cannot offer an explanation other than that during the first years of development other factors than IGF I or II may play a more important role or that growth hormone itself may act directly on growing tissues. Another possibility is that cells of newborns and children are more sensitive to IGF than cells from adults. On the other hand, the pubertal growth spurt might be related to the increase in IGF I levels. However, a causal relationship between the elevated IGF I levels and the increase

Growth rate and dependence of serum IGF I levels on age and pubertal stage in normal children.

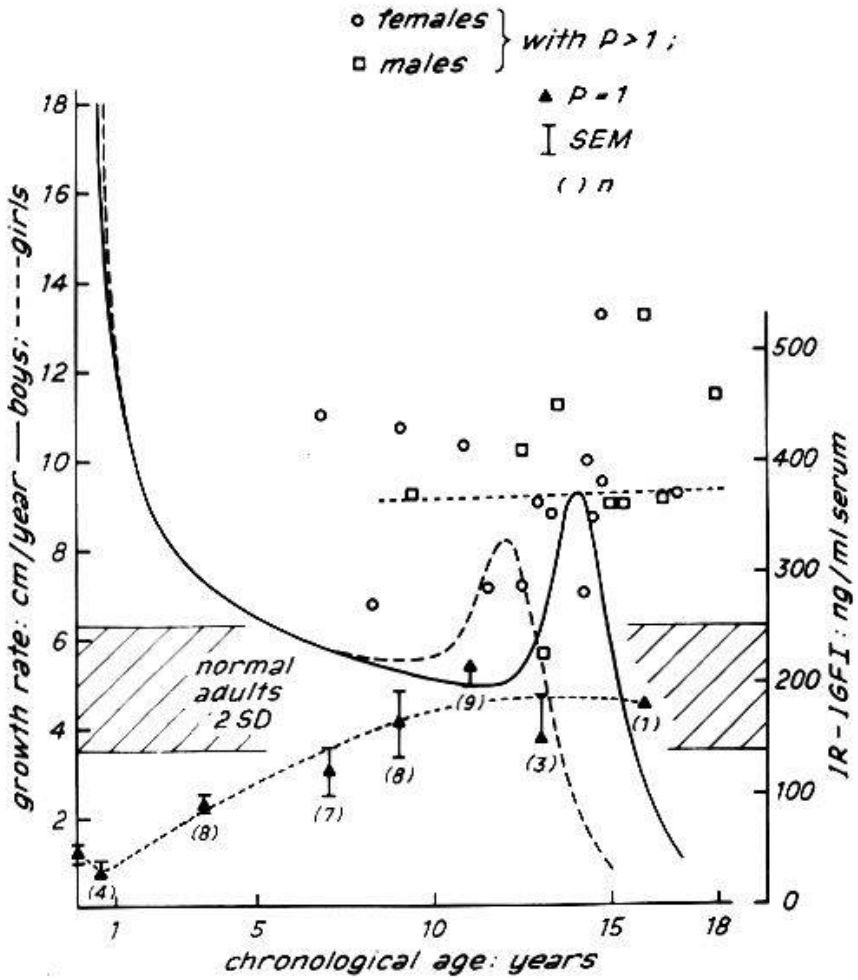

Fig. 6. Growth rate and dependence of serum IR-IGF I levels on age and pubertal stage in normal children. Solid line: growth rate curve in boys. Broken line: growth rate curve in girls. Stippled lines: IR-IGF I in prepubertal children (closed triangles) and children with a pubertal stage 1 (open squares and circles).

in the growth rate at the onset of puberty has not yet been proven. Any such conclusion would certainly be premature.

One of the much discussed clinical situations characterized by hypoglycemia without a concomitant rise of immunoreactive insulin is observed in patients with large extrapancreatic tumors. The sometimes dramatic symptoms of hypoglycemia in these patients have been reported to be due to an elevation of IGF or IGF-like substances (14, 15). We have checked more than 20 patients with extrapancreatic tumor hypoglycemia and found neither an elevation of biologically active IGF (16) nor of immunoreactive IGF I or II (fig. 7). Whereas in most of the patients IR-IGF I was decreased below normal IGF II levels were within the normal range.

Serum IR-IGF I and II levels in patients with extrapancreatic tumor hypoglycemia before and after operation.

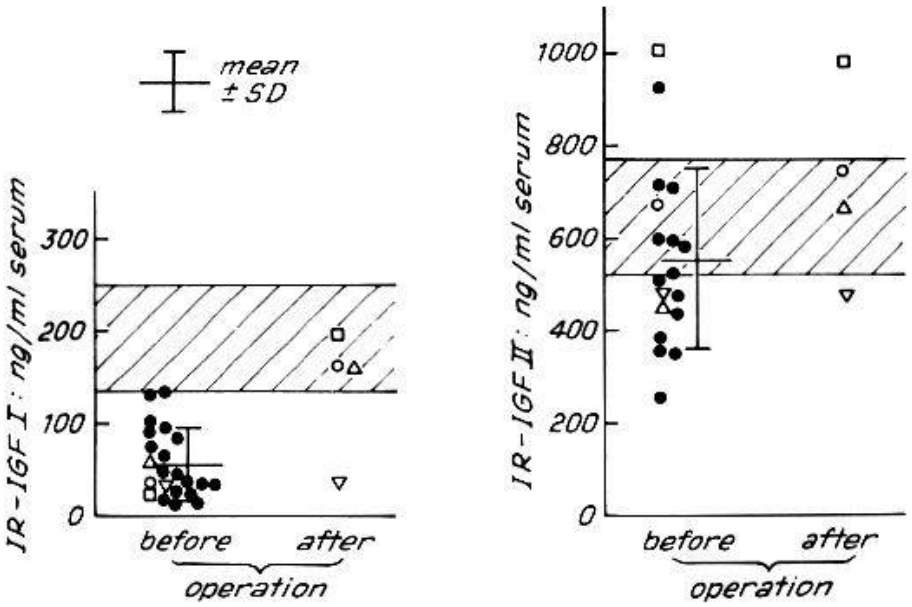

Fig. 7. Serum IR-IGF I and II levels in patients with extrapancreatic tumor hypoglycemia before and after successful removal of the tumor. From ref. 9.

All of the studies in which elevated IGF levels were detected in patients with extrapancreatic tumor hypoglycemia were carried out with a radioreceptor assay using rat liver membranes as a matrix. Therefore, we applied the same radioreceptor assay to determine receptor reactive IGF in the sera of our patients. The tracer used was ^{125}I-IGF II. In none of our patients could we demonstrate an increase of receptor reactive IGF (Widmer et al., in preparation). In agreement with the explanation offered above regarding the absence of acute insulin-like effects of IGF in the form it is present in the circulation, we believe that IGF is not responsible for the development of hypoglycemia in patients with extrapancreatic tumors.

The third question of whether increased or diminished growth in certain clinical situations is correlated with increased or decreased IGF levels is answered by fig. 8. IGF I levels are increased in acromegalic patients and decreased in patients with isolated growth hormone deficiency. This is, of course, not new and has been reported for somatomedin C (17) as well as for somatomedin A (18). However, elevated growth hormone levels in acromegalic patients do not cause a significant elevation of IGF II. In fact, the mean IGF II level is identical to that of normal subjects. In contrast, growth hormone deficiency is accompanied by significantly decreased IGF II levels. Apparently, IGF II production is already stimulated maximally by normal growth hormone concentrations and decreases only when the growth hormone level falls below normal. Similar results have been reported by the group of Hintz et al. (19) who used a specific IGF II RIA with an antibody directed against the IGF II C-peptide, and by the

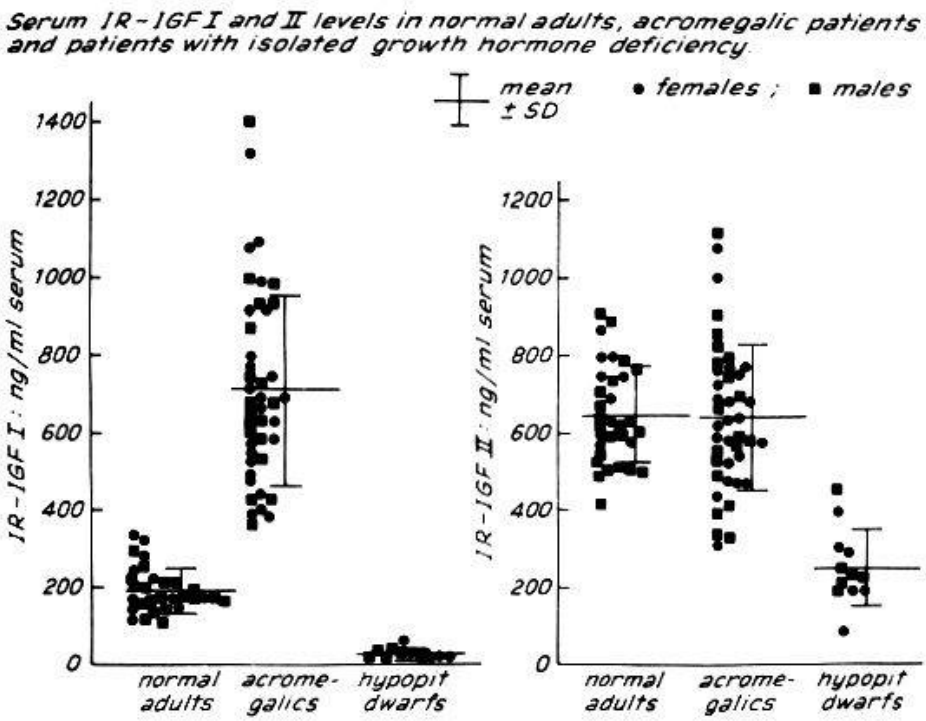

Fig. 8. Serum IR-IGF I and II levels in normal adults, acromegalic patients and patients with isolated growth hormone deficiency. From ref. 9.

group of Daughaday (20) who used an IGF II-specific radioreceptor assay with rat placental membranes.

The results of fig. 8 demonstrate that IGF I is clearly dependent on the growth hormone status, but they do not yet prove a growth hormone dependence of IGF II. The latter has recently been demonstrated by Merimee et al. (in preparation.) Growth hormone treatment for 5 days of patients with isolated growth hormone deficiency does not only result in a dramatic increase of IGF I, but also in a significant increase of IGF II. These results now make it possible to classify also IGF II as a somatomedin. Its possible physiological role, however, appears to be much less appreciable so far than that of IGF I.

The data in acromegalic patients and in patients with isolated growth hormone deficiency are again compatible with our view that IGF is not involved in the acute regulation of glucose metabolism: Despite the increased IGF levels acromegalic patients show no signs of hypoglycemia. On the contrary, hyperglycemia and diabetes often accompany the acromegalic state. On the other hand, decreased IGF levels in patients with growth hormone deficiency are not at all associated with hyperglycemia, but rather with hypoglycemia and with an increased sensitivity to insulin.

Let us now turn to the last and most intriguing question: However relevant are all our radioimmunological measurements of IGF? Or asked in a more direct and provocative manner:

Do IGF I or II have at all anything to do with growth in vivo? A few months ago a preliminary answer to this question was obtained: Hypophysectomized rats were treated for 8 days with a partially purified preparation of IGF (containing both IGF I and II) which was constantly infused from implanted minipumps. Two control groups of hypophysectomized rats received either human growth hormone (86 mU/day) or saline as an infusion. At a dose of IGF corresponding to 150 µg/day of the pure peptides an increase in body weight and in the tibial epiphyseal cartilage width was observed, which was around 50 % of the increase in body weight and tibial width obtained by the growth hormone infusion (21). When compared with the saline-infused group of hypophysectomized rats the increase of these two growth indices was statistically highly significant. These results constituted a big challenge to carry out the crucial experiments with pure IGF I and II.

During the preparation of this manuscript the first results of such experiments have been obtained: a constant infusion of 150 µg/day of pure IGF I over a period of 6 days led to an increase in body weight, in the tibial epiphyseal cartilage width and in the DNA-synthesizing activity of rib cartilage of hypophysectomized rats which was similar to the increase of these indices elicited by infusion of 50 mU/day of human growth hormone. IGF II, at a similar dose, was also, but less effective than IGF I (Schoenle et al., in preparation).

Therefore, the answer to our initial question clearly is yes. Our in vitro findings on IGF bioactivity and our radioimmunological measurements of IGF levels are of physiological and clinical relevance. Nevertheless, we are still far from understanding the extent to which IGFs are involved in the intriguing and complex physiology of growth. It would be too simple to invoke IGFs wherever we are confronted with the phenomenon of growth. One of the latest textbooks on the physiology of growth by R.J. Goss, which appeared in 1978 (22) does not even mention the word "somatomedin" or "insulin-like growth factor" on any of its 400 pages. Apparently, growth can occur without IGF. This does, however, not mean that we should stop trying to find out where such potent growth factors as IGF I and II come into play in the concerted process of growth.

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