Increased weight gain, nitrogen retention and muscle protein synthesis following treatment of diabetic rats with insulin-like growth factor (IGF)-I and des(1–3)IGF-I

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We have examined the effects of infusing recombinant human growth hormone (hGH), insulin-like growth factor-I (IGF-I), the truncated IGF-I analogue, des(1–3)IGF-I, and insulin over a 7-day period in streptozotocin-induced diabetic rats. IGF-I at a dose of 1.05 or 1.08 mg/kg per day in two experiments increased body weight and nitrogen retention above those of vehicle-infused controls to about 30 % of the improvement achieved with 25 or 30 units of insulin/kg per day, but only in the second experiment were the differences statistically significant ($P < 0.05$). A 2.5-fold higher IGF-I dose, or des(1–3)IGF-I at 1.08 mg/kg per day, gave effects that were approx. 70 % of those obtained with insulin. hGH at 1.38 mg/kg per day was not effective. The IGF peptides, unlike insulin, did not ameliorate the diabetic glucosuria. The improvements in nitrogen balance could be accounted for in part by increases in muscle protein synthesis. Muscle protein breakdown, as assessed by 3-methylhistidine excretion, was inhibited by insulin, but not by the IGF peptides. Carcass fat increased substantially following insulin administration. This did not occur with the IGF peptides, suggesting that IGF predominantly stimulates the growth of lean tissue. IGF-I concentrations and IGF-I-binding proteins in plasma were increased by IGF-I, especially at the higher dose, whereas hGH produced only a transient increase in IGF-I. Des(1–3)IGF-I induced binding proteins, but had only a slight effect on measured IGF-I concentrations. We conclude that IGF peptides stimulate muscle protein synthesis and improve nitrogen balance in diabetes without obviously influencing the abnormal carbohydrate metabolism. Moreover, des(1–3)IGF-I is at least as potent as the full-length IGF-I.

INTRODUCTION

Induction of diabetes in young rats by streptozotocin (STZ) leads to hyperglycaemia, glucosuria and, despite an increased food intake, a cessation of growth [1]. Muscle protein metabolism is altered, with decreased rates of synthesis and generally increased rates of breakdown observed [2–5]. These effects are reversed by the administration of insulin [1–5]. The plasma concentrations of insulin-like growth factor-I (IGF-I), as well as the main circulating IGF-binding protein, are also decreased by STZ [6,7]. A role for IGF-I independent of insulin has been suggested because the chronic administration of IGF-I to STZ-diabetic animals leads to a substantial increase in the growth rate without producing any changes in blood glucose or insulin concentrations [8,9]. Similar effects are not produced by growth hormone, presumably because this hormone does not increase blood IGF-I, unlike the situation in normal animals [8]. One implication of these results is that IGF-I may restore the protein metabolism and nitrogen balance of the diabetic rats to normal without having effects on carbohydrate metabolism. In the present investigation we have tested this hypothesis. Specifically, we have examined the effects of IGF-I and the truncated IGF analogue, des(1–3)IGF-I, on growth rate, nitrogen balance, muscle protein metabolism and glucosuria in STZ-diabetic rats.

MATERIALS AND METHODS

Peptides

Recombinant human growth hormone with *N*-terminal methionine (hGH), recombinant human IGF-I and recombinant

des(1–3)IGF-I were supplied by Genentech Inc., South San Francisco, CA, U.S.A. The peptides were administered to animals via Alzet model 2001 osmotic pumps that delivered 0.92 μ l/h (Alza, Palo Alto, CA, U.S.A.). The hGH was dissolved in water to give a concentration of 10 mg/ml, and the IGF peptides were dissolved in 0.1 M-acetic acid to give concentrations of 7.6 or 18.9 mg/ml as indicated in the individual experiments. Pumps for control animals were filled with 0.1 M-acetic acid. Isophane insulin, obtained from CSL-Novo Pty. Ltd., North Rocks, N.S.W., Australia, was injected subcutaneously.

Animals

Male Hooded-Wistar rats were bred in the CSIRO Division of Human Nutrition. From 100 g body wt. they were maintained in Techniplast metabolism cages at 25 °C with lighting controlled over a 12 h-light/12 h-dark cycle. The animals had continued access, unless indicated otherwise, to water and a high-carbohydrate diet containing 180 g of casein and 2.5 g of methionine/kg as the nitrogen source [10]. The protocols for both experiments were approved by the Divisional Animal Care and Ethics Committee following the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Expt. 1

After 3 days acclimatization in the metabolic cages, the animals were anaesthetized by an intraperitoneal injection of 45 mg of sodium methohexital/kg and 30 mg of sodium pentobarbital/kg for the insertion of a jugular cannula. Subsequently the animals were given buprenorphine analgesia as well as an intramuscular injection of Procaine penicillin, and kept warm until fully

Abbreviations used: IGF-I, insulin-like growth factor I; hGH, human growth hormone; STZ, streptozotocin.

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conscious. The animals were allowed access to food for 6–8 h, after which food was withdrawn for 12–16 h. The STZ was then injected intravenously at a dose of 65 mg/kg, and the animals were given free access to food for the remainder of the protocol. On the next day a blood sample was taken for glucose analysis, and those animals with blood glucose exceeding 15 mM were included in the experiment. On this day (day 1) and day 2 all the diabetic animals were injected subcutaneously with isophane insulin (25 units/kg). Insulin-maintained control animals continued to receive the same dose daily throughout the experiment. Each animal was anaesthetized on day 3 by intravenous injection of 9 mg of alphaxolone/kg and 3 mg of alphacalone acetate/kg, a filled osmotic pump was inserted subcutaneously in the scapular region and the wound sutured. The average body weight of the animals on this day was 160 g. From day 1 to day 10 inclusive, 24 h collections of urine and faeces and measurements of food intake and body weight as well as daily blood samples from the jugular cannula were obtained. At the end of the last daily collection period, each animal was restrained gently and 10 ml (per kg body wt.) of a solution containing 150 mM-M-phenylalanine, 77 mM-NaCl and 50 μ Ci of L-[2,6- 3 H]phenylalanine/ml was injected via the jugular cannula. Then 15 min later the animal was stunned and decapitated, and blood was collected into a heparinized tube. The gastrocnemius plus plantaris muscles from one leg were immediately excised and frozen in a clamp precooled in liquid N₂.

The dose rates based on animal weights at the time of pump insertion and the number of animals in each group were: vehicle (7); insulin-maintained, 25 units/kg per day (6); IGF-I, 1.05 mg/kg per day (8); hGH, 1.38 mg/kg per day (8).

Expt. 2

This experiment differed from Expt. 1 in the following ways: (a) STZ was administered by intraperitoneal injection at a dose of 70 mg/kg 7 days before insertion of the osmotic pumps; (b) insulin was not injected into any animals before pump insertion; (c) only those animals that had a weight change over the 7-day period after STZ administration between +10 g and –10 g were included in the experiment; (d) the jugular vein was not cannulated; hence blood samples for IGF-I measurements were taken on days 3 and 7 from the tail vein, osmotic pumps were inserted under light ether anaesthesia and the labelled phenylalanine was injected into the tail vein while animals were restrained gently in open-weave cloth; (e) the animals weighed an average of 155 g on day 3; (f) the pelt was stripped from the carcass, the viscera, feet and tail were discarded, and the carcass was kept for nitrogen and fat analyses; (g) the dose rates based on day-3 animal weights were: insulin-maintained, 30 units/kg per day; IGF-I, 1.08 mg/kg per day; IGF-I, 2.69 mg/kg per day; des(1–3)IGF-I, 1.08 mg/kg per day; and (h) there were six animals in each group.

Analytical methods

The nitrogen contents of food, faeces, dried carcass and urine were measured by the Dumas procedure using a Carlo Erba NA1500 Nitrogen Analyser (Milan Italy). Carcass fat was determined gravimetrically after chloroform/methanol extraction of the dried carcass. Urinary 3-methylhistidine was measured by an automated method [11] after initial hydrolysis and ion-exchange-chromatography steps [12]. Muscle RNA contents were measured as described by Munro & Fleck [13]. Muscle protein-synthesis rates were calculated from the incorporation of labelled phenylalanine on day 10 [14] and are expressed both as the fractional synthesis rate, K_s (%/day), and as the synthesis rate per unit of RNA (g of protein/g of RNA per day). Urinary glucose

was measured with a glucose oxidase method supplied by Skalar Analytical BV, Breda, The Netherlands, for use with the Continuous Flow Analyser.

IGF-I was measured in acid/ethanol extracts of plasma by a modification [15] of the original procedure [16]. With the antiserum used, des(1–3)IGF-I and rat IGF-I cross-reacted to about 50 % the extent of human IGF-I. The standard used in the assay was recombinant human IGF-I. In addition to these assays, plasma obtained on day 10 was chromatographed under acid gel-permeation conditions to separate IGF-binding proteins from the growth factors [17]. Samples of the growth factor and binding-protein regions were evaluated in the IGF-I radioimmunoassay. Activity in the latter region was expressed in 'IGF-I equivalents' because the binding proteins are detected as IGF-I, since they compete with antibody for the binding of radioligand [17]. IGF-binding proteins in unfractionated plasma were also detected by the ligand-blot procedure [18] with 125 I-labelled IGF-I as probe. Molecular masses of binding proteins were estimated from the migration of 14 C-labelled Rainbow markers (Amersham International, Amersham, Bucks., U.K.) in an adjacent lane.

Statistics

Values are presented as means on each measurement day. The S.E.M. values are given for all treatment groups in Tables, bar graphs and in the text, as well as for the vehicle and insulin-treatment groups in all Figures, except where they are smaller than the symbols used. The S.E.M. values are not presented for the other treatment groups, to preserve clarity. Between-treatment significance was assessed initially by one-way analysis of variance. Where significance ($P < 0.05$) was achieved, the means have been compared by Student's t test using the pooled estimate of the standard error.

RESULTS

The two experiments examined different aspects of the response to IGF peptides in diabetic rats. In Expt. 1 diabetes was induced by insulin withdrawal at the same time as IGF-I or hGH was administered. Hence the abilities of IGF-I or hGH to prevent diabetes were being investigated. In Expt. 2 the diabetic state had been established before the IGF peptides were administered. Accordingly, this was an investigation of the abilities of IGF-I or des(1–3)IGF-I to reverse the diabetic conditions. Both experiments included vehicle-treated and insulin-treated groups.

IGF-I concentrations and IGF-binding proteins

The IGF-I concentration in insulin-maintained diabetic animals was approx. 650 μ g/l (Fig. 1a) before the insertion of osmotic pumps, and was maintained at this level if the insulin treatment was continued. However, plasma IGF-I fell upon insulin withdrawal in the animals with vehicle pumps, to reach 400 μ g/l by day 10. Addition of hGH from day 3 led to a transient increase in IGF-I on days 5 and 6 before falling to 320 μ g/l on day 10 (Fig. 1a). IGF-I at a dose rate of 1.05 mg/kg per day increased the circulating level of the growth factor to a plateau at approx. 900 μ g/l. These differences between measurements made on acid/ethanol extracts of plasma were reflected in the day-10 analyses after the more-rigorous removal of IGF-binding proteins by acid gel-permeation chromatography (Fig. 1a, inset).

The IGF-I measurements in Expt. 2 (Fig. 2b) were similar when equivalent treatment groups were compared. Thus the plasma IGF-I levels in the insulin-treated group were intermediate between those animals with vehicle in the pumps and

Fig. 1. IGF-I concentrations in plasma from animals in (a) Expt. 1 and (b) Expt. 2

The graphs represent IGF-I concentrations measured in acid/ethanol extracts of plasma collected on the indicated days from animals with osmotic pumps inserted on day 3. The osmotic pumps delivered vehicle ($\circ$), 25 (Expt. 1) or 30 (Expt. 2) units of insulin/kg per day ($\square$), 1.05 (Expt. 1) or 1.08 (Expt. 2) mg of IGF-I/kg per day ($\bullet$), 2.69 mg of IGF-I/kg per day (Δ), 1.38 mg of hGH/kg per day ($\blacksquare$), or 1.08 mg of des(1-3)IGF-I/kg per day ($\blacktriangle$). Values are means with the S.E.M. values presented for the vehicle and insulin groups. The bar graphs reflect IGF-I concentrations (means + S.E.M.) measured in day-10 plasma that had first been chromatographed to remove binding proteins. The symbols beneath the bars indicate the animal groups.

those where IGF-I was delivered at 1.08 mg/kg per day. Pre-treatment levels of the growth factor were slightly lower in Expt. 2 and did not fall significantly, presumably reflecting the continuing diabetic state rather than insulin withdrawal. Substantially high IGF-I concentrations were achieved with the higher dose of IGF-I, whereas the animals treated with des(1-3)IGF-I

Fig. 2. IGF-binding proteins measured in plasma collected on day 10 from animals in (a) Expt. 1 and (b) Expt. 2

The bar graphs indicate binding proteins (means + S.E.M.) measured in the IGF-I radioimmunoassay after first removing the IGF-I by acid chromatography. Binding proteins in plasma pools from each treatment group were also detected by the ligand-blotting technique. The molecular masses of protein standards are shown. The different animal groups are indicated by the following symbols: ($\circ$) vehicle; ($\square$) 25 (Expt. 1) or 30 (Expt. 2) units of insulin/kg per day; ($\bullet$) 1.05 (Expt. 1) or 1.08 (Expt. 2) mg of IGF-I/kg per day; (Δ) 2.69 mg of IGF-I/kg per day; ($\blacksquare$) 1.38 mg of hGH/kg per day; ($\blacktriangle$) 1.08 mg of des(1-3)IGF-I/kg per day.

had plasma IGF-I levels only modestly above the vehicle group on both day 7 and day 10. Removal by acid gel-permeation chromatography of binding proteins in plasma samples from day 10 (Fig. 1b, inset) reflected the measurements made on acid-ethanol extracts, but, as with Expt. 1, higher values were obtained.

IGF-I-binding proteins were assessed on day 10 in the two experiments by inclusion of separated binding-protein fractions in the IGF-I radioimmunoassay as well as by the ligand-blotting technique. Both methods showed a decrease in IGF-binding protein in the hGH-treated group of Expt. 1, as well as increases with the low dose of IGF-I (Fig. 2). Even higher levels of binding protein occurred in those animals in Expt. 2 that had been treated with the high dose of IGF-I. Binding proteins were also increased above values for the vehicle group in the insulin-treated animals in Expt. 2 and in those where des(1-3)IGF-I was administered (Fig. 2). The lower amount of binding protein in the vehicle group of Expt. 2 relative to Expt. 1 may reflect the extra week of diabetes in the second experiment. Ligand blots of plasma pools from each group of animals showed that the changes occurred mostly with binding proteins of molecular masses 40–50 kDa, presumably IGFBP-3 forms (see [19] for nomenclature).

Fig. 3. Body-weight changes (means with S.E.M. for the vehicle and insulin groups) of animals in (a) Expt. 1 and (b) Expt. 2 from the commencement of treatments on day 3

The treatment groups were: vehicle (○); 25 (Expt. 1) or 30 (Expt. 2) units of insulin/kg per day (□); 1.05 (Expt. 1) or 1.08 (Expt. 2) mg of IGF-I/kg per day (●); 2.69 mg of IGF-I/kg per day (△); 1.38 mg of hGH/kg per day (■); 1.08 mg of des(1-3)IGF-I/kg per day (▲).

Body-weight changes and food intake

The insulin-maintained diabetic rats in Expt. 1 lost weight progressively once insulin was withdrawn (Fig. 3a). This loss was not altered by hGH, and although IGF-I treatment at 1.05 mg/kg per day prevented the weight loss, the average weight change over 7 days, at 2.58 ± 8.40 g, was not significantly different from the vehicle group, at -8.52 ± 4.50 g (Fig. 3a). These effects contrasted with a 7-day weight gain of 36.32 ± 2.11 g in animals where the insulin-maintenance regime was continued.

All groups in Expt. 2 gained weight (Fig. 3b), including those with vehicle pumps. Over the treatment period these changes were 17.67 ± 4.65 g (vehicle), 53.91 ± 3.98 g (insulin), 31.22 ± 3.60 g (low IGF-I), 38.91 ± 2.51 g (high IGF-I) and 37.07 ± 1.33 g [des(1-3)IGF-I]. Clearly, a dose of IGF-I similar to that used in the first experiment led to a similar relative response. Thus by day 10 the animals treated with 1.08 mg/kg per day had gained on average 14 g compared with the vehicle group, in this case a statistically significant ($P < 0.05$) difference. With the higher IGF-I dose of 2.69 mg/kg per day in Expt. 2 and with des(1-3)IGF-I the weight gains were significantly more ($P < 0.01$) than in the vehicle group, but not compared with the low dose of IGF-I. The increases in body weight produced by insulin in the two experiments were approximately the same as the 45 g/7 days occurring in normal animals of the same strain and initial weight and fed on the same diet (results not shown).

The food intakes showed no differences that could account for the treatment effects after IGF-I or des(1-3)IGF-I administration. When expressed as g/day over the 7 days, the food intakes

Fig. 4. Urinary glucose excretion (means with S.E.M. for the vehicle and insulin groups) of animals in (a) Expt. 1 and (b) Expt. 2

The treatment groups from day 3 were: vehicle (○); 25 (Expt. 1) or 30 (Expt. 2) units of insulin/kg per day (□); 1.05 (Expt. 1) or 1.08 (Expt. 2) mg of IGF-I/kg per day (●); 2.69 mg of IGF-I/kg per day (△); 1.38 mg of hGH/kg per day (■); 1.08 mg of des(1-3)IGF-I/kg per day (▲).

in Expt. 1 were 16.0 ± 0.8 (vehicle), 17.6 ± 0.4 (insulin), 15.8 ± 1.0 (IGF-I) and 15.6 ± 0.6 (hGH). Food intakes (g/day) in Expt. 2 were 30.4 ± 1.2 (vehicle), 24.9 ± 0.6 (insulin), 30.7 ± 0.7 (low IGF-I), 29.7 ± 1.0 (high IGF-I) and 31.3 ± 0.8 [des(1-3)IGF-I]. The higher food intakes of all groups in the second experiment were probably a consequence of the additional week without insulin, because food intakes progressively increased throughout Expt. 1 after insulin was withdrawn on day 3. It should also be noted that in both experiments the insulin-maintained groups had the lowest food intakes.

Glucose excretion, water intakes and urine volumes

As expected, the urine volumes were much higher in the diabetic animals of both experiments than in those animals where a maintenance dose of insulin was injected daily. The urine volumes of the diabetic rats were only slightly decreased by IGF administration. For example, over the last 3 treatment days, the urine volumes (ml/day) in Expt. 1 were 110 ± 13 , 32 ± 2 , 96 ± 19 and 117 ± 13 for the vehicle, insulin, IGF-I and hGH groups, and in Expt. 2 were 235 ± 9 , 58 ± 7 , 234 ± 5 , 196 ± 19 and 217 ± 9 for the vehicle, insulin, low-dose IGF-I, high-dose IGF-I and des(1-3)IGF-I groups respectively. The water intakes were slightly higher than the urine volumes in all groups (results not shown).

Glucose excretion followed a similar pattern to fluid intake and urine volume. In Expt. 1 (Fig. 4a) glucose excretion was low on day 3 and gradually increased upon insulin withdrawal. By day 10 the excretion rates (mmol/day) had increased to 46 ± 8 , 38 ± 10 and 50 ± 8 in the vehicle, IGF-I and hGH groups, but remained very low at 2 ± 1 in the insulin-maintained animals.

Fig. 5. Cumulative nitrogen retention (means with S.E.M. for the vehicle and insulin groups) of animals in (a) Expt. 1 and (b) Expt. 2 from the commencement of treatments on day 3

The treatment groups were: vehicle (○); 25 (Expt. 1) or 30 (Expt. 2) units of insulin/kg per day (□); 1.05 (Expt. 1) or 1.08 (Expt. 2) mg of IGF-I/kg per day (●); 2.69 mg of IGF-I/kg per day (△); 1.38 mg of hGH/kg per day (■); 1.08 mg of des(1-3)IGF-I/kg per day (▲).

Glucose-excretion rates clearly reflected the altered experimental protocol in Expt. 2. Thus the introduction of insulin treatment led to a decrease in glucose excretion, from 89 ± 4 mmol/day on day 3 to 13 ± 4 mmol/day by day 10. The vehicle and IGF treatment groups, on the other hand, actually increased their glucose output during the 7 treatment days. No significant differences occurred between these four groups on any day (Fig. 4b). It should be noted that, although the animals

receiving the high dose of IGF-I had the lowest rate of glucose excretion among the groups not receiving insulin, this distinction also applied at the pretreatment stage (Fig. 4b).

Nitrogen balance

We have expressed nitrogen retention as the cumulative nitrogen balances (Fig. 5). A trend towards improved retention was seen for the animals treated with the low dose of IGF-I in both experiments. Over the full 7-day treatment period this value in Expt. 1 was 855 ± 160 mg, compared with 619 ± 108 in the vehicle group (not significant). The equivalent values in Expt. 2 were 708 ± 110 mg and 380 ± 100 mg respectively ($P < 0.05$). This trend was greater for the high dose of IGF-I and the des(1-3)IGF-I treatment groups, which retained 980 ± 142 mg and 908 ± 89 mg respectively over the treatment period.

Muscle protein synthesis and breakdown

Muscle protein synthesis was measured at the end of the treatment period after injection of a bolus of labelled phenylalanine. In Expt. 1, the fractional synthesis rates of protein in the gastrocnemius plus plantaris muscles were 5.15 ± 0.42 , 6.95 ± 0.42 , 4.75 ± 0.38 and 8.01 ± 0.20 %/day for the vehicle, IGF-I, hGH and insulin groups respectively (Table 1). The insulin- and IGF-I-treated animals had significantly higher ($P < 0.001$ and $P < 0.01$ respectively) synthesis rates than either the vehicle or hGH-treated animals. When synthesis was expressed relative to tissue RNA to give a measure of the efficiency of protein synthesis, the rates were similar in all four groups (Table 1).

In Expt. 2, the fractional synthesis rates of hind-limb muscle were 6.03 ± 0.52 , 7.05 ± 0.32 , 7.83 ± 0.44 , 7.99 ± 0.12 and 9.05 ± 0.47 %/day for the vehicle, low-dose IGF-I, high-dose IGF-I, des(1-3)IGF-I and insulin treatment groups respectively (Table 1). The des(1-3)IGF-I, high-dose IGF-I and insulin treatment groups had significantly higher ($P < 0.01$) synthesis rates than the vehicle group. As in Expt. 1, the rate of protein synthesis expressed relative to muscle RNA was not significantly different between groups.

Muscle protein breakdown rates can be estimated daily from the excretion rate of 3-methylhistidine. In Expt. 1 this excretion rate averaged $6.5 \mu\text{mol/kg}$ per day on the day before insertion of the pumps and increased gradually in all except the insulin-maintained group, as shown by the upward change in slope of the excretion plots (Fig. 6a). No significant differences, or even

Table 1. Muscle protein synthesis after 7 days treatment of STZ-induced diabetic rats

Values are means $\pm$ S.E.M. for the numbers of animals indicated in parentheses: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ versus vehicle-treated rats.

Treatment group	Protein content (mg/g)	RNA content (mg/g)	Protein synthesis	
			(<i>K</i> _s , %/day)	(g of protein/ g of RNA per day)
Expt. 1				
Vehicle (6)	178 ± 2	1.40 ± 0.18	5.15 ± 0.42	6.81 ± 0.62
IGF-I (1.05 mg/kg per day) (6)	179 ± 3	1.83 ± 0.12*	6.95 ± 0.42†	6.84 ± 0.37
hGH (1.38 mg/kg per day) (7)	176 ± 3	1.39 ± 0.17	4.75 ± 0.38	6.34 ± 0.66
Insulin (25 units/kg per day) (6)	175 ± 5	2.01 ± 0.05†	8.01 ± 0.20‡	6.98 ± 0.20
Expt. 2				
Vehicle (6)	195 ± 2	1.42 ± 0.08	6.03 ± 0.52	8.27 ± 0.49
IGF-I (1.08 mg/kg per day) (6)	189 ± 3	1.60 ± 0.05*	7.05 ± 0.32	8.36 ± 0.36
IGF-I (2.69 mg/kg per day) (6)	192 ± 2	1.79 ± 0.10*	7.83 ± 0.44†	8.46 ± 0.39
Des(1–3)IGF-I (1.08 mg/kg per day) (6)	192 ± 2	1.69 ± 0.07*	7.99 ± 0.12†	9.13 ± 0.22
Insulin (30 units/kg per day) (6)	190 ± 3	2.18 ± 0.10‡	9.05 ± 0.47‡	7.92 ± 0.40

Fig. 6. Muscle protein breakdown measured as the cumulative 3-methylhistidine-excretion rate (means with the S.E.M. shown only for the insulin-treated groups) of animals in (a) Expt. 1 and (b) Expt. 2 from the commencement of treatments on day 3

The treatment groups were: vehicle (○); 25 (Expt. 1) or 30 (Expt. 2) units of insulin/kg per day (□); 1.05 (Expt. 1) or 1.08 (Expt. 2) mg of IGF-I/kg per day (●); 2.69 mg of IGF-I/kg per day (△); 1.38 mg of hGH/kg per day (■); 1.08 mg of des(1-3)IGF-I/kg per day (▲).

clear trends, could be discerned between the vehicle, IGF-I and hGH groups, which excreted 67.5 ± 2.3 , 65.9 ± 4.7 and 72.6 ± 5.0 $\mu\text{mol/kg}$ respectively over the 7 days, although the insulin-treated animals excreted less ($P < 0.01$), at 52.7 ± 1.7 $\mu\text{mol/kg}$.

In Expt. 2, the addition of insulin to frankly diabetic animals after day 3 decreased the 3-methylhistidine excretion rate to give a cumulative rate of 50.5 ± 1.6 $\mu\text{mol/kg}$, compared with 57.1 ± 1.5 in the vehicle group ($P < 0.01$; Fig. 6b). The other groups in Expt. 2 showed no significant differences. They excreted 58.4 ± 2.2 (low IGF-I), 58.7 ± 3.4 (high IGF-I) and 60.8 ± 4.0 [des(1-3)-IGF-I] $\mu\text{mol/kg}$ over the 7 days.

Carcass composition

The carcass water content was significantly ($P < 0.01$) lower in the insulin-treated animals than in the other groups (Table 2). This difference was accompanied by a 2-fold increase in the lipid content of the carcass without any difference in protein or the residual dry matter of the carcass (Table 2).

DISCUSSION

The first experiment reported here was designed to examine effects of IGF-I and hGH on growth parameters in previously insulin-maintained STZ-treated rats over a 7-day period after withdrawal of insulin. Our second experiment extended these findings by the inclusion of a 2.5-fold higher dose of IGF-I as well as a low dose of the truncated IGF-I analogue, des(1-3)IGF-

I, that has been shown to be more potent than IGF-I in cell-culture growth studies [20-22]. The second experiment was also different because at the beginning of the 7-day treatment period the animals were not insulin-maintained. This latter distinction is clearly evident from the glucose-excretion measurements (Fig. 4), where glucosuria was low at the pretreatment stage of Expt. 1 and increased once insulin was withdrawn. On the other hand, glucosuria was high immediately before insertion of the pumps in Expt. 2, and remained high in the all but the insulin-treated group.

Notwithstanding the different design of the two experiments, the effects of the similar dose of IGF-I (1.05 mg/kg per day in Expt. 1; 1.08 mg/kg per day in Expt. 2) were consistent. Thus the body-weight changes by day 10 produced by this dose of IGF-I were 11 and 14 g higher than in the respective vehicle group, but well short of the 45 and 36 g differences between the insulin and vehicle groups (Fig. 3). A very similar situation occurred with nitrogen retention, as illustrated by the cumulative retention data in Fig. 5. Here the equivalent IGF-I doses in the two experiments led to nitrogen retentions by day 10 that were about 30% of the difference between the vehicle and insulin-treated groups. Our low dose of IGF-I was a little less than the daily dose of 150 μg per 120-130 g rat employed by Scheiwiller *et al.* [8]. In that study a significant weight gain was achieved, but less than found in the same report and in a more recent experiment [9] where 300 μg of IGF-I was administered to 120-135 g rats. This latter amount is comparable with the 2.69 mg/kg per day used as the higher dose in our second experiment. With the higher level of IGF-I administration, the relative weight gains of the animals in all three studies were similar, at about 70% of those achieved with insulin replacement.

Nitrogen-balance data have not been reported previously for rats treated with IGF-I. Hence it was conceivable that the weight gains achieved with the growth factor could have been a consequence of additional fluid retention. Indeed, Carlsson *et al.* [23] found that the rise in body weight over the first 2 days of IGF-I infusion to STZ-diabetic rats could be accounted for by changes in water balance. However, in both the experiments reported here we observed a close concordance between body-weight change (Fig. 3) and cumulative nitrogen retention (Fig. 5), indicating that true tissue growth had occurred. Moreover, the equal protein content of the muscles taken on day 10 in both experiments and the unchanged carcass water contents in Expt. 2 argue strongly against fluid retention.

We have sought to explain the improvements in nitrogen balance produced by IGF-I through changes in muscle protein synthesis or breakdown. In both experiments we found that protein synthesis was low in the untreated diabetic groups and substantially higher upon insulin treatment, in agreement with earlier studies [3-5]. IGF-I administration also led to higher rates of muscle protein synthesis, a result that is consistent with effects of the hormone on cultured muscle cells [21]. These effects were mediated by an increase in the synthetic capacity of the muscle tissue, because no changes were detected if synthesis was measured per unit of muscle RNA. We found no significant effect of IGF-I on 3-methylhistidine excretion in either experiment, unlike responses to protein breakdown in cultured muscle cells [21]. In contrast with IGF-I, the effects of insulin were mediated by a co-ordinated inhibition of protein breakdown and stimulation of protein synthesis, consistent with previous reports [3-5, 24]. This lack of an IGF-I effect on protein breakdown contrasts with the response to insulin, which did decrease 3-methylhistidine excretion.

We find that IGF-I, unlike insulin, did not decrease glucose excretion or the associated hyperphagia in the diabetic rats. This result suggests that carbohydrate metabolism may be insensitive

Table 2. Carcass composition of animals after 7 days treatment of diabetic rats in Expt. 2

Values are means $\pm$ S.E.M. for measurements on six animals in each group: * $P < 0.001$ versus vehicle-treated rats. The protein content has been calculated by multiplying nitrogen by 6.25.

Treatment group	Percentage of carcass weight as			
	Water	Protein	Fat	Residue
Vehicle	72.4 $\pm$ 0.2	20.3 $\pm$ 0.2	2.83 $\pm$ 0.17	4.49 $\pm$ 0.19
IGF-I (1.08 mg/kg per day)	72.3 $\pm$ 0.1	20.9 $\pm$ 0.3	2.97 $\pm$ 0.05	4.72 $\pm$ 1.30
IGF-I (2.69 mg/kg per day)	72.6 $\pm$ 0.3	20.3 $\pm$ 0.3	2.80 $\pm$ 0.15	4.27 $\pm$ 0.27
Des(1-3)IGF-I (1.08 mg/kg per day)	72.9 $\pm$ 0.2	20.2 $\pm$ 0.2	2.68 $\pm$ 0.07	4.25 $\pm$ 0.20
Insulin (30 units/kg per day)	69.9 $\pm$ 0.4*	19.5 $\pm$ 0.4	5.77 $\pm$ 0.39*	4.79 $\pm$ 0.14

to IGF-I, a conclusion supported by the lack of any effect of IGF-I administration on blood sugar concentrations in STZ-diabetic rats [8,9,23]. Perhaps the inability of IGF-I to decrease glucosuria or blood glucose is a consequence of the greatly enhanced glucose production rate in STZ-diabetic rats, because an acute increase in blood IGF-I in non-diabetic animals provokes a substantial hypoglycaemic response [25-27]. It is also possible that adaptation during chronic IGF-I administration contributed to the lack of a hypoglycaemic response. Thus Zapf *et al.* [7] have argued that the acute effect is caused by an increase in unbound circulating IGF-I, whereas chronic treatment with the growth factor induces IGF-binding proteins and hence leads to a decrease in the free peptide. This interpretation is supported by our confirmation that IGF-binding proteins with molecular masses of 40-50 kDa (IGFBP-3) are markedly increased in the IGF-treated animals.

Another difference between the IGF and insulin responses is the pronounced increase in carcass fat only after insulin treatment (Table 2). Analogous results have recently been reported in depancreatized dogs, where insulin had a much greater antilipolytic effect than IGF-I at doses where glucose utilization rates were more stimulated by IGF-I [28]. A conclusion consistent with both studies is that IGF-I exerts a selective anabolic effect on muscle, whereas insulin exerts a selective effect on adipose tissue. These differences could be explained mechanistically by the presence of relatively more IGF-I receptors in muscle than in adipose tissue, with the reverse for insulin receptors.

The high dose of hGH utilized in the first experiment produced no metabolic effects or weight changes relative to the vehicle-treated group. The only responses obtained were a transient increase in plasma IGF-I on the days 2 and 3 after insertion of the osmotic pumps (days 5 and 6), followed by a lower concentration of IGF-binding proteins at the end of the experiment, and a trend towards lower IGF-I levels at the same time. Scheiwiller *et al.* [8] have also reported the lack of an hGH stimulus of growth in diabetic rats. It would seem that hGH does not alleviate the diabetic state because this hormone does not produce a sustained increase in IGF-I, and also does not have any direct insulin-like effects. The lack of any IGF-I increase after GH administration to diabetic rats has been described previously and ascribed to a post-receptor defect, because GH receptor numbers and affinities are normal [29].

A feature of our second experiment was the potency of des(1-3)IGF-I. Des(1-3)IGF-I produced quantitative effects very similar to those of a 2.5-fold higher dose of the full-length molecule. This assessment applies to body-weight change, nitrogen balance and muscle protein synthesis. It should be stressed, however, that the responses produced by des(1-3)IGF-I or the higher dose of IGF-I were not significantly greater than those

obtained with the lower IGF-I dose. Hence any conclusion that des(1-3)IGF-I is more potent than IGF-I in diabetic rats, as in cultured muscle cells [20-22], must await more complete dose-response studies.

We thank K. Edson, K. Lymn, A. Collins, S. Quinn, M. Pearce and J. Burgoyne for technical assistance. The IGF-I, des(1-3)IGF-I and hGH were kindly provided by Genentech Inc., South San Francisco, CA, U.S.A.

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Received 6 November 1990/14 January 1991; accepted 5 February 1991