Insulin-like growth factor-I and more potent variants restore growth of diabetic rats without inducing all characteristic insulin effects

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The effects of graded doses of insulin-like growth factor-I (IGF-I) and two variants which bind poorly to IGF-binding proteins were investigated in 160 g streptozotocin-induced diabetic rats. The two variants were the truncated form, des(1-3)IGF-I, and another with arginine at residue 3 and an N-terminal extension, termed LR^3-IGF-I. The peptides were infused via mini-osmotic pumps. Reference groups received either vehicle or insulin (30 i.u. per day). Treatment led to a marked dose-dependent increase in growth rate and nitrogen balance. The highest dose (695 μg/day) of IGF-I increased body weight by 48.1 $\pm$ 1.7 g/7 days, compared with 11.0 $\pm$ 2.8 g/7 days for the vehicle-treated group. The two variants were 2.5–3 times more potent than IGF-I in restoring growth. The insulin-treated group gained more weight (64.5 $\pm$ 1.6 g/7 days), but the added gain was fat (92.5 $\pm$ 4.8 g of fat/kg carcass wet wt., compared with 32.2 $\pm$ 2.1 for all other groups) rather than protein. All peptides increased muscle protein-synthesis rates and RNA levels by up to 50%, with IGF-I the least potent. These high doses of IGFs did not decrease either the glucosuria or the daily excretion rate of N^{τ}-methylhistidine (N^{τ}-MH). On the other hand, insulin treatment markedly decreased both glucosuria (from 82.7 $\pm$ 5.4 to 4.5 $\pm$ 3.3 mmol/day) and N^{τ}-MH excretion (from 9.3 $\pm$ 0.3 to 7.1 $\pm$ 0.4 μmol/day per kg). This experiment shows that, although IGF-I and variants can restore growth in diabetic rats, other insulin-dependent metabolic processes in liver, muscle and adipose tissue are not restored.

INTRODUCTION

Impaired insulin production and resultant diabetes induced in young rats by streptozotocin (STZ) treatment is accompanied by a decrease in circulating levels of insulin-like growth factor-I (IGF-I) and IGF-I-binding proteins (IGFBPs) in plasma [1–3]. Administration of IGF-I to STZ-diabetic rats restores growth, nitrogen balance and muscle protein-synthesis rates, but does not ameliorate the hyperglycaemia or glucosuria associated with the deranged carbohydrate metabolism [4–6]. Acute studies in normal and diabetic animals have indicated that IGF-I treatment does increase glucose uptake by the peripheral tissues [7,8], but is much less effective in the inhibition of hepatic glucose production. Also, the dose of IGF-I used in these acute experiments is relatively high compared with those used in the chronic infusion studies which report growth promotion.

Recently, our laboratory has reported the higher potency of the variant des(1-3)IGF-I in cultured cells [9] and in STZ-diabetic rats [6,10]. This increased potency appears related to the decreased binding of the peptide to IGFBPs [11], which presumably results in higher availability to the receptors of target cells. It was suggested that even higher levels of such variants may decrease glucose production in the liver of diabetic rats by virtue of maintaining high unbound local concentrations, which would act through either specific IGF receptors or the insulin receptor. Thus, in addition to the restoration of protein accretion and growth of diabetic rats achieved by the continuous infusion of relatively low doses of des(1-3)IGF-I [6], we considered that the administration of higher doses may also moderate the defects in carbohydrate metabolism. To answer this question, and also to establish relative potencies of IGF-I variants, we have examined the effects of IGF-I, des(1-3)IGF-I, and a further variant with even lower affinity for IGFBPs, LR^3-IGF-I [12], in a range of doses on the growth, carcass composition, protein metabolism and glucosuria in STZ diabetic rats.

EXPERIMENTAL

Peptides and chemicals

Recombinant human IGF-I and recombinant des(1-3)IGF-I were supplied by Genentech Inc., South San Francisco, CA, U.S.A. Recombinant LR^3-IGF-I, which has a 13-residue N-terminal extension and Glu-3 replaced by Arg in the IGF sequence [12], was obtained from GroPep Pty. Ltd., Adelaide, Australia. The peptides were administered to animals by using mini-osmotic pumps (Alza, Palo Alto, CA, U.S.A.) which delivered 0.92 μl/h. The peptides were dissolved in 0.1 M acetic acid to the concentrations required for the pumps to deliver 44, 111, 278 or 695 μg/day according to the allocated treatment. Pumps for control animals were filled with 0.1 M acetic acid. Protamine zinc insulin [Isophane insulin (N.P.H.); 100 units/ml] was obtained from CSL–Novo Pty. Ltd., North Rocks, N.S.W., Australia. The insulin was diluted in a vehicle consisting of 0.2% Na_2HPO_4 and 1.6% glycerol (pH 7.4) before once-daily subcutaneous injection. Streptozotocin was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and dissolved in 20 mM sodium citrate buffer immediately before administration to the animals. L-[*ring*-2,6-^{3}H(n)]Phenylalanine (2.22 TBq/mmol) was obtained from Du Pont (Australia) Ltd., North Ryde, N.S.W., Australia.

Animals

Male Hooded-Wistar rats were obtained from the CSIRO Division of Human Nutrition colony and held in cages at 25 °C under controlled lighting (12 h-dark/12 h-light cycle). At 140 g body weight, food was withdrawn for 6–8 h, after which the rats

Abbreviations used: IGF-I, insulin-like growth factor I; STZ, streptozotocin; IGFBP, IGF-I-binding protein; N^{τ}-MH, N^{τ}-methylhistidine.
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were injected intraperitoneally with 65 mg of STZ/kg body wt. The rats were then transferred into individual metabolism cages and given a diet which contained 180 g of casein and 1.25 g of methionine/kg and was free of N^{τ}-methylhistidine (N^{τ}-MH) [13]. Only rats showing polydipsia, polyuria, glucosuria and near growth stasis were used in the experiment. Experimental treatments commenced 7 days after STZ injection. The protocol for the experiment was approved by the Animal Care and Ethics Committee of the CSIRO Division of Human Nutrition, following the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Experimental protocol

The rats were given free access to food and water and were weighed daily throughout the study. Daily urine and faeces collection and measurement of food intake commenced 2 days before the insertion of the osmotic pumps. Excreta samples were stored at −20 °C until analysed. The pumps containing peptide were implanted subcutaneously in the supra-scapular region with the rat under ether anaesthesia. Body weight averaged 162 ± 1.4 g at this time. There were 11 groups of 6 rats, comprising a control group, an insulin-treated group and 9 IGF treatment groups to which the rats were randomly allocated. Control rats received a pump containing vehicle only. The insulin-treated group received a daily subcutaneous injection of insulin (30 i.u./day per kg body wt.) as well as a vehicle pump. IGF-treated groups received either IGF-I at 111, 278 or 695 μg/day or one of the two variants at 44, 111 or 278 μg/day. After 7 days of treatment, the rats were restrained in an open-weave cloth while a solution containing 150 mM phenylalanine, 77 mM NaCl and 50 μCi of L-[*ring*-2,6-^{3}H]phenylalanine/ml was injected (10 ml/kg body wt.) via a lateral tail vein. Then 15 min later the rats were stunned and decapitated, and trunk blood was collected into a heparinized tube for 10 s. A consistent section of mixed hind-limb muscles (chiefly gastrocnemius, biceps femoris and plantaris) was rapidly excised and frozen in tongs precooled in liquid N_2. The pelt and visceral organs were then removed and weighed before being discarded. The remaining carcass was frozen for later analysis. Because of caging and other constraints the experiment was done in three stages, each with two rats per treatment group.

Analytical methods

The nitrogen contents of food, faeces, urine and dried carcass were measured by the Dumas procedure by using a Carlo Erba NA1500 Nitrogen Analyser (Milan, Italy). Urinary N^{τ}-MH was measured by continuous-flow analysis [14] after initial hydrolysis and ion-exchange chromatography [15]. Muscle total RNA contents were measured as described by Munro and Fleck [16]. Rates of muscle protein synthesis were determined essentially as described by Garlick et al. [17] and expressed as a fractional rate (K_s; % of protein pool synthesized/day) and as a rate per unit of RNA (g of protein synthesized/day per g of RNA). The rationale for the use of the 'flooding-dose' method for measurement of protein synthesis and validation for its use in muscle have been presented elsewhere [17,18]. Urinary glucose was measured by a glucose oxidase method with a continuous-flow analyser (Skalar Analytical, Breda, The Netherlands). Plasma glucose levels were measured with glucose test strips (BM-Test-Glycaemie 20-800 glucose; Boehringer Mannheim Australia Pty. Ltd., Sydney, Australia) read with a blood glucose monitor ('Omniscan', Newcrest Diagnostics Ltd., Hong Kong).

IGF-I was measured in acid/ethanol extracts of plasma [6], and IGFBPs were analysed in untreated plasma by the ligand-blot procedure [10].

Statistics

Values are presented as means with the S.E.M. indicated. Treatment effects were initially assessed by using two-way analysis of variance, partitioning variance due to stages and treatments. Where the treatment effect was significant ($P < 0.05$) (NS, not significant), multiple comparisons among means were made by the Student–Newman–Keul's test and comparisons with the vehicle control group were made by Dunnett's test. Dose–responses and relative potencies were examined by regression analysis (Systat 5.0; Systat Inc., Evanston, IL, U.S.A.), log dose being used as the independent variable and growth factor and stage as categorical variables. The vehicle group was not included in analyses where log dose was used.

RESULTS

Growth responses and food intake

Each of the growth factors increased growth rate in a dose-dependent manner between that of the vehicle group (1.6 ± 0.40 g/day) and the insulin-treated group (9.2 ± 0.23 g/day) (Figure 1). An increase in growth rate was obvious from day 1 of treatment with growth factors (see inset, Figure 1) and was sustained throughout the treatment period. The variants were 2.5–3-fold more potent than was IGF-I ($P < 0.001$), with LR^3-IGF-I consistently more potent than des(1-3)IGF-I ($P = 0.1$, NS).

Except for the insulin-treated group, food intakes remained virtually constant throughout the experiment. Averaged over the 7 treatment days, they ranged from 31.1 to 34.1 g/day, with only partial compensation for changes in body size due to differential growth rates between treatment groups. Thus the daily food consumption averaged over those groups receiving the low, intermediate and high dose of a growth factor was 1.84, 1.78 and 1.71 g/kg body wt. [S.E. of difference (SE_D) = 0.037; $P < 0.01$] respectively. Administration of insulin caused a substantial

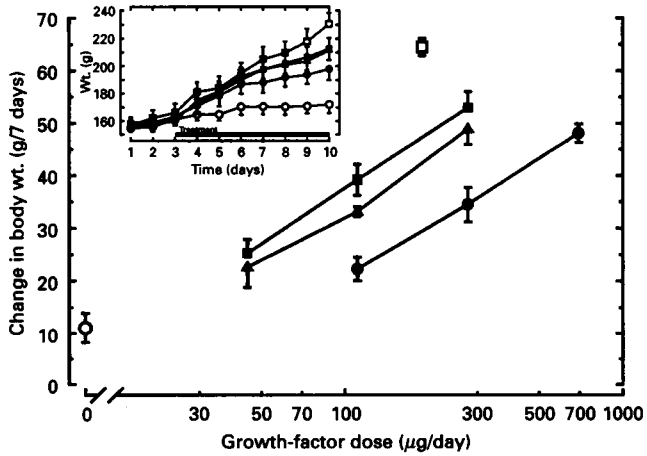

Figure 1 Dose–response for the change in body weight of groups of STZ-diabetic rats receiving infusions of vehicle (○), IGF-I (●), des(1-3)IGF-I (▲), LR^3-IGF-I (■), or daily subcutaneous insulin injection (□), over 7 days

The inset shows the growth curve for the vehicle- and insulin-treated rats and those groups receiving 278 μg of IGF-I, des(1-3)IGF-I and LR^3-IGF-I/day. Each point is the mean of six rats, with S.E.M. indicated by vertical bars.

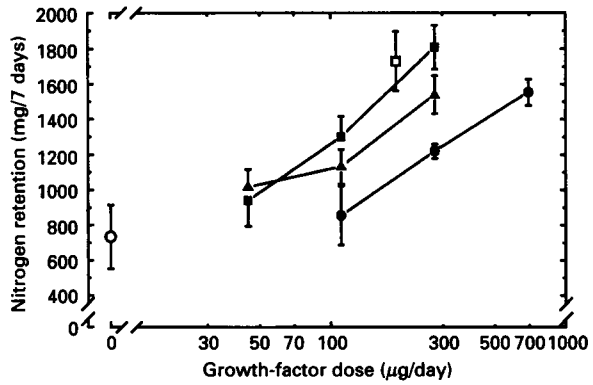

Figure 2 Dose–response curves for cumulative nitrogen retention during treatment

Symbols and treatments are given in Figure 1 legend.

decrease in daily food intake to an average of 26.6±0.9 g, or 1.30±0.46 g/kg body wt. ($P < 0.001$).

Nitrogen balance

The nitrogen retention over the treatment period is an indicator of somatic growth. Cumulative nitrogen balances increased in a dose-related manner during IGF treatment ($P < 0.001$, Figure 2), with IGF-I about 2–3 times less potent than the two variants ($P < 0.01$). However, unlike the response pattern for growth rate, insulin treatment was not more effective than the highest dose of IGFs. Thus the insulin-treated group retained 1730±167 mg of nitrogen over 7 days, compared with 1809±123 mg for the group receiving 278 μg of LR³-IGF-I/day.

Glucose excretion

Average glucose excretion was not decreased by growth-factor treatment (Figure 3a). However, there was a small but statistically significant dose effect of the growth factors evident in a 10% lowering of glucose excretion, from an average of 97.0 mmol/day at the lowest dose of each to 89.2 mmol/day at the highest dose ($P < 0.02$). The dose effect is illustrated by comparing the change in average glucose excretion rate from the initial 2-day control collections after treatment (Figure 3b). LR³-IGF-I tended to be

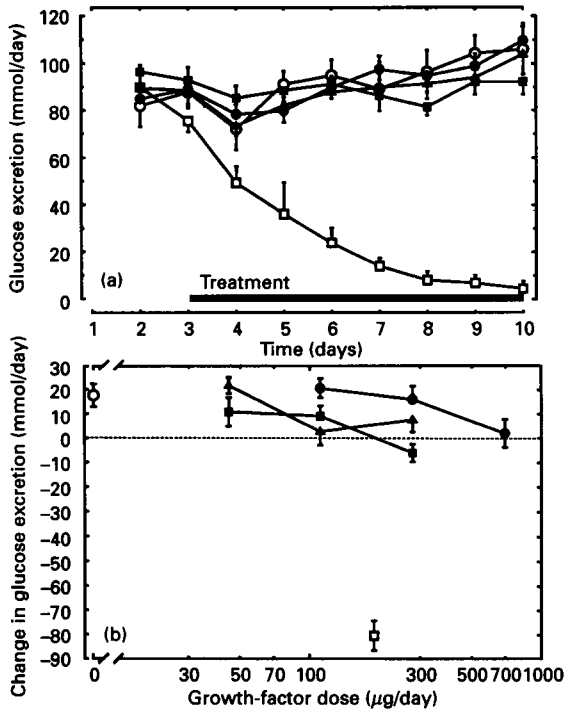

Figure 3 (a) Daily glucose excretion by rats receiving either infusions of vehicle, IGF-I, des(1-3)IGF-I and LR³-IGF-I at 278 μg/day or daily subcutaneous insulin injection at 1.25 mg/day per kg, and (b) dose–responses for the change in the rate of glucose excretion by groups of STZ-diabetic rats receiving infusions of vehicle, IGF-I, des(1-3)IGF-I, LR³-IGF-I or subcutaneous daily insulin injection over 7 days

Symbols and treatments are given in Figure 1 legend.

Table 1 Muscle protein and RNA contents and protein-synthesis rates after 7 days tratment of STZ-induced diabetic rats

Values are means±S.E.M. for six animals in each group, with the peptide doses (μg/day) shown in parentheses: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle-treated control rats.

Treatment group		Protein content (mg/g wet wt. of muscle)	RNA content (μg/g of protein)	Protein synthesis (K_s, %/day)†	Protein synthesis (g of protein/day per g of RNA)
Vehicle	(0)	203±4	5.52±0.26	6.31±0.45	11.4±0.44
Insulin	(225)	198±7	8.68±0.46***	10.31±1.01***	11.8±0.76
IGF-I	(111)	201±4	6.17±0.21	7.30±0.39	11.8±0.60
	(278)	200±4	6.67±0.19**	7.84±0.40	11.8±0.56
	(695)	191±8	7.79±0.34***	8.67±0.51**	11.2±0.67
Des(1-3)IGF-I	(44)	200±8	6.25±0.16	7.07±0.27	11.3±0.49
	(111)	204±5	6.22±0.11	7.53±0.23	12.1±0.31
	(278)	196±6	7.15±0.24***	8.83±0.61**	12.4±0.89
LR³-IGF-I	(44)	206±4	6.22±0.23	6.77±0.27	10.9±0.37
	(111)	197±5	6.60±0.15*	7.49±0.21	11.4±0.34
	(278)	197±6	7.11±0.14***	8.13±0.41*	11.4±0.48
SE_D		5.13	0.238	0.500	0.662

† K_s, proportion of protein pool synthesized per day.

Table 2 Molar and fractional excretion rate (K_{MH}) of N^{τ}-MH and concentrations in plasma and in muscle intracellular water after 6–7 days treatment of STZ-induced diabetic rats

Values are means ± S.E.M. for six animals in each group, with the peptide doses (μg/day) shown in parentheses. K_{MH} (proportion of carcass N^{τ}-MH pool excreted per day) is calculated from average N^{τ}-MH excretion on days 6 and 7 and the carcass N^{τ}-MH content. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle-treated control rats.

Treatment group		Urine N^{τ}-MH (μmol/day per kg)	K_{MH} (%/day)	Plasma N^{τ}-MH (mM)	Muscle N^{τ}-MH (mM)†
Vehicle	(0)	9.1 ± 0.54	3.44 ± 0.17	1.63 ± 0.14	0.82 ± 0.16
Insulin	(225)	7.1 ± 0.45**	2.68 ± 0.14*	2.93 ± 0.35***	0.69 ± 0.13
IGF-I	(111)	8.8 ± 0.41	3.44 ± 0.12	1.38 ± 0.20	0.54 ± 0.07
	(278)	9.3 ± 0.54	3.55 ± 0.24	1.63 ± 0.15	0.60 ± 0.09
	(695)	10.2 ± 0.47	4.01 ± 0.20	1.63 ± 0.18	0.71 ± 0.08
Des(1-3)IGF-I	(44)	9.1 ± 0.36	3.50 ± 0.17	1.52 ± 0.25	0.59 ± 0.14
	(111)	9.9 ± 0.44	3.91 ± 0.18	1.49 ± 0.06	0.77 ± 0.14
	(278)	9.4 ± 0.24	3.77 ± 0.11	1.81 ± 0.20	0.66 ± 0.14
LR^3-IGF-I	(44)	9.3 ± 0.57	3.62 ± 0.26	1.61 ± 0.11	0.80 ± 0.11
	(111)	9.7 ± 0.24	3.93 ± 0.10	2.28 ± 0.25	0.88 ± 0.14
	(278)	9.2 ± 0.48	3.77 ± 0.19	1.66 ± 0.13	0.72 ± 0.09
SE_D		0.063	0.238	0.273	0.140

† Concentration in muscle water was estimated as [(nmol/g of muscle) − 0.2(nmol/ml of plasma)]/0.6. It was assumed that muscle contains 200 g of extracellular and 600 g of intracellular water per kg wet wt. and that the interstitial space (150 g/kg) is at equilibrium with plasma. No other corrections were made.

the most potent of the three IGF peptides for lowering of glucose excretion, with the effect more pronounced when glucose excretion was expressed per unit body weight or food intake. Nonetheless, insulin treatment was very much more effective than the growth factors and decreased glucose excretion to 4.5 ± 3.3 mmol/day, less than 4% of that in the other groups by the last day of the experiment.

At the end of the treatment period, plasma glucose levels of insulin-treated rats were 9.6 ± 3.25 mM, less than half the combined average for all the other groups (21.7 ± 0.16 mM) which did not respond to growth-factor treatment.

Muscle protein synthesis and breakdown

Fractional synthesis rates of muscle protein (K_s, %/day) were increased up to 50% in rats treated with growth factor compared with the control group (Table 1). The effect of dose was highly significant ($P < 0.001$). Although both variants were on average more potent than IGF-I in promoting synthesis, this difference was only statistically significant ($P < 0.05$) for des(1-3)IGF-I. Insulin-treated rats showed a significantly higher increment in protein-synthesis rates than was obtained with the growth factors ($P < 0.05$), even though the last insulin injection was about 24 h before the measurement. Treatment did not affect muscle protein concentration, but tissue RNA levels, expressed relative to protein content, showed a marked dose-related increase ($P < 0.001$), such that the rate of protein synthesized per g of RNA remained virtually constant (Table 1).

N^{τ}-MH excretion, an index of total myofibrillar protein breakdown, was not changed from control levels by any of the IGFs (Table 2). On the other hand, insulin treatment led to a 20% decrease in N^{τ}-MH excretion ($P < 0.001$). The average rate of excretion of N^{τ}-MH over the last 2 days of treatment, expressed as a fraction of the carcass N^{τ}-MH pool excreted per day (K_{MH}, %/day, equivalent to the fractional rate of breakdown of myofibrillar protein), tended to be higher than the control value in groups receiving growth factors (NS; $P > 0.05$) but was significantly lower in the insulin-treated group ($P < 0.02$; Table 2).

Free N^{τ}-MH levels in the intracellular fluid of muscle tissue are also an index of the rate of breakdown of myofibrillar protein in many conditions. The results in Table 2 show that intracellular levels of N^{τ}-MH in both growth-factor- and insulin-treated rats were not significantly different. However, plasma N^{τ}-MH levels were higher in the insulin-treated group ($P < 0.05$) and suggest that both N^{τ}-MH clearance and production rates may have been lower.

Carcass composition

The proportional contribution of water, protein and fat to the carcass wet weight was significantly altered in insulin-treated groups ($P < 0.001$) with fat increased more than 2-fold (Table 3). On the other hand, carcass fat concentration significantly declined with increasing doses of each IGF peptide ($P < 0.05$), reflecting a lack of growth in fat mass as body weight increased. Protein concentration of the fat-free carcass was not affected by IGF or insulin treatment. The absolute amount of carcass protein was significantly increased above that of control animals by the intermediate ($P < 0.05$) and high ($P < 0.001$) doses of growth factors and by insulin ($P < 0.001$; results not shown).

IGF-I concentrations and IGFBPs

The plasma IGF-I concentration just before insertion of the osmotic pumps was approx. 500 μg/l. IGF-I infusion increased average IGF-I levels progressively with increasing dosage, to achieve up to 4-fold those of the vehicle-treated controls. Treatment with des(1-3)IGF-I, which has about half the potency of IGF-I in the radioimmunoassay, showed a relatively minor rise in measured IGF-I levels compared with controls, whereas treatment with LR^3-IGF-I, which has only 10% of the potency of IGF-I in the radioimmunoassay, caused no change in assayed IGF-I levels (results not shown). Insulin injections led to a sustained 1.5–2-fold increase in circulating IGF-I levels above the diabetic control rats.

The higher-molecular-mass IGFBPs (presumably IGFBP-3) in the final plasma samples were increased after treatment with

Table 3 Carcass composition of diabetic rats after 7 days treatment

Values are means ± S.E.M. ($n = 6$) with the peptide dose (μg/day) shown in parentheses: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle-treated control rats. Protein values are nitrogen × 6.25.

Treatment group		Content in carcass (g/kg)			
		Water	Protein	Fat	Residue
Vehicle	(0)	706 ± 3.1	210 ± 3.3	37.6 ± 2.8	46.1 ± 5.2
Insulin	(225)	669 ± 3.1***	192 ± 1.7***	92.5 ± 4.8***	46.6 ± 3.2
IGF-I	(111)	710 ± 3.1	206 ± 2.4	37.3 ± 1.9	46.8 ± 3.6
	(278)	715 ± 4.7	206 ± 1.4	35.0 ± 3.3	44.2 ± 3.0
	(695)	722 ± 4.6***	200 ± 2.9	30.3 ± 2.5	46.7 ± 4.1
Des(1-3)IGF-I	(44)	711 ± 3.0	207 ± 3.3	37.0 ± 2.5	44.6 ± 3.6
	(111)	714 ± 3.1	204 ± 2.0	33.6 ± 2.0	48.3 ± 2.5
	(278)	717 ± 3.9*	205 ± 3.8	36.2 ± 3.8	41.3 ± 4.2
LR^3-IGF-I	(44)	710 ± 4.1	211 ± 2.3	36.3 ± 1.7	42.9 ± 3.3
	(111)	716 ± 3.4*	202 ± 2.2	35.3 ± 2.4	46.1 ± 3.1
	(278)	719 ± 3.1**	202 ± 2.9	34.6 ± 2.4	44.2 ± 3.1
SE_D		3.33	3.36	3.36	3.62

either insulin or IGF-I as reported previously [6]. Lesser increases were indicated by the ligand blots for rats treated with IGF variants.

DISCUSSION

Continuous infusion of IGF-I to diabetic rats produced growth responses in proportion to the infused dose, as previously described by us [6] and by others [4,5]. We now illustrate that the dose–response in diabetic rats extends substantially beyond that defined previously. The highest dose rates used here were about 2.5 times those of the previous report [6] and induced growth rates almost equal to that observed for the insulin-treated rats. In fact, in terms of somatic growth the highest doses of des(1-3)IGF-I and LR^3-IGF-I were equipotent with the insulin treatment used here (1.25 mg/day per kg body wt.) and stimulated lean growth to the normal range for these rats. Unlike insulin, the growth factors did not stimulate fat deposition, and in fact fat mass did not increase in proportion to the body mass. This difference could be explained by the paucity of type 1 IGF receptors in adipose tissue [19] and also argues against the IGF peptides acting via the insulin receptor to stimulate glucose uptake or other processes in this study. The difference between the relative responses of body weight and nitrogen retention for insulin and the growth factors can be explained by their relative effects on lipid deposition.

Despite normalization of nitrogen balance and growth, there was virtually no effect of growth-factor treatment on hepatic glucose production as assessed by urinary glucose output. Although there was an indication of a small dose effect, glucose excretion continued to rise during treatment in all except the insulin-treated group. Thus, despite the higher dosage of IGFs and the higher potency of these variants for tissue and organ growth, the effect on glucose excretion was similar to that in our previous study [6]. The highest level of IGF-I infused (695 μg/day) gave a dose ranging from 3.2 to 2.5 μg/min per kg during the course of the infusion. Acute studies have generally used even higher doses up to 16.3 μg of IGF-I/min per kg [7,8,20,21]. The reported acute inhibition of hepatic glucose production was dependent on the dose and chosen model, but appears to be about half of that obtained with insulin at a dose equipotent for plasma glucose decrease or glucose disposal. The large differences in the responsiveness to IGF-I between the various rat models may reflect metabolic differences. For example, rats made diabetic with STZ display marked insulin resistance despite increased binding capacity of the insulin receptors, implying a post-receptor defect [22–25]. These changes in the insulin-receptor/signalling processes may be important, as the lack of hepatic type-I IGF-I receptors require IGF-I to act via the insulin receptors [26].

One potentially important difference between the acute studies and our chronic infusion experiment is the possible role of the binding proteins. In acute studies there would be little change in the levels of IGFBPs. In contrast we found a substantial rise in the levels of IGFBPs in response to the IGF infusions, which would blunt the ultimate increase in the levels of free IGF-I available for receptor binding. This argument does not explain the lack of effect of the variants on glucose excretion, however, as LR^3-IGF-I especially does not bind to the IGFBPs in the rat [9,10,12]. The explanation may simply be that extraordinarily high levels of free IGF-I (as may be generated in acute experiments) are needed to produce responses via the insulin receptor. Thus levels of IGF-I and variants required to produce biological responses in H35 cells [12], a hepatoma line that has abundant insulin receptors but no IGF-I receptors [27], are about 100- and 1000-fold higher respectively than for insulin. The small but statistically significant effect of LR^3-IGF-I on glucose excretion by the diabetic rats indicates that this peptide, which binds very poorly to the principal IGFBPs in the rat, may have almost reached effective concentrations for action via the insulin receptor.

The observed improvement in nitrogen balance and somatic growth with growth-factor treatment could only arise through co-ordinate changes in protein turnover which lead to an increased positive difference between protein synthesis and breakdown. Our data confirm a dose-related increase in muscle protein-synthesis rates with IGF treatment as reported previously for both diabetic [6] and dexamethasone-treated [10] rats. The increase could be entirely explained by concomitant changes in the concentration of RNA in the tissue, in agreement with our previous report [6]. In contrast, the N^{τ}-MH-excretion data indicate that the IGFs do not decrease the rate of muscle protein

breakdown in diabetic rats and point to another difference in the actions of insulin and IGFs.

Both Goodman [28] and Pain et al. [29] have shown that rates of muscle protein breakdown are increased during acute diabetes and subsequently decreased below normal with chronic diabetes. Our rats received the peptide infusions from 8 to 15 days after STZ treatment, which may be a transition period between acute and chronic phases, and in fact the rates of N^{τ}-MH excretion were close to those expected for non-diabetic rats of equivalent age and weight [10]. The contribution of non-skeletal muscle sources of N^{τ}-MH, in particular gut smooth muscle [28], must be considered when evaluating the data. Relative gut weight in rats is elevated by diabetes, further increased by growth-factor treatment and returned to normal by insulin treatment [30]. However, gut protein-synthesis rates are not increased by IGFs [30–32], pointing to a lower breakdown rate (and N^{τ}-MH release) to account for the growth. Furthermore, the N^{τ}-MH-excretion data are supported by alternative estimates of protein breakdown based on the data for protein synthesis and carcass protein accretion (results not shown), as well as by the intracellular levels of N^{τ}-MH in skeletal muscle, which indicate no major effect of the IGFs on N^{τ}-MH release.

On balance, we believe that our data indicate that IGF-I and the tested variants exert little influence on muscle protein breakdown in diabetic rats, at least where rates of protein breakdown are near normal. The failure to produce an insulin-like effect, even at high doses of variants, may be related to the continuing high rate of hepatic gluconeogenesis and coincident distortion of the normal amino acid pattern.

In conclusion, we have found that with the chronic infusion of a high dose of IGF-I to diabetic rats there is no evidence of a decrease in hepatic glucose production, despite a marked increase in somatic growth rate. This was also the case for the more potent variants of IGF-I tested, which presumably maintained even higher levels of free peptide, as they do not bind to the principal IGFBPs in the rat. These results differ from those obtained by others from acute studies. Our data show that long-term administration of IGFs appears to induce counter-regulatory mechanisms which modify IGF actions and indicate that the prediction of longer-term effects from acute studies may be unreliable. The IGFs also failed to decrease the rate of excretion of N^{τ}-MH or to affect the rate of carcass fat deposition, which contrast with the effects of insulin. Further work is needed to investigate whether these effects may be related to the continuing hepatic gluconeogenesis or to altered tissue sensitivities towards insulin and IGF.

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