Serum levels of insulin-like growth factor (IGF) and its carrier protein in various metabolic disorders

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Abstract. The levels of insulin-like growth factors (IGF), two somatomedin-like polypeptides of human serum and of their carrier protein were determined in sera of patients with various metabolic disorders. IGF was measured by 4 different methods (fat pad and fat cell assay and competitive protein binding assay measuring total IGF, and a radioimmunoassay for IGF I) after extraction by acidic gel filtration on Sephadex G-50. This procedure is necessary to separate IGF from the carrier protein, which interferes with all of these assays.

1) In normal serum, immunoreactive IGF I accounts for one third of total IGF determined by the fat pad assay, but only for one fifth to one sixth of immunoreactive IGF I + II.

2) In acromegalics total IGF was increased 1.5- (protein binding and fat cell assay) to 2-fold (fat pad assay), but the increase was solely due to immunoreactive IGF I, which was ~5-times above normal. The IGF binding activity was not elevated. Total IGF and IGF binding were decreased in hypopituitarism, Laron-type dwarfism and in liver cirrhosis. Immunoreactive IGF I was more drastically reduced in these diseases than total IGF. Apparently, only IGF I is under growth hormone control. The liver seems to be involved in the production of IGF.

3) No elevation of total IGF was found in patients with extrapancreatic tumour hypoglycaemia, but IGF binding was reduced. Immunoreactive IGF I was decreased in 5 of 10 patients. These results suggest that tumour hypoglycaemia in our patients is unlikely to be caused by increased IGF levels.

4) In patients with hyperprolactinaemia neither total IGF nor immunoreactive IGF I were elevated, and IGF binding was unchanged.

5) In newly detected insulin-deficient juvenile diabetics total IGF and immunoreactive IGF I levels were within the normal range, although the variation was greater than in normal subjects. However, IGF binding was markedly decreased.

Non-suppressible insulin-like activity extracted by acid/ethanol from human serum (NSILA-S): for review see Oelz et al. 1972; Zapf et al. 1978a) consists of two distinct polypeptides of a mol. wt. of 7500. They have been termed insulin-like growth factors (IGF) I and II (Rinderknecht & Humbel 1978a,b). Their amino acid sequences display ~50% structural homology to the A- and B-chain of human insulin. Their biologic actions comprise insulin-like effects in the presence of excess insulin antibodies on adipose tissue (Zapf et al. 1978a), heart (Meuli & Froesch 1975) and skeletal muscle (Poggi et al. 1979) as well as growth promoting and sulphation activities (Zapf et al. 1978a). The two polypeptides are closely related to the somatomedins (Zapf et al. 1978a), a family of polypeptides which are held responsible for mediating the actions of growth hormone on some of its target tissues (Daughaday et al. 1972; Chochinov & Daughaday 1976; van Wyk & Underwood 1978).

1 NSILA-S: small molecular weight non-suppressible insulin-like activity extracted from serum with acid/ethanol (Oelz et al. 1972) or by acidic Sephadex chromatography (Schlumpf et al. 1976; Zapf et al. 1977). In this manuscript the new term insulin-like growth factor (IGF) is used instead of NSILA-S. The term NSILA is used as a general term for the overall insulin-like activity of serum or serum fractions, which is not suppressible by an excess of insulin antibodies.
When total non-suppressible insulin-like activity in whole serum is measured by the fat pad assay conflicting results are obtained. For instance, activities in whole serum of normal subjects, acromegalic and hypopituitary patients were found to be the same (Schlumpf et al. 1976). In contrast, marked differences became apparent when sera were gel-filtered at acidic pH and determinations carried out in the small mol. wt. fractions (Schlumpf et al. 1976; Zapf et al. 1977). The reason for these discrepancies rests in the fact that native serum contains two different mol. wt. species of NSILA: 1) IGF (IGF I and II) which is almost completely bound to a specific carrier protein (Zapf et al. 1975); 2) a large mol. wt. species of NSILA (Poffenbarger 1975; Zapf et al. 1978c) not dissociable into IGF I or II by acid or urea treatment. The IGF-carrier complex does not exert acute biologic effects on insulin target tissues (Meuli et al. 1978; Zapf et al. 1979), and the in vitro effects of whole serum on adipose tissue appear to be due to large mol. wt. NSILA (Zapf et al. 1979).

In the present work we have used a one-step chromatographic procedure (gel filtration on Sephadex G-50 in 1 mol/l acetic acid) to separate IGF from its carrier (Zapf et al. 1977; Megyesi et al. 1975; Schalch et al. 1979). IGF was then determined in small mol. wt. serum fractions by 4 completely different methods: 2 biological assays (fat pad and fat cell assay) and a competitive protein binding assay (Zapf et al. 1977), which measure total IGF, and a recently developed radioimmunoassay (RIA) for IGF I (= immunoreactive IGF I). The results shed light on the levels of IGF and of the IGF carrier protein in various metabolic diseases and on the regulation of the levels of IGF I and II. Preliminary aspects of this work have been presented in a recent review article (Zapf et al. 1978a).

Materials and Methods
Pure IGF I (prep. 3 SE II) and II (prep. 10 SE IV) were kindly provided by Dr. R. E. Humbel. Somatomedin A (SM-A) and somatomedin B (SM-B) was a generous gift from Dr. L. Fryklund, Stockholm, somatomedin C (SM-C) from Dr. J. J. van Wyk, Chapel Hill.
All blood samples were drawn after an overnight fast. After clotting (1 h at 4°C) they were centrifuged (15 min at 4°C and 1500 g) and the ‘serum’ was pipetted off and stored at −20°C. One ml serum samples were chromatographed on Sephadex G-50 (medium: bed volume 100 ml; column Ø 2 cm) in 1 mol/l acetic acid. Fractions were pooled between 50−55, 55−60 and 60−80% bed vol, lyophilized, taken up in 0.1 mol/l Na4HCO3 and re-lyophilized. In each of the 3 pools IGF was determined by the competitive protein binding assay (Zapf et al. 1977).

Equal aliquots of the 3 pools were re-combined, re-lyophilized, taken up in Krebs-Ringer bicarbonate buffer containing 2 g/l of human serum albumin (HSA), and IGF was measured in the fat pad assay (Froesch et al. 1963), the fat cell assay and in the IGF I-RIA (see below).

The pools eluting between 30 an 50% bed volume, which contain the large mol. wt. NSILA (Zapf et al. 1978c) and the IGF carrier protein devoid of endogenous IGF (Zapf et al. 1978c) were ultrafiltered to a volume of 5 ml, dialyzed extensively against 0.1 mol/l phosphate buffer, pH 7.0, and assayed for [125I]IGF binding activity at different dilutions (see below).

**Assays**
1) **Fat pad assay.** The fat pad assay was performed as described in detail by Froesch et al. (1963). Pooled segments of epididymal adipose tissue from 12 male Zb Caras (formerly Osborne-Mendel) rats weighing 130−140 g were incubated in Warburg flasks in 1 ml of Krebs-Ringer bicarbonate buffer containing 2 mg of HSA (from the Swiss Red Cross, Bern: devoid of intrinsic insulin-like activity), 2 mg of glucose and 1 µl of guinea pig anti-insulin serum (insulin-neutralizing capacity 1 µl). Manometric readings were taken after 30 and 60 min of incubation and compared with a 4-point (10, 30, 90 and 270 µU/ml) insulin standard curve (waxl insulin, identical amino acid sequence to porcine insulin, kindly supplied by Dr. R. E. Humbel). Activities were expressed as µU of insulin equivalents. In the fat pad assay 1 µg of IGF I or II is equivalent to 330 and 310 µU of insulin, respectively (Zapf et al. 1978b).

2) **Fat cell assay.** Isolated fat cells from epididymal fat pads of normal male Zb Caras weighing between 100 and 120 g were prepared by the method of Rodbell (1964). The cells (~ 2 × 109) were incubated for 1 h at 37°C in 1 ml of Krebs-Ringer bicarbonate buffer containing 2 mg of HSA, 0.18 mg of glucose, 0.2 µCi of U[14C]glucose (Radiochemical Centre, Amersham), different concentrations of insulin (standard) or unknown concentrations of IGF (from the above re-combined serum pools) with 1 µl of guinea pig anti-insulin serum.

14C-incorporation into total lipids was determined in 300 µl aliquots of the cell suspension centrifuged (10 000 g, 20 s, Beckman microfuge B) through a layer of dinonylphthalate (50 µl) in 400 µl plastic microtubes as described by Gliemann et al. (1975).

The tops of the microtubes containing the packed cell layer were cut off and counted in 5 ml of Instagel.
(Packard) in a liquid scintillation counter (Tricarb, Packard). IGF values were calculated from the insulin standard curve in μU of insulin equivalents. In the fat cell assay 1 μg of IGF I or II is equivalent to ~180 μU and ~540 μU of insulin, respectively (Zapf et al. 1978b), giving a potency ratio between IGF I and II of 1:5.

3) Protein binding assay. The experimental procedure was identical to that described in detail earlier (Zapf et al. 1977). [125I]IGF I was used as a tracer (see below). The assay was standardized with a partially purified preparation of IGF, containing a 1:1 (wt./wt.) mixture of IGF I and II. This preparation was itself standardized in the fat pad assay and had a specific biological activity of 4.5 mU of insulin equivalents/mg protein. The results of the protein binding assay are, therefore, expressed as μU of fat pad insulin equivalents (see results and discussion). When compared with the standard used in the protein binding assay, 1 μg of IGF I or II is equivalent to ~200 and ~600 μU, respectively.

4) Radioimmunoassay (RIA) for IGF I. Iodination of IGF I was carried out as described previously (Zapf et al. 1978b). The tracer had a specific radioactivity of 50–100 μCi/μg and contained between 0.3 and 0.6 atoms of iodine per molecule of IGF I. Between 90 and 95% of the tracer was precipitable with 10% trichloroacetic acid.

Unlabelled IGF I (prep. 2 SF II, the same as used earlier by Zapf et al. 1978b) served as standard (concentration range between 0.2 and 125 ng/ml corresponding to 0.21–42 μU/ml of insulin equivalents in the fat pad assay; see below).

Rabbit antiserum against IGF was kindly supplied by Dr. K. Reber, Hoffmann-La Roche, Basel. Some of the characteristics of this antiserum have been described recently (Reber & Liske 1976). We found that it contained antibodies which were directed against IGF I (see results). The assay mixture contained 0.2 ml of the IGF I antiserum (diluted 1/10 000 in 0.1 mol/l phosphate buffer/0.2% HSA, pH 7.0), 0.1 ml (~20 000 cpm, ~0.2 ng) of [125I]IGF I and different concentrations (0.1 ml of the same buffer) of unlabelled IGF I or of the pooled small mol. wt. serum fractions obtained by the procedure described above. Incubations were carried out for 20 h at 4°C. 0.5 ml of a charcoal suspension (2 g of charcoal pre-equilibrated in 100 ml of 0.1 mol/l phosphate buffer, pH 7.0, containing 2 g of HSA) was added for 20 min at 4°C and the mixture was centrifuged for 10 min at 4°C and 1500 × g. 0.5 ml of the supernatant was counted in a gamma counter (Packard). At this dilution the antiserum bound ~17% of the total counts added. Binding determined in the presence of 125 ng/ml of unlabelled IGF I was ~5% of the total radioactivity added and was close to the non-specific binding in the absence of antiserum (~4%). The intra-assay variation was 6.0 ± 1.1% (n = 15), the inter-assay variation 13.3 ± 2.2% (n = 20).

5) Determination of relative [125I]-IGF binding activities. Binding of [125I]IGF I to protein in dialyzed ultrafiltrates of 30–50% bed vol pools obtained after acid Sephadex G-50 gel filtration of the sera (see above) was determined at different dilutions of the ultrafiltrate in order to determine the linear range of specific binding (difference between total binding and binding in the presence of 50 μU/ml of unlabelled IGF).

A partially purified preparation of unlabelled IGF (1.5% pure, containing a 1:1 mixture of IGF I and II) was used to determine radioimmunologically served to determine non-specific binding. To 0.2 ml of the ultrafiltrate diluted in 0.1 mol/l phosphate buffer/0.2% HSA, pH 7.0, was added 0.1 ml of [125I]IGF I (~100 000 cpm ~1 ng; in the same buffer) and 0.1 ml of the above buffer containing either no (total binding) or 50 μU/ml of unlabelled IGF (non-specific binding). After equilibration of the mixture for 2 h at room temperature (Zapf et al. 1977), 0.5 ml of a charcoal suspension (same as used for the RIA and for the protein binding assay) was added for 20 more min. After centrifugation for 10 min at 1500 × g, 0.5 ml of the supernatant was counted in a gamma counter (Packard).

Binding activity has been expressed as cpm [125I]-labelled IGF I specifically bound/μl stripped serum calculated per 100 000 cpm added. According to the Scatchard equation

\[ \frac{[HR]}{[H]} = R_0 - K \cdot \frac{[HR]}{[H]} \]

where [HR] is the concentration of the hormone-receptor complex corresponding to specific binding, [H] the concentration of free hormone, \( R_0 \) the receptor (binding protein) concentration and K the affinity constant. Within the linear range of specific binding as used here the latter is negligible (1–2%) as compared to the total amount of labelled hormone added. Therefore, since

\[ [H] \leq [H]_{\text{total}} \]

the Scatchard equation is expressed as a function of \( R_0 \) and \([H]\) replaced by \( [H]_{\text{total}} \) then

\[ \frac{[HR]}{[H]} = R_0 \cdot \frac{K}{[H]_{\text{total}}} \]

this means that [HR] is directly proportional to \( R_0 \), since

\[ \frac{K}{[H]_{\text{total}}} \]

is constant. So far, we have no evidence that K of the stripped carrier protein is different for different diseases. Under these assumptions, the specific binding activity as defined here may be taken as a relative measure of the concentration \( R_0 \) of the total stripped binding protein present in the gel-filtered sample. Native serum contains 2 forms of binding protein (Zapf et al. 1975, 1978c), a large mol. wt. form (MW ~200 000, which carries most of the IGF present in serum, and a small mol. wt. form (MW ~50 000), which is largely devoid of IGF (Zapf et al. 1978c). The physiological significance of the 2 forms of carrier protein and their inter-relationship are not at all clear, at present. Gel-filtration at acidic pH converts the large mol. wt. into a small mol. wt. form (Zapf et al. 1975, 1978c). Since the latter does not re-associate into the former at neutral pH, acidic gel filtration may alter the binding characteristics of the native carrier pro-
tein(s). The specific binding activity measured in our samples, therefore, reflects the over-all concentration of both the small mol. wt. binding protein plus the stripped and dissociated, originally large mol. wt. IGF-carrier obtained after acidic gel filtration, and not the binding capacity of original serum.

Patients

Healthy normal donors (40 males, 22 females) were between 22 and 50 years old.

Acromegalic patients (age between 27 and 55 years) all had elevated serum phosphate (4.7–6.1 mg/100 ml) and growth hormone levels (10–250 ng/ml by RIA). GH levels were not suppressed in the oral glucose tolerance test. The determination of immunoreactive GH was kindly performed by Dr. Zahnd, Geneva.

In hypopituitary patients (8–40 years) GH was not measurable and no GH response to insulin hypoglycaemia or to arginine was observed. Six of the patients had idiopathic panhypopituitarism, 2 had panhypopituitarism after hypophysectomy and 10 had isolated GH-deficiency.

Patients with Laron type dwarfism (Laron 1974) had normal to elevated GH levels. Administration of exogenous GH did not induce growth in these patients.

The age of the cirrhotic patients was between 34 and 71 years. In 15 of them cirrhosis was caused by chronic alcoholism, the others were in the final stage of chronic active hepatitis (8 patients) and of biliary obstruction (2 patients). Most of them had portal hypertension, ascites and oesophageal varices. Relevant laboratory tests were as follows: pseudocholinesterase 1055 ± 400 U/l (normal 1900–3800 U/l); alkaline phosphatase 242 ± 140 U/l (normal 60–290 U/l); bilirubin 4.1 ± 4.2 mg/100 ml; gamma globulins 29.3 ± 11.8%; albumin 2.85 ± 0.39 g/100 ml.

Of the 15 patients with extrapancreatic tumour hypoglycaemia (age between 13 and 63 years) 9 had a fibrosarcoma, 2 a leiomyosarcoma, 1 an undifferentiated carcinoma, 1 a mesothelioma, 1 a congenital mesoblastic nephroma and 1 a malignant haemangio-endothelioma. Blood sugar values at the time of blood sampling ranged between 20 and 45 mg/100 ml.

Hyperprolactinaemia in 10 patients (28–42 years, 8 females, 2 males) was caused by 'chromophobic adenomas' (prolactinomas) of the pituitary gland. Serum prolactin levels (by RIA) were between 50 and 800 ng/ml.

The diabetic patients (age 11–25) were all newly discovered juvenile type patients before initiation of insulin therapy. Fasting blood sugar values at that time ranged between 210 and 790 mg/100 ml. Two of the patients were ketoacidotic.

Fig. 1.

Correlation between total IGF as determined in the fat cell and in the competitive protein binding assay. IGF was determined by the fat cell and competitive protein binding assays in small molecular weight material (‘biological material’) obtained by acidic Sephadex G-50 chromatography from sera of normal subjects, acromegalic and hypopituitary patients, Laron dwarfs, patients with tumour hypoglycaemia, hyperprolactinaemia and newly discovered juvenile onset diabetes. Details of the fat cell and the competitive protein binding assay are described in the text.

Cross-bars give the mean ± SD for both assays in normal (N), acromegalic (A) and Laron type dwarfism (LTD) sera. Correlation coefficient r = 0.85; P < 0.001.
Results

Fig. 1 shows that the IGF values obtained in the protein binding assay for small mol. wt. fractions of sera from different groups of patients correlate with those obtained in the fat cell assay (r = 0.85) over a wide range of IGF concentrations. In general, the two assays give similar results (see also Table 1), although discrepancies occur.

In Fig. 2 the competitive potencies of IGF I and II in the IGF I-RIA are compared with those of other somatomedins, and of insulin, proinsulin and human growth hormone. Half-maximal inhibition of \[^{[125}\text{I}]\text{IGF I}\) binding to the antiserum is caused by

| Table 1. IGF values determined by different methods and \[^{[125}\text{I}]\text{IGF}\) binding activities in normal subjects and in patients with various metabolic disorders (means ± s.d). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Fat pad assay [\(\mu U/ml\)] | Fat cell assay [\(\mu U/ml\)] | Protein binding assay [\(\mu U/ml\)] | IGF-RIA ng/ml [\(\mu U/ml\)] fat pad insulin equivalents | \[^{[125}\text{I}]\text{IGF binding activity CPM} \times 10^{-3}\) specifically bound/\(\mu l\) stripped serum per \(10^8\) CPM added |
| Normal          | 148 ± 43         | 337 ± 74         | 317 ± 65         | 153 ± 47 [51 ± 16] (n = 16) | 9.0 ± 2.9 (n = 22) |
| (n = 36)        |                  |                  |                  |                  |                  |
| Acromegalis     | 282 ± 50         | 533 ± 114        | 480 ± 73         | 808 ± 306 [270 ± 102] (n = 25) | 8.1 ± 3.8 (n = 14) |
| (n = 25)        |                  |                  |                  |                  |                  |
| Hypopituitary dwarfs | 61 ± 13         | 151 ± 67         | 153 ± 47         | 34 ± 22 [11 ± 7] (n = 11) | 2.4 ± 1.4 (n = 11) |
| (n = 10)        |                  |                  |                  |                  |                  |
| Laron dwarfs    | not det.         | 119 ± 64         | 71 ± 40          | 25 ± 18 [8 ± 6] (n = 12) | 2.1 ± 0.9 (n = 11) |
| (n = 11)        |                  |                  |                  |                  |                  |
| Liver cirrhosis | 42 ± 22          | not det.         | 83 ± 48          | 25 ± 20 [8 ± 7] (n = 12) | 3.8 ± 3.2 (n = 25) |
| (n = 20)        |                  |                  |                  |                  |                  |
| Extrapancreatic tumour hypoglycaemia | 132 ± 44      | 232 ± 113        | 183 ± 63         | 66 ± 35 [22 ± 12] (n = 10) | 2.7 ± 1.4 (n = 11) |
| (n = 15)        |                  |                  |                  |                  |                  |
| Hyperprolactinaemia | 189 ± 59       | 364 ± 75         | 355 ± 111        | 225 ± 70 [75 ± 23] (n = 7) | 6.2 ± 3.7 (n = 8) |
| (n = 8)         |                  |                  |                  |                  |                  |
| Insulin-deficient | 124 ± 37        | 306 ± 125        | 247 ± 137        | 234 ± 160 [78 ± 53] (n = 7) | 1.5 ± 0.7 (n = 13) |
| (n = 11)        |                  |                  |                  |                  |                  |

The methodology is described in Materials and Methods. All values are the mean ± s.d. In parentheses, the number of patients investigated is listed. \(\mu U\) in the protein binding assay refer to an IGF reference standardized with insulin in the fat pad assay (S.A. 4.5 \(\mu U/mg\) protein). For reasons of easier comparison with the fat pad assay the results of the RIA have also been calculated in \(\mu U\) of fat pad insulin equivalents (shown in brackets). The relative specific \[^{[125}\text{I}]\text{IGF}\) binding activity was measured in the void volume pool (30–50\% bed volume) obtained from 1 ml serum samples on acidic Sephadex G-50 gel filtration and dialyzed extensively against 0.1 mol/l phosphate buffer, pH 7.0. Binding activity is expressed in CPM \(\times 10^{-3}\) specifically bound per \(\mu l\) of stripped serum calculated per 100 000 CPM (\(\sim 0.3 \mu U; \sim 1 \text{ng}\) of \[^{[125}\text{I}]\text{IGF}\) added.|

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2.2 ng/ml (~3 × 10^{-10} M) of unlabelled IGF I. SM-A and SM-C are nearly as potent as IGF I. In contrast, IGF II is only a weak competitor (cross-reactivity ~ 3.5%). No cross-reaction is observed with somatomedin B, insulin, proinsulin and human growth hormone up to concentrations of 1 μg/ml. Fig. 3 shows the dilution curves in the IGF I-RIA of small mol. wt. fractions (termed 'biological material') obtained by acidic Sephadex G-50 gel filtration of normal serum (pool of 10 healthy subjects, 25–40 years of age) and of sera from 3 acromegalic patients. These dilution curves are parallel to that for unlabelled IGF I. In the insert the IGF I values determined from this figure have been calculated in μU of fat pad insulin equivalents (1 ng ~ 0.33 μU) and compared with the total IGF values measured in the fat pad assay. According to this calculation, immunoreactive IGF I in normal serum accounts for approximately one third of total biologically active IGF (see also Table 1). In contrast, immunoreactive IGF I in the 3 acromegalic patients is 5–7 times higher than normal and makes up 84–108% of the total IGF determined in the fat pad assay (see also Table 1).

The mean levels of total IGF determined in the fat pad, the fat cell and the competitive protein binding assay and the mean levels of immunoreactive IGF I for healthy subjects and for patients with different metabolic disorders are summarized in Table 1. Comparing the results of the different assays within one and the same group it becomes apparent that the IGF values obtained in the fat cell and protein binding assay are ~2-fold higher than those of the fat pad assay. This discrepancy observed for many years when partially purified IGF preparations from different purification steps were tested in the fat pad and fat cell assay, is likely to be due to the following reasons: whereas IGF I and II are equipotent in the fat pad assay, IGF II is ~3-fold more potent than IGF I in the fat cell and in the protein binding assay (using [125I]IGF I as a tracer) (Zapf et al. 1978b). Since all sera contain both factors (assuming that the difference between total IGF determined in the fat pad assay and immunoreactive IGF I in Table 1 represents IGF II; see Discussion) the IGF II activity prevails in the latter two assays, which results in relatively higher total IGF activities than determined in the fat pad assay.

As reported earlier, total IGF is elevated in
acromegalic and decreased in hypopituitary patients (Schlumpf et al. 1976; Zapf et al. 1977; Heinrich et al. 1978). However, the increase of total IGF in acromegalics is exclusively due to an increase in immunoreactive IGF I. The mean IGF I level in 25 acromegalic patients was 808 ± 306 ng/ml serum. This corresponds to 270 ± 102 μU/ml of fat pad insulin equivalents and is close to the total IGF level in these patients as determined by the fat pad method. In 9 of the acromegalic patients IGF I even accounted for more than 100% (between 113 and 180%) of total IGF active on the fat pad. Interestingly, the [125I]IGF binding activity is not elevated in acromegaly and thus, does not follow the increase in IGF I.

No correlation was found between the level of immunoreactive GH in acromegalic patients and the level of total IGF or of immunoreactive IGF I (P > 0.01; not shown). As shown in Table 2, GH levels can even be normal when IGF I levels are drastically elevated. These patients had a pituitary tumour, typical clinical symptoms of acromegaly and an elevated serum phosphate level. On the other hand, elevated GH levels need to necessarily be accompanied by elevated IGF I levels. In one of our acromegalic patients GH levels remained elevated after hypophysectomy, but IGF I was decreased (Table 2).

In hypopituitary and Laron dwarfs and in patients with liver cirrhosis immunoreactive IGF I is more drastically decreased than total IGF. In these diseases a concomitant decrease of the [125I]IGF binding activity is observed.

Elevated levels of prolactin, which is structurally

<table>
<thead>
<tr>
<th>IGFI ng/ml</th>
<th>IGFI total IGF ng/ml</th>
<th>found in pad assay u,U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>142</td>
<td>47</td>
</tr>
<tr>
<td>acro 1</td>
<td>1030</td>
<td>343</td>
</tr>
<tr>
<td>acro 2</td>
<td>749</td>
<td>250</td>
</tr>
<tr>
<td>acro 3</td>
<td>689</td>
<td>230</td>
</tr>
</tbody>
</table>

Fig 3.

Competitive inhibition of the binding of [125I]-labelled IGF I to IGF I antiserum (diluted 1:10 000) by unlabelled IGF I and different dilutions of small molecular weight material obtained by acidic Sephadex G-50 chromatography of 1 ml of a normal serum pool and of 3 acromegalic sera. The methodology is described in Materials and Methods. The lyophilized small molecular serum fractions (50–80% bed volume on an acidic Sephadex G-50 column) were re-constituted to 1 ml with 0.1 mol/l phosphate buffer/0.2% HSA, pH 7.0. 0.1 ml of the indicated dilutions were used in the assay. Points are the means of duplicates (intra-assay variation 6.0 ± 1.1%). Comparison between IGF I levels determined from Fig. 3 and the total IGF levels determined in the fat pad assay: IGF I levels read from the figure have been calculated in μU of insulin equivalents according to the following index: 1 ng ~ 0.33 μU of insulin equivalents in the fat pad assay (Zapf et al. 1978b).
Table 2.
IGF levels in 3 acromegalic patients with normal GH and in an acromegalic patient with elevated GH before and 5 years after removal of the pituitary tumour.

<table>
<thead>
<tr>
<th>Patient</th>
<th>GH ng/ml</th>
<th>Fat pad assay μU/ml</th>
<th>Binding assay μU/ml</th>
<th>IGF I-RIA ng/ml [± μU/ml of fat pad insulin equivalents]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. B.</td>
<td>1.5</td>
<td>275</td>
<td>467</td>
<td>590 [197] elevated</td>
</tr>
<tr>
<td>K. F.</td>
<td>1.0 normal</td>
<td>230</td>
<td>494</td>
<td>505 [168] elevated</td>
</tr>
<tr>
<td>V. M.</td>
<td>2.9</td>
<td>238</td>
<td>376</td>
<td>780 [260] elevated</td>
</tr>
<tr>
<td>A. I.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before op.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years after op.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are the means of duplicate determinations within the various assays. Small molecular weight material was obtained from 1 ml serum by Sephadex G-50 gel filtration in 1 M acetic acid (see Materials and Methods). In brackets RIA values are also calculated as μU of fat pad insulin equivalents.

Elevated IGFI binding activity is statistically not significant (P > 0.05).

In 15 patients with extrapancreatic tumour hypoglycaemia total IGF levels were within the normal range or even decreased, whereas the [¹²⁵I]IGF binding activity was decreased. Immunoreactive IGF I was decreased in 5 of 10 patients. In 5

Table 3.
IGF values in patients with tumour hypoglycaemia before operation and after successful removal of the tumour (normoglycaemia).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fat pad assay μU/ml</th>
<th>Fat cell assay μU/ml</th>
<th>Binding assay μU/ml</th>
<th>IGF I-RIA ng/ml [± μU/ml of fat pad insulin equivalents]</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. M. ♂: fibrosarcoma</td>
<td>184</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H. B. ♂: mesothelioma</td>
<td>45</td>
<td>119</td>
<td>-</td>
<td>133</td>
</tr>
<tr>
<td>R. R. ♂: congenital mesoblastic nephroma</td>
<td>167</td>
<td>250</td>
<td>150</td>
<td>400</td>
</tr>
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All values are the means of duplicate determinations within the various assays. Small molecular weight material was obtained from 1 ml serum by Sephadex G-50 gel filtration in 1 M acetic acid (see Materials and Methods). In brackets RIA values are also calculated as μU of fat pad insulin equivalents.

Closely related to GH, do not cause a significant rise of total IGF (P > 0.1) nor of IGF I (P > 0.05), and there is no correlation between IGF and prolactin levels (not shown). The change in [¹²⁵I]IGF binding activity is statistically not significant (P > 0.05).
Patients IGF levels did not change or rose slightly after removal of the tumour, when blood sugar values had returned to normal (Table 3).

Insulin-deficient juvenile-type diabetics at the onset of their disease showed a rather wide variations of their serum IGF levels. The mean total IGF and IGF I concentrations in this group lay within the normal range ($P > 0.05$). However, there was a most conspicuous reduction of the [125I]IGF binding activity.

No correlation between the level of total IGF (fat pad, fat cell or protein binding assay) and that of immunoreactive IGF I is observed within each individual group of patients. However, when a similar number of patients with low, medium and high IGF levels is examined a significant correlation between the two parameters becomes apparent (not shown).

Discussion

Four different assays based on completely different methodology were used to determine the mean IGF levels and their range of variation in different metabolic disorders. The fat pad, the fat cell and the competitive protein binding assay cannot distinguish between IGF I and II and, therefore, measure both factors, i.e. total IGF. Each of these 3 assays is standardized in µU of insulin equivalents. In the fat pad and fat cell assay insulin itself serves as a standard, and the biological effect obtained with the sample containing IGF is quantitated from corresponding insulin standard curves. However, this 'direct' standardization cannot be applied to the competitive protein binding assay, since insulin does not compete with IGF for binding to the IGF carrier protein. Therefore, a partially purified IGF preparation has to be used as a standard, which is itself first standardized with insulin in the fat pad assay.

The differences in the absolute values of the 3 assays are explained by potency differences between IGF I and II (see Results). Nevertheless, the reasonably good correlation between the 3 assays (Zapf et al. 1977; Fig. 1) allows to use them as a measure for total serum IGF. However, it is important to re-emphasize that determinations have to be carried out in small mol. wt. serum fractions obtained after separation by acidic gel filtration of the IGF binding protein, which heavily interferes with all of these assays, and of the large mol. wt. NSILA (Poffenbarger 1975; Zapf et al. 1978c). This 'conditio sine qua non' also applies to the IGF I-RIA, which cannot be carried out in whole serum by the method described here. Radioimmunological determinations of IGF I in whole serum should be interpreted with caution even if the assay is carried out under disequilibrium conditions and if the double antibody technique is used for the separation of antibody-bound and free IGF. IGF I values obtained under these conditions in whole serum are 30–40% lower than after separation of IGF from the carrier protein (Walter et al. in preparation). The use of equilibrium conditions for radioimmunological IGF determinations in whole serum leads to erroneous results, as also shown by Furlanetto et al. (1977) for SM-C. This also explains why the results of Reber & Liske (1976) are completely different from ours, although they used the same antisera.

In normal subjects immunoreactive IGF I accounts for approximately one third (50 µU/ml) of total biologically active IGF measured by the fat pad method (≈ 150 µU/ml, Table 1). The difference (≈ 100 fat pad µU corresponding to ≈ 300 ng; see Methods) would then represent IGF II. However, if the latter is determined separately by IGF II-RIA, one finds between 600 and 1000 ng/ml corresponding to ≈ 180–300 fat pad µU/ml (manuscript in preparation). The reason for this discrepancy is not clear at present. If one calculates the results of the IGF I and II RIA in fat cell µU (1 ng IGF I ≈ 0.18 fat cell µU; 1 ng IGF II ≈ 0.54 fat cell µU; see Methods) this discrepancy becomes much smaller. Similar as yet unexplained discrepancies between immunoreactive and biologically active IGF are found in acromegalic sera.

The elevation of immunoreactive IGF I in serum of acromegalic patients and its reduction in hypopituitary and Laron dwarfs is consistent with reports on serum somatomedin levels in these patients (Chochinov & Daughaday 1976; van Wyk & Underwood 1978; Furlanetto et al. 1977; Hall et al. 1979). Thus, besides all the other properties shared with the somatomedin, IGF I resembles the latter also with respect to its GH-dependence.

The finding that total IGF levels are increased in acromegaly, which confirms and extends earlier observations (Schlumpf et al. 1976; Zapf et al. 1977; Heinrich et al. 1978), is in contrast to the report of Megyesi et al. (1975, 1977), who found normal IGF levels in acromegalic patients. This
discrepancy may not only be due to the different methodology (radioreceptor assay with rat liver membranes) applied by these investigators, but to the fact that they used $^{[125]}$MSA (multiplication stimulating activity) instead of $^{[125]}$NSILA-S (IGF) in one of their studies (Megyesi et al. 1977) and that the $^{125}$I-labelled IGF used by them in another study (Megyesi et al. 1975) was only $\sim 20\%$ pure and contained mostly IGF II (as recently determined by RIA). The latter, like MSA, binds much better to rat liver membranes than IGF I and competes much more potently for $^{[125]}$IGF II and $^{[125]}$MSA binding (Rechler et al. 1977 and in press). Therefore, Megyesi et al. (1975, 1977) may have determined IGF II rather than IGF I in their assay. Immunoassay of IGF II is not elevated in acromegaly, but is in the same range as in normal subjects (600–1000 ng/ml; manuscript in preparation). This would explain why Megyesi et al. (1975, 1977) found normal IGF levels in acromegalic sera.

The finding that only IGF I, but not IGF II is elevated in acromegalic indicates that it is mainly IGF I which is under GH control. However, in hypopituitary patients immunoassay of IGF II is significantly decreased (manuscript in preparation).

The lack of correlation between GH and IGF I levels in acromegalic as well as the observation that IGF I can be normal despite elevated GH (Table 2) or that it can be increased despite normal GH (Table 2) does not speak against the GH dependence of IGF I. It may rather point to a divergence between the immunoreactivity and the biological potency of GH as has recently been reported (Lewis et al. 1978). A similar lack of correlation between immunoassay of GH and GH activity in rats has already been reported by Yde (1969). This fits the clinical observation that the severity of the acromegalic symptoms is not adequately reflected by the level of immunologically measured GH.

The decrease of the IGF binding activity in hypopituitary dwarfs is in accord with results obtained in rats (Kaufmann et al. 1978; Schalch et al. 1979). It might suggest that the IGF carrier protein may be under the control of GH also in the human. The decrease of the IGF binding activity in Laron dwarfs despite normal or increased GH levels (Laron 1974) would not be contradictory to this concept, since in the latter patients the impairment of the GH receptor of the liver (Chochinov & Daughaday 1976) would explain both the decreased production of IGF and its binding protein. However, the observation that IGF binding is not elevated in acromegalic patients and decreased in liver cirrhosis, insulin-deficiency and tumour hypoglycaemia suggests that additional factors like the functional state of the liver, insulin and other, still unknown mechanisms also play a role in the regulation of the IGF carrier protein.

With respect to the IGF I-RIA, it should be mentioned, that both somatomedin A and somatomedin C cross-react remarkably (Fig. 2). Hall et al. (1979) reported that IGF I was even 10-times more potent than somatomedin A in their somatomedin A-RIA. Furthermore, the displacement curves of IGF I and somatomedin C in the somatomedin C-RIA were superimposable (van Wyk et al. 1979). Antibodies directed against the synthesized C-region of IGF I cross-react with IGF I and somatomedin C in an identical manner (Hinz et al. 1980). All of these observations suggest that IGF I and somatomedin C may be identical and that somatomedin A either contains IGF I or shares it with a similar immunoreactive site. The results of Furlanetto et al. (1977) obtained with their somatomedin C-RIA in whole sera of normal, acromegalic and hypopituitary patients under disequilibrium conditions underline this reasoning: the somatomedin C values determined by these investigators were between 75 and 200 ng/ml in normal sera, which is close to our IGF I levels. Furthermore, Furlanetto et al. (1977) reported a greater than 30-fold difference between the somatomedin C level in acromegalic and hypopituitary dwarfs. We find a similar difference for IGF I. The similarity between IGF I and the somatomedins is further underlined by the results obtained in sera of cirrhotic patients. IGF I levels are as low as in hypopituitary patients and Laron dwarfs. Somatomedin measured by bioassay (sulphation assay) or by radioreceptor assay has also been found to be decreased in cirrhotics (Chochinov & Daughaday 1976; Takano et al. 1977; Schimpff et al. 1977). The decrease of total IGF and of IGF I in this disease may be taken as indirect evidence that the liver is involved in the production of both IGF I and IGF II, although the latter has not yet been determined separately. Evidence for the production of somatomedins or IGF by the liver has been obtained by several workers in this field (for review see Chochinov & Daughaday 1976; van Wyk & Underwood 1978; Schalch et al. 1979; Schwander & Hauri 1979; Rechler et al. 1979; Haselbacher et
al., in press). The decrease of the IGF-binding activity in cirrhotic patients may point to the liver also as the site of production of the IGF carrier protein. More direct evidence for this stems from rat liver perfusion and cell and tissue culture experiments (Schalch et al. 1979; Schwander & Hauri 1979; Moses et al. 1979; Binoux et al., in preparation) where a time-dependent production of somatomedin carrier protein has been measured in the perfusate and culture medium. GH levels were elevated above normal in 10 of our 25 cirrhotic patients, as also reported by Conn & Daughaday (1970), but they did not correlate with total IGF nor with IGF I.

Hypoglycaemia caused by extrapancreatic tumours has recently been attributed to an elevation of NSILA-S (IGF) (Megyesi et al. 1974; Hyodo et al. 1977). However, in none of our patients could this finding be confirmed. Total IGF levels were within the normal range or even decreased in the biological assays as well as in the protein binding assay. Immunoreactive IGF I was decreased in 5 of 10 patients. In 5 patients, whose blood sugar levels had become normal following removal of the tumour, IGF values remained unchanged or increased after the operation (Table 3). The reason for this discrepancy between the results of Megyesi et al. (1974) and our results is not clear. As mentioned above, the determinations of Megyesi et al. (1974) were carried out in a radioreceptor assay using rat liver membranes and a [125I]IGF preparation which was only 20% pure. It may have contained a labelled impurity cross-reacting with some unknown factor present in these patients' sera. It is unlikely, however, that this factor was large mol. wt. non-suppressible insulin-like protein (NSILP); (Poffenbarger 1975), which has been reported to be elevated in tumour hypoglycaemia (Ganda et al. 1978), since Megyesi et al. (1974) performed the radioreceptor assay with small mol. wt. serum fractions obtained by acidic gel filtration. Like IGF, somatomedin C determined by the somatomedin C radioreceptor assay (van Wyk & Underwood 1978) or by the somatomedin C-RIA has not been found to be elevated in extrapancreatic tumour hypoglycaemia (van Wyk, personal communication). Although the increased ratio between total IGF levels and IGF binding protein as observed in patients with tumour hypoglycaemia would appear to be associated with increased levels of free IGF, this is unlikely to explain hypoglycaemia. Otherwise, hypoglycaemia or normoglycaemia might be expected in acromegaly where IGF is increased without a concomitant elevation of the binding activity. Contrary to this expectation, acromegalis have glucose intolerance or are even hyperglycaemic. Therefore, our results are not compatible with the concept that tumour hypoglycaemia is caused by an elevation of IGF.

The finding that patients with hyperprolactinaemia have normal total IGF and immunoreactive IGF I levels is in accord with the results of Furlanetto et al. (1977) who reported normal somatomedin C as determined by RIA, in 3 patients with hyperprolactinaemia.

Insulin-deficient juvenile-type diabetics showed a relatively wide variation of their serum IGF levels, the mean lying within the normal range. This may be surprising in view of earlier findings obtained in pancreatectomized dogs (Eigenmann et al. 1977), whose IGF levels fell after pancreatectomy and remained low until insulin therapy restored IGF levels to near normal. It is conceivable that, in contrast to pancreatectomy, residual B-cell function, which prevails in most juvenile-type diabetics after the detection of their disease (Madsbad et al. 1978) is still sufficient to maintain their IGF levels within the normal range. The physiologic significance of the most conspicuous decrease of IGF binding in the sera of our diabetic patients remains unclear until methods are available which permit to determine the bioavailability of serum IGF in vivo.

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