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Effects of recombinant insulin-like growth factor I on insulin secretion and renal function in normal human subjects

(growth hormone/glomerular filtration rate/C peptide)

HANS-PETER GULER*, CHRISTOPH SCHMID, JÜRGEN ZAPF, AND E. RUDOLF FROESCH

Metabolic Unit, Department of Medicine, University Hospital, 8091 Zürich, Switzerland

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ABSTRACT **Insulin-like growth factor I (IGF-I) is an important mediator of growth hormone (GH) action and it appeared tempting to evaluate possible clinical applications. Recombinant IGF-I was infused s.c. at a dose of 20 μg/kg of body weight per hour during 6 days in two healthy adult subjects. Blood glucose and fasting insulin levels remained within normal limits and IGF-II levels were suppressed. In contrast to insulin, fasting C peptide levels were decreased. GH secretion was also suppressed by IGF-I. Our preliminary data allow us to distinguish between the effects of GH *per se* and those of IGF-I: GH causes hyperinsulinism, whereas IGF-I leads to decreased insulin secretion. Glomerular filtration rate, as estimated by creatinine clearance, increased to 130% of preinfusion values during the IGF-I infusion. Total creatinine and urea excretion remained unchanged. We conclude that IGF-I influences kidney function and, in contrast to GH, exerts an insulin-sparing effect. It may be speculated that the therapeutic spectrum of IGF-I is quite different from that of GH.**

Recombinant growth hormone (GH) is increasingly used in clinical medicine because of its anabolic properties. Most, but not all, of its effects appear to be mediated by the somatomedins/insulin-like growth factors (IGFs). The administration of IGF-I has been documented to stimulate growth in several pathophysiological states characterized by low IGF-I levels—e.g., hypophysectomized rats (1), diabetic rats (2), and Snell dwarf mice (3). In addition, prolonged s.c. infusions of IGF-I to hypophysectomized rats also led to a marked weight gain of the kidneys surpassing that observed under GH infusions (4). Similar findings have been reported in Snell dwarf mice (3). IGF-I has recently been shown to lower blood glucose in man after i.v. bolus injection (5).

In this study we investigated hormonal and metabolic changes in normal healthy adults during constant infusions of high doses of IGF-I not leading to hypoglycemia. We report inhibition of insulin secretion by IGF-I during a 6-day infusion period and an increase of glomerular filtration rate.

METHODS

Subjects and Experimental Protocol. Two of the authors (ages 38 and 34 years) served as normal subjects in this clinical trial. Their body weights and heights were 65 kg/172 cm and 59 kg/172 cm, respectively. They had no clinical evidence of illness and did not take any medication. Routine hematology, blood chemistry, and endocrine parameters were within normal limits. Baseline values were obtained during an initial control period after which IGF-I was administered by continuous s.c. infusion over 6 days. This method and duration of administration were selected to reach constant serum levels of IGF-I. The study was concluded with a second control period. Food intake was strictly controlled during the whole study and consisted of 2500 kcal/day (1 kcal = 4.18 kJ); 25% protein—i.e., 1.9 g of protein/kg of body weight, 20% fat, and 55% carbohydrate. The protocol was approved by the Ethics Committee of the University Hospital of Zürich. Venous blood was obtained every morning between 6 and 7 a.m. It was immediately placed on ice and centrifuged 1 hr later. Serum or plasma was stored in 1-ml portions at −20°C. All assays were done in samples that had not been thawed before. Twenty-four-hour urine collections were obtained throughout the study (6 a.m. to 6 a.m.). Several aliquots were stored at −20°C.

In subject 1 both control periods lasted for 3 days. On the first day of treatment, IGF-I was started at an arbitrary dose of 32 μg/kg of body weight per hour. This dose of IGF-I caused hypoglycemia (see *Results*). Twenty micrograms per kg of body weight per hour over the next 5 days was found to be safe, and blood glucose remained normal. The total amount of IGF-I infused over 6 days was 184 mg. In subject 2 both control periods were 5 days. IGF-I was infused at the same dose as in subject 1 (20 μg/kg of body weight per hour) over 6 days. The total amount of IGF-I infused was 167 mg.

GH Secretory Capacity. GH secretory capacity was assessed by three stimulation tests with GH-releasing factor [GRF-(1–44); Sanofi, Basel, Switzerland]. The first test was done before the infusion; the second test was done on the sixth day of IGF-I infusion; the third stimulation test was performed 14 and 16 days after the end of the infusion in subject 1 and subject 2, respectively. During the test, the subjects were in supine position. After an overnight fast, 80 μg of GRF was injected i.v. Blood samples were drawn at −15, 0, +15, 30, 45, 60, and 120 min.

Nocturnal GH Surges. Nocturnal GH surges were evaluated in subject 2 during the sixth night from 10 p.m. to 6 a.m. under IGF-I infusion and on a second occasion 5 weeks later. Blood samples were drawn every 20 min.

Recombinant Human IGF-I. Recombinant human IGF-I was a generous gift of W. J. Rutter (Emeryville, CA) and J. Nüesch (Basel). It has been characterized chemically and biologically and found to be identical to highly purified extracted human IGF-I (R. E. Humbel, Zürich, batch I/4). The same material had been used in a previous study in man (5). IGF-I was dissolved in 0.1 M acetic acid. Twenty-five microliters was infused per hour by a miniaturized insulin infusion device (MRS 1 infusor/Disetronic AG, Burgdorf, Switzerland). The infusor cartridge containing the IGF-I was refilled after 3 days. A microcatheter was placed under the skin of the abdomen. It was changed after 3 days and placed at a location distant from the first one.

Assays. Total IGF-I and IGF-II and free IGF-I were measured by RIA as described (6, 7). Blood glucose was determined by a YSI 23A glucose analyzer. Commercially

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Abbreviations: IGF, insulin-like growth factor; GH, growth hormone; GRF, GH-releasing factor.

*To whom reprint requests should be addressed.

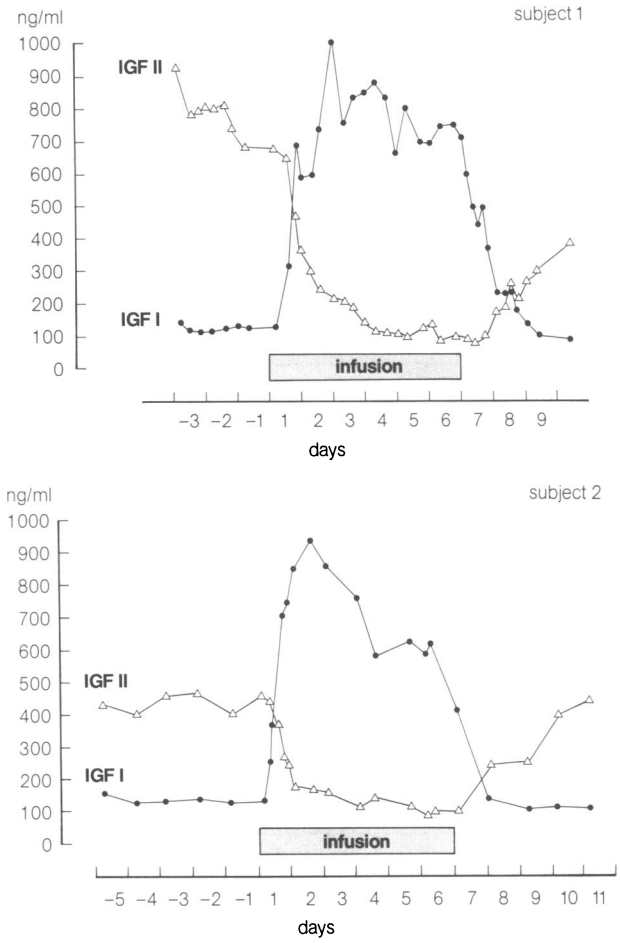

FIG. 1. Serum levels of total IGF-I and IGF-II in two subjects before, during, and after 6 days of constant s.c. infusion of recombinant IGF-I in a dose of 20 μg/kg of body weight per hour.

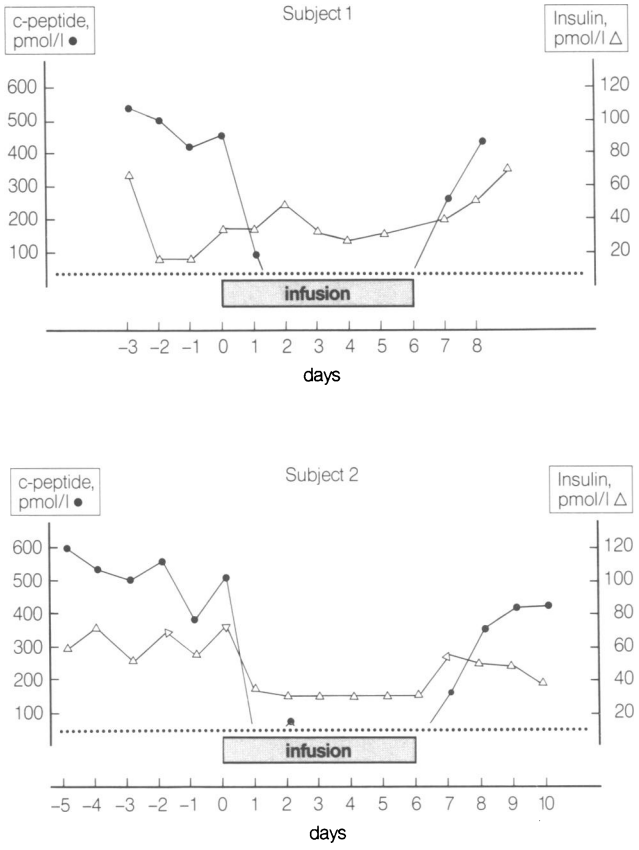

FIG. 2. Serum levels of C peptide and insulin in two subjects before, during, and after 6 days of constant s.c. infusion of recombinant IGF-I in a dose of 20 μg/kg of body weight per hour. The dotted line represents the detection limit of the assay.

available kits were used to determine GH (human GH-RIA-Kit, Medipro, Teufen, Switzerland), insulin (RIA-GNOST Insulin, Behringwerke AG, Marburg, Germany), and C peptide (RIA kit for human C peptide, Medgenix, Fleurus, Belgium). All other analyses were performed in the Department of Clinical Chemistry of the University Hospital of Zürich.

Comparison with Human GH. A similar experiment with recombinant human GH (gift from Nordisk Gentofte A/S, Gentofte, Denmark) had been performed in subject 1 6 months before. After an initial control period of 3 days, GH was injected s.c. in a dose of 3 mg twice daily over 5 days.

RESULTS

After 3 control days (without any hormone) the IGF-I infusion was started at 6:30 a.m. at a rate of 32 μg/kg of body weight per hour. Blood glucose was 4.4 mmol/liter, the serum level of total IGF-I was 120 ng/ml, and that of free IGF-I was 20 ng/ml. Thirteen and one-half hours later, after infusion of a total of 28.1 mg of IGF-I and 8 hr after the last meal, blood glucose had fallen to 2.6 mmol/liter without any clinical signs of hypoglycemia. By that time the serum level of total IGF-I had reached 683 ng/ml, and the serum level of free IGF-I was 123 ng/ml. The infusion was stopped overnight and resumed again on the next morning at 6:30 a.m. at a rate of 20 μg/kg of body weight per hour. This dose was kept constant during the subsequent 5 days in subject 1 and was also used during the whole 6-day infusion period in subject 2.

Apart from the hypoglycemic episode in subject 1 on the first day of the IGF-I infusion, no other such event was recorded. Both subjects felt normal throughout the study. Blood pressure, pulse rate, and body temperature remained stable. Body weight was in the range of 64.8–65.5 kg in subject 1 and of 58.8–59.2 kg in subject 2, respectively.

Blood glucose was monitored daily after overnight fasting (at least 12 hr) and remained between 3.7 and 4.4 mmol/liter throughout the study. In subject 2 blood glucose levels measured every hour during one night of IGF-I infusion were between 3.6 and 4.4 mmol/liter.

Within 2–4 hr after starting the infusion, IGF-I levels rose and reached levels of 700 ng/ml after 13–14 hr (Fig. 1). Peak levels in the two subjects were 980 and 920 ng/ml, respectively. When the infusion was stopped, IGF-I levels fell in the normal range within 1 day. Serum levels of IGF-II were suppressed to 100 ng/ml during high IGF-I serum concentrations in both subjects and returned to normal only 4–5 days after the infusion was stopped. Free IGF-I levels during the control days were between 15 and 20 ng/ml and between 50 and 80 ng/ml during continuous IGF-I infusion.

Serum Levels of Insulin and C Peptide. Serum levels of insulin and C peptide were measured every morning in fasting serum samples (Fig. 2) and in subject 2 also every hour during the sixth night of IGF-I infusion as well as during a night 5 weeks after the infusion. All fasting insulin values before, during, and after the infusion were between 17 and 77 pmol/liter. Similar values were found in subject 2 during the sixth night and 5 weeks after the infusion in blood samples taken every hour (51–77 pmol/liter). Fasting insulin levels in subject 2 tended to be slightly lower during the infusion. Fasting serum levels of C peptide drawn every morning before and after the infusion were 450 ± 80 pmol/liter (mean

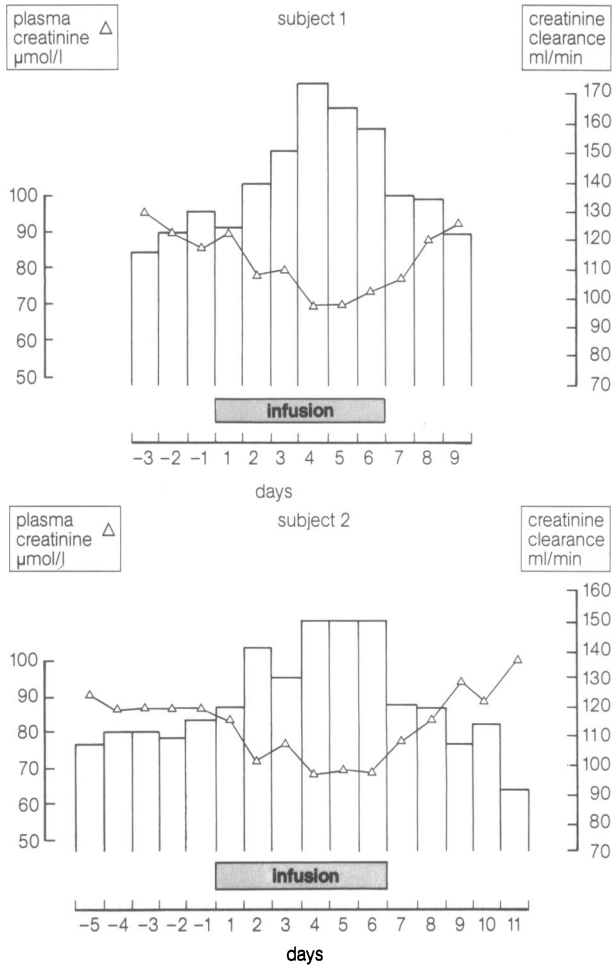

FIG. 3. Plasma levels of creatinine and creatinine clearance (represented by bars) in two subjects before, during, and after 6 days of constant s.c. infusion of recombinant IGF-I in a dose of 20 μg/kg of body weight per hour.

± SD), whereas during the infusion period all but two values (95 and 69 pmol/liter) were below the detection limit of the assay (50 pmol/liter). Serum levels of C peptide were also measured during the sixth night of the infusion in subject 2: all but one value at 10 p.m. (98 pmol/liter) were below 50 pmol/liter. During a control night 5 weeks after the infusion, C peptide levels were 650 ± 440 pmol/liter.

Plasma Levels of Creatinine and Creatinine Clearance. Initially, the two subjects had plasma creatinine levels of 90 and 87 μmol/liter, respectively (Fig. 3). Creatinine clearances were 122 and 111 ml/min. From day 2 through day 6 of the IGF-I infusion plasma creatinine was reduced to 73 μmol/liter in both subjects (corresponding to 81% and 84% of the baseline values), and creatinine clearances rose to 157 and 144 ml/min (corresponding to 129% and 130% of control) and returned to preinfusion levels within 1–2 days after the infusion was stopped. Urinary creatinine excretion per 24 hr remained constant throughout the whole study [15.8 ± 1.1 and 14.2 ± 1.3 mmol (mean ± SD) in subjects 1 and 2, respectively].

Plasma levels of urea were 5.8 and 7.6 mmol/liter in subjects 1 and 2, respectively, during the initial control period. They fell to 3.4 and 4.2 mmol/liter (corresponding to 59% and 55% of baseline) within 2 days and remained at these levels until the infusion was stopped. Plasma levels of uric acid decreased from 268 and 311 μmol/liter to 137 and 180 μmol/liter (51% and 58% of baseline, respectively) (Fig. 4). Urinary excretion of urea was constant before, during, and

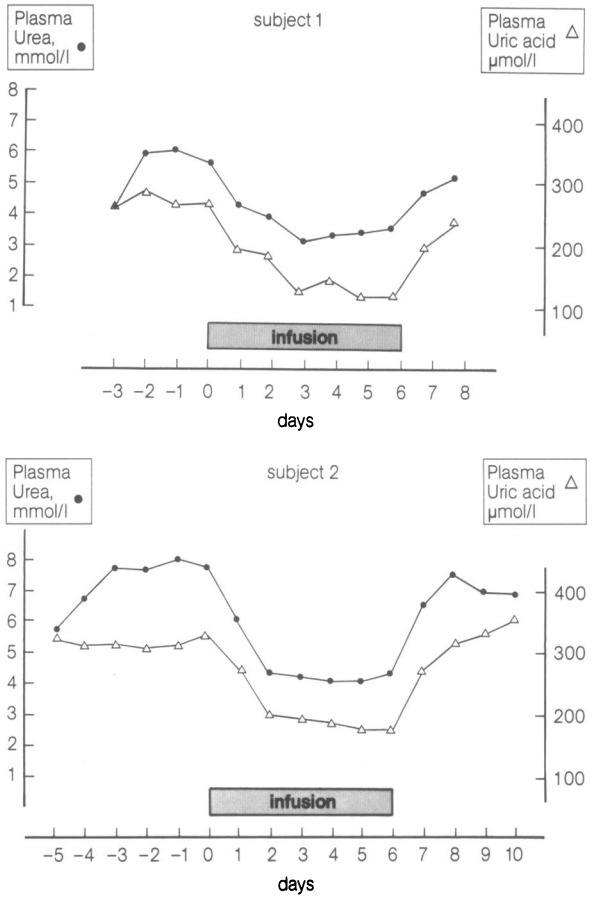

FIG. 4. Plasma levels of urea and uric acid in two subjects during constant s.c. infusion of recombinant human IGF-I in a dose of 20 μg/kg of body weight per hour.

after the infusion [477 ± 66, 467 ± 46, and 463 ± 56 mmol/24 hr (mean ± SD), respectively].

Changes of GH. In subject 1 GH curves obtained after GRF stimulation before and during the IGF-I infusion were similar (Fig. 5). However, 14 days after the infusion was stopped, the peak level at 45 min was 49.8 ng/ml—i.e., three times higher than in the two other tests. A clear inhibition of the GH response was observed during the IGF-I infusion in subject 2: maximal GH peak responses at 30 min were 33.5, 3.2, and 76.6 ng/ml before, during, and 6 days after the infusion, respectively. During the sixth night of IGF-I infusion one single surge of GH was recorded as compared to three much higher spikes during a control night 5 weeks later. The area under the GH curve during infusion was 19% of that during the control night (Fig. 6).

The results of the GH experiment in subject 1 are summarized in Table 1. Fasting serum levels of insulin and C peptide before treatment with GH were 92 ± 2 pmol/liter and 450 ± 46 pmol/liter (mean ± SD), respectively. During GH treatment respective values were 678 ± 452 pmol/liter and 2509 ± 820 pmol/liter. Body weight increased 2 kg.

DISCUSSION

Infusions of IGF-I s.c. in a dose of 20 μg/kg of body weight per hour to healthy adult subjects were safe, did not influence general well being, blood pressure, pulse rate, and body temperature, and did not lead to hypoglycemia. Morning fasting serum levels of insulin remained in the normal range but C peptide levels were suppressed during the infusion of IGF-I. Creatinine clearance was increased, suggesting renal hyperfiltration.

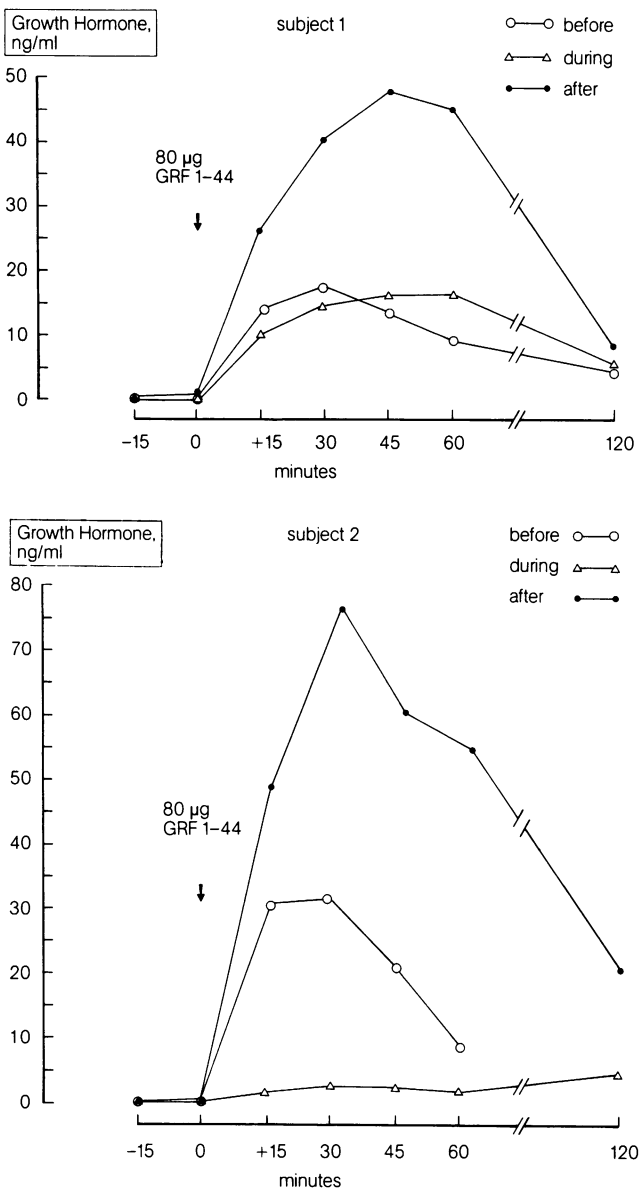

FIG. 5. GH levels after stimulation with 80 μg of GRF [GRF-(1–44)] before, during, and after constant s.c. infusion of recombinant IGF-I in a dose of 20 μg/kg of body weight per hour in two subjects over 6 days.

Half-life and serum levels of C peptide are not decreased by renal hyperfiltration in acromegaly or during GH treatment (8, 9). Therefore, normal insulin levels in the presence of decreased C peptide levels, as observed in the present study, must indicate a decrease of insulin degradation and consecutively a prolongation of its half-life. In fact, IGF-I has been shown to interfere with insulin degradation in cultures of human hepatoma cells (HepG2) (10), preparations of rat liver plasma membranes (11), and preparations of rat kidney plasma membranes (12).

The results of these preliminary experiments clarify the still contradictory issue about which metabolic alterations of acromegalic patients must be attributed to GH itself and which attributed to IGF-I. During GH administration in subject 1, insulin levels increased rapidly. In acromegalic patients, insulin secretion is always found to be increased, presumably because GH in excess leads to insulin resistance (8, 9).

One can speculate that under the infusion of IGF-I insulin sensitivity may actually increase for two main reasons: (*i*) low GH secretion is usually associated with increased insulin

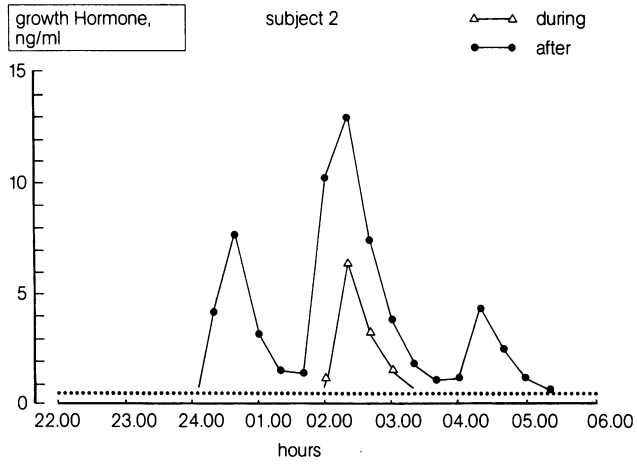

FIG. 6. GH levels in subject 2 from 10 p.m. to 6 a.m. during the sixth night of IGF-I infusion and during a control night 5 weeks later. The area under the curve during IGF-I infusion is 19% as compared with the control night. The dotted line represents the detection limit of the assay.

sensitivity (13), and (*ii*) low insulin levels are also supposed to increase insulin sensitivity at receptor and postreceptor levels (14). Therefore, IGF-I may be of interest for investigation of conditions characterized by insulin resistance such as type II diabetes, obesity, and hyperlipidemia.

IGF-I had effects on renal function. Creatinine clearance increased by 30% above preinfusion values in both subjects. This increase of glomerular filtration rate resulted in a fall of creatinine plasma levels and decreased plasma levels of urea and of uric acid. Daily urinary creatinine and urea excretion remained constant. The clearance of inulin and *p*-aminohippuric acid was not determined in the present study. It is known that glomerular filtration rate is increased in acromegaly (15). Treatment of healthy adults for 4 days with large doses of GH results in an increase of glomerular filtration rate and renal plasma flow (16). Administration of GH s.c. in subject 1 led to a low increase of endogenous IGF-I levels that paralleled the rise of the creatinine clearance. During the infusion of IGF-I, glomerular filtration rate rose and remained steady from the second day onward. We conclude that the increase of glomerular filtration rate after GH administration is due to the rise of IGF-I serum levels. Hyperfiltration may, in the long run, be harmful for the kidney, as suspected in the case of diabetics (17). Whether or not IGF-I may be of any use for patients in renal failure remains to be seen.

There was no increase in body weight during IGF-I infusion, indicating that IGF-I does not cause any significant fluid retention. In contrast, sodium retention and expansion of extracellular volume are well known side effects of GH

Table 1. Effects of two s.c. injections of 3 mg of recombinant human GH per day in subject 1 from day +1 to day +5

Day	IGF-I, ng/ml	IGF-II, ng/ml	Creatine clearance, ml/min	N intake − urinary N excretion, g/24 hr
−3	107	742	120	+1.3
−2	103	627	115	+0.7
−1	109	646	135	−0.4
+1	137	646	123	+3.8
+2	277	452	122	+6.2
+3	497	532	128	+9.5
+4	519	491	160	+7.5
+5	546	517	145	+7.5

The control period was from day −3 to day −1.

treatment (18, 19). It appears, therefore, that IGF-I is not responsible for fluid retention in acromegaly and that fluid accumulation may be a direct effect of GH.

IGF-II is another peptide of the somatomedin family with similar but less potent *in vivo* actions than IGF-I (1). It shares common carrier proteins with IGF-I. After 2 days of IGF-I infusion, IGF-II serum levels were depressed. In contrast, IGF-II levels are decreased only to a minor extent in acromegaly (6) and GH-treated healthy adults. The rates at which IGF-II levels fell and rose again at the end of the IGF-I infusion were quite different. The rapid fall of IGF-II may be due to its displacement from the specific binding proteins by the excess of infused IGF-I, followed by rapid degradation. The subsequent slow rise of IGF-II must be attributed to *de novo* synthesis. One could speculate that high levels of IGF-I exert a negative feedback on the biosynthesis of IGF-II either directly and/or by means of suppression of GH secretion. In fact, the results of the present study indicate that during prolonged IGF-I infusions GH secretion is impaired, suggesting a negative feedback of IGF-I on GH secretion. In subject 2 the GH response to GRF stimulation was almost completely abolished during IGF-I infusion, and the number and amplitude of nocturnal GH spikes as well as the area under the GH curve were reduced. In both subjects GRF-stimulated GH release rebounded after cessation of IGF-I infusion. GH secretion of pituitary cells *in vitro* and in animals *in vivo* was shown to be inhibited by IGF-I, suggesting a negative feedback loop (20–23).

IGF-I infusions over a 6-day period had no effect on total urinary nitrogen excretion—i.e., no apparent protein-sparing effect. In contrast, GH treatment of healthy adults results in a positive nitrogen balance within 2 days. This discrepancy between GH and IGF-I treatment may have several roots. (*i*) IGF-I infusions do not only suppress GH secretion but also lead to a decrease of IGF-II levels. It can be argued that GH and/or IGF-II may be required for a full anabolic response to IGF-I. (*ii*) GH probably has direct anabolic effects on muscle that are not mediated by IGF-I. A positive nitrogen balance would be expected only if muscle, the major organ of protein storage and synthesis besides the liver, were affected. In an earlier study in hypophysectomized rats, IGF-I stimulated weight gain of the gastrocnemius and soleus muscles to a lesser degree than GH (4).

In an initial dose-finding trial, free IGF-I levels rose to 123 ng/ml, which is six times as high as the basal free IGF-I level. In an earlier study we demonstrated that the i.v. injection of 100 μg of IGF-I per kg of body weight in healthy adults causes acute hypoglycemia (5). In those experiments, the highest serum levels of free IGF-I were around 350 ng/ml. Serum levels of free IGF-I above 100 ng/ml are likely to cause hypoglycemia.

We tried to define the margins of safety in which IGF-I can be administered over a prolonged period of time to healthy subjects. So far, the major apparent "side effect" of IGF-I was hypoglycemia. Future investigations will clarify the clinical potential of recombinant human IGF-I in disease conditions characterized by decreased insulin sensitivity and in renal failure.

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