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Insulin-like growth factor-I (IGF-I) and especially IGF-I variants are anabolic in dexamethasone-treated rats

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The administration of insulin-like growth factor-I (IGF-I) via subcutaneously implanted osmotic pumps partially reversed a catabolic state produced by the co-administration of 20 μg of dexamethasone/day to 150 g male rats. Marked dose-dependent effects on body weight and nitrogen retention were produced, with the highest IGF-I dose, 695 μg/day, giving a 6 g increase in body weight over 7 days, compared with a 19 g loss in the dexamethasone-only group and an 18 g gain in pair-fed controls. Two IGF-I analogues that bind poorly to IGF-binding proteins, the truncated form, des(1–3)IGF-I, and a variant with an *N*-terminal extension as well as arginine at residue 3, LR^{3}IGF-I, were approx. 2.5-fold more potent than IGF-I. The response with LR^{3}IGF-I was particularly striking because this peptide binds 3-fold less well than IGF-I to the type 1 IGF receptor. The increased potencies of the IGF-I variants may relate to the substantially increased plasma levels of IGF-binding proteins, particularly IGFBP-3, produced by the combined treatment of dexamethasone with IGF-I or the variants. These binding proteins would be expected to decrease the transfer of IGF-I, but not that of the variants, from blood to tissue sites of action. Measurements of muscle protein synthesis at the end of the treatment period and muscle protein breakdown by 3-methylhistidine (3MH) excretion throughout the experiment indicated co-ordinate anabolic effects of the IGF peptides on both processes. Thus 3MH excretion was decreased at the highest IGF-I dose from 83.5±4.2 (S.E.M.) μmol/kg per 7 days to 65.1±2.2, compared with 54.9±1.2 in the pair-fed controls. Part of this response in 3MH excretion may have reflected a decrease in gut protein breakdown, because IGF-I and especially the IGF analogues increased the gut weight by up to 45%. Notwithstanding the effects on protein synthesis and breakdown, the fractional carcass weights remained low in the IGF-treated groups, although the increase in total carcass weight reflected nitrogen rather than fat gain. The dexamethasone-induced changes in liver, spleen and heart weight were restored towards normal by the IGF treatment. The experiment demonstrates the potential of IGF-I treatment of catabolic states and especially the value of modified forms of growth factors that bind weakly to IGF-binding proteins.

INTRODUCTION

Circulating levels of glucocorticoids are elevated as part of the acute or chronic response to infection, physical stress and other traumatic conditions. These changes, as well as increases produced by the exogenous administration of natural or synthetic glucocorticoids such as dexamethasone, lead to atrophy of the musculature as a consequence of decreased protein synthesis and increased protein breakdown (Goldberg, 1969; Tomas *et al.*, 1979, 1984*a*; Odedra & Millward, 1982). The concentration of insulin-like growth factor-I (IGF-I) in blood is decreased during polytrauma or after glucocorticoid administration (Coates *et al.*, 1981; Frayn *et al.*, 1984; Clemmons *et al.*, 1985; Unterman & Phillips, 1985; Mosier *et al.*, 1987). This observation, taken together with experiments demonstrating that IGF-I stimulates protein synthesis and inhibits protein breakdown in cultured muscle cells (Ballard *et al.*, 1986), suggest that the administration of IGF-I to glucocorticoid-treated rats may partially reverse the catabolic state, through effects on muscle protein metabolism. Preliminary reports of improved nitrogen balance following IGF-I treatment of rats by Fagin *et al.* (1989, 1990) support this inference.

In the present investigation we have measured the effects of IGF-I on nitrogen balance and muscle protein metabolism in rats given catabolic doses of dexamethasone. Parallel experiments have been carried out with the truncated variant, des(1–3)IGF-I, and with LR^{3}IGF-I (an extended, modified, form of IGF-I), because these analogues bind weakly to IGF-binding proteins and produce more potent anabolic responses than IGF-I does in cultured cells (Walton *et al.*, 1990).

MATERIALS AND METHODS

Growth factors

Recombinant human IGF-I and recombinant human des(1–3)IGF-I were supplied by Genentech Inc., South San Francisco, CA, U.S.A. Recombinant human LR^{3}IGF-I was supplied by GroPep Pty. Ltd., Adelaide, S.A. 5000, Australia. This growth factor has arginine replacing glutamate at position 3 of human IGF-I and the *N*-terminal extension Met-Phe-Pro-Ala-Met-Pro-Leu-Ser-Ser-Leu-Phe-Val-Asn. The growth factors were dissolved in 0.1 M-acetic acid and administered to animals via Alzet model 2001 osmotic pumps that delivered 0.92 μl/h (Alza, Palo Alto, CA, U.S.A.). The concentrations used were 31.5, 12.6 or 5.0 mg/ml for IGF-I, and 12.6, 5.0 or 2.0 mg/ml for des(1–3)IGF-I and LR^{3}IGF-I.

Animals

Male Hooded Wistar rats were bred in the CSIRO Division of Human Nutrition. From approx. 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 continuous access to water and a high-carbohydrate diet containing 180 g of casein plus 2.5 g of methionine per kg as the nitrogen source (Tomas *et al.*, 1984*b*).

Abbreviations used: IGF, insulin-like growth factor; 3MH, 3-methylhistidine.

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Experimental protocol

After 4 days' acclimatization in the metabolism cages, quantitative collection of urine and faeces as well as measurement of food intake was carried out for a further 3 days. After the third collection, each animal was anaesthetized with ether, two Alzet model 2001 osmotic pumps were inserted subcutaneously in the supra-scapular region, the wound was sutured and approx. 0.1 ml of blood was withdrawn from a tail vein. One pump contained either a growth factor at one of the indicated doses or 0.1 M-acetic acid as vehicle, and the other contained either dexamethasone sodium phosphate (Intervet, Boxmeer, Netherlands) dissolved in 0.15 M-NaCl or saline alone. The dexamethasone concentration of 0.9 mg/kg body wt. was calculated to deliver 20 μg/day. The animals for all groups averaged 150 g body wt. on the day the pumps were inserted. Urine and faeces were collected and food intake was measured daily for the next 7 days.

On day 4 after insertion of the osmotic pumps, approx. 0.1 ml of blood was withdrawn from a tail vein of each animal while the animal was restrained gently in open-weave cloth.

After 7 days' treatment, 10 ml of a solution comprising 150 mM-L-phenylalanine, 77 mM-NaCl and L-[2,6-^{3}H]phenylalanine (50 μCi/ml)/kg body wt. was injected into a tail vein of each animal while the animal was restrained gently in open-weave cloth. Then 15 min later the animal was stunned, decapitated, and blood was collected into a heparinized tube. The gastrocnemius plus plantaris muscles were immediately excised and frozen in a clamp pre-cooled in liquid N_2. The gut from stomach to caecum was also removed immediately, transferred to an ice/water mixture, and the combined weight was measured after removal of gut contents. Subsequently the pelt was stripped off, the remaining viscera were removed, the feet, tail and head were discarded and the carcass was frozen for subsequent water, nitrogen and fat measurements.

The experiment was conducted in three parts. Sections 1 and 2 contained two animals in each control and treatment group, except that there were no pair-fed control animals. Section 3 of the experiment also contained two animals in each group, bringing the total number to six per group. In addition a second control group of six animals was included. These were pair-fed on the average food intake of the four animals in the dexamethasone-only group in sections 1 and 2 of the experiment. For this purpose the daily food intake was calculated on the basis of the body weight at the beginning of each 24 h period. The experimental protocol was 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.

Analytical methods

Methods for the measurement of nitrogen in urine, faeces, food and carcass, the analysis of urine 3-methylhistidine (3MH) and creatinine, carcass fat, muscle protein synthesis, IGF-I in acid-chromatographed portions of plasma and IGF-binding proteins have been described by Tomas *et al.* (1991*b*). Plasma IGF-I was measured by a radioimmunoassay that utilized a monoclonal antibody kindly provided by Dr. R. Baxter, University of Sydney, Sydney, N.S.W., Australia, as well as with the polyclonal antiserum adopted for the study reported by Tomas *et al.* (1991*b*). The monoclonal-antibody assay was identical with the polyclonal method, except for the use of sheep anti-mouse Ig (Silenus Laboratories, Hawthorn, Vic., Australia) as the second antibody. The monoclonal assay was included as it detects endogenous rat IGF-I only weakly. Thus rat IGF-I purified to homogeneity by the method described for bovine and ovine IGF-I (Francis *et al.*, 1988, 1989) cross-reacted only 2.5% relative to human IGF-I. The equivalent value with the polyclonal antiserum was 45%. Des(1–3)IGF-I and LR^{3}IGF-I cross-reacted respectively 70% and 10% relative to human IGF-I with the polyclonal antiserum and 100% and 2.9% with the monoclonal antiserum.

Statistics

Values are presented as means ± S.E.M. Between-treatment significance was assessed initially by two-way analysis of variance, partitioning variance due to sections and treatments. Where significance ($P < 0.05$) was achieved, the treatment means have been compared to the dexamethasone-only group by Student's t test using the pooled estimate of the standard error. Dose–response curves have been compared by regression analysis. The dexamethasone-treated control group (dexamethasone-only) was excluded because log dose was used. The slopes and intercepts (at a dose of 165 μg/day) were compared by using t tests with appropriate corrections for multiple comparisons.

RESULTS

The present study was designed to evaluate the relative abilities of IGF-I and IGF-I variants to restore body-weight gain in the presence of a catabolic dose of dexamethasone, and to investigate the respective roles of muscle protein synthesis and degradation in the process. To this end a preliminary experiment (results not shown) established that dexamethasone doses between 10 and 40 μg/day given to 150 g male rats caused effects ranging from growth stasis to severe weight loss. A dose of 20 μg/day induced an intermediate yet progressive weight loss of 20–25 g over 7 days, with the animals showing no apparent distress.

In the main investigation, IGF-I was administered at three doses, 695, 278 and 111 μg/day. The amounts of the two variants were set 2.5-fold lower, in recognition of the greater potencies of each in cultured cells (Walton *et al.*, 1990) and, in the case of des(1–3)IGF-I, in nitrogen-restricted (Tomas *et al.*, 1991*a*) and diabetic rats (Tomas *et al.*, 1991*b*). The daily body-weight changes over the 7-day period as well as for 3 pretreatment days are shown in Fig. 1 for the reference groups and the group receiving the highest dose of IGF-I. Daily data for the other treatment groups are excluded for clarity and ease of presentation. The

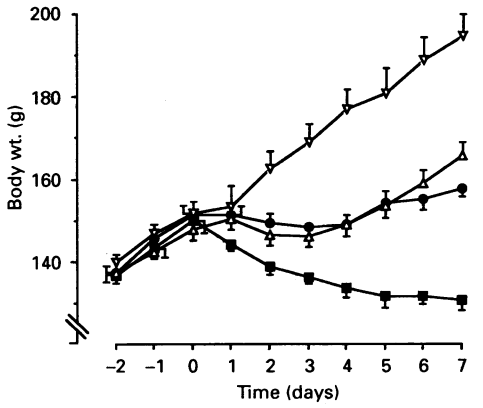

Fig. 1. Changes in body weight in reference animals and in those treated with 695 μg of IGF-I/day

Body weights in control animals fed *ad libitum* (▽), dexamethasone-only animals (■), control animals pair-fed with reference to the dexamethasone-only group (△) and dexamethasone-treated animals also receiving 695 μg of IGF-I/day (●). Pumps delivering dexamethasone, IGF-I or the appropriate vehicle solutions were inserted on day 0. Values are means ± S.E.M. for six animals in each group.

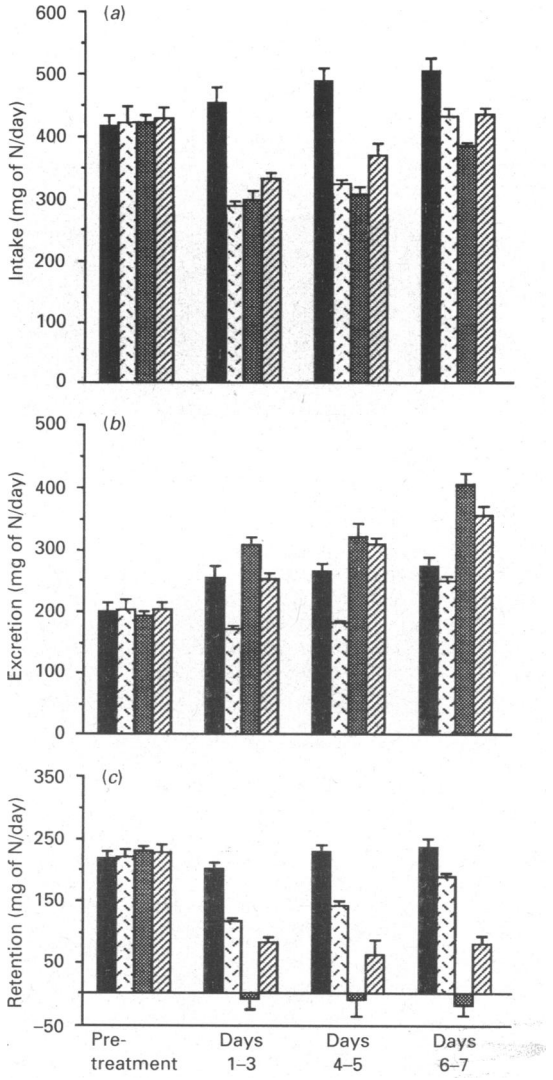

Fig. 2. Nitrogen balance in reference animals and in those treated with 695 μg of IGF-I/day

Nitrogen (*a*) intake, (*b*) excretion and (*c*) retention were expressed as mg of N/day in control animals fed *ad libitum* (■), dexamethasone-only animals (▨), control animals pair-fed with reference to the dexamethasone-only group (■) and dexamethasone-treated animals also receiving 695 μg of IGF-I/day (▨). Data have been calculated from intake and excretion pools on the 3 pre-treatment days as well as on days 1–3, 4–5 and 6–7 of treatment. Values are means ± S.E.M. for six animals in each group.

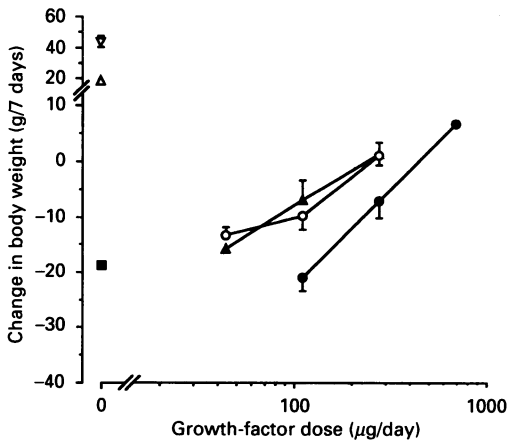

Fig. 3. Dose–response curves for body-weight change over 7 days of treatment

The body-weight changes over the 7 treatment days are shown for control animals fed *ad libitum* (▽), dexamethasone-treated animals receiving an infusion of vehicle (■), IGF-I (●), des(1–3)IGF-I (▲) or LR^{3}IGF-I (○), and control animals pair-fed with reference to the dexamethasone-only group (△). Values are means ± S.E.M. for six animals in each group.

weights of untreated normal rats increased from an average of 152 g at the time the pumps were inserted to 197 g after 7 days, whereas the dexamethasone-treated control group (dexamethasone-only) lost an average of 19 g over the same period. Those control animals pair-fed on the intake of the dexamethasone-only group maintained their pretreatment weight over the first 4 days, when food intake was low (see below), and then gained weight to achieve a net average gain of 18 g. The animals treated with 695 μg of IGF-I/day plus dexamethasone gained 6 g over the 7-day period, thus restoring 25 of the 37 g difference between the dexamethasone-only and pair-fed control groups.

Nitrogen-balance results from the same four groups described in Fig. 1 are displayed in Fig. 2. Before nitrogen analysis the urine and faeces were pooled over the 3 pretreatment days, as well as on days 1–3, 4–5 and 6–7. Before the treatment began, the nitrogen intakes (Fig. 2*a*), excretions (Fig. 2*b*) and retentions (Fig. 2*c*) were similar between the groups. Nitrogen intake was subsequently decreased in the animals treated with dexamethasone only and gradually increased through the 7 days. No significant differences in intake between the dexamethasone-only, pair-fed or dexamethasone–IGF-I groups were found, although the average intake in those animals treated with 695 μg of IGF-I/day was higher than the vehicle group unless nitrogen intake was expressed per kg body wt. rather than per animal. Nitrogen excretion (Fig. 2*b*) was decreased in the pair-fed animals relative to those fed *ad libitum* and was markedly increased ($P < 0.001$) on all three occasions by dexamethasone above the pair-fed group. Animals treated with 695 μg of IGF-I/day also excreted more nitrogen than the pair-fed controls, but the increases were less than in the dexamethasone-only group.

The amount of nitrogen retained was decreased in the pair-fed group below that found for the control animals fed *ad libitum* (Fig. 2*c*) and even further to a slightly negative balance in the dexamethasone-only group. At each pooled collection period the animals treated with 695 μg of IGF-I/day retained an amount of nitrogen which was below that of the pair-fed group, but significantly more ($P < 0.01$) on days 1–3 and days 6–7 than that found for the dexamethasone-only group.

The data for the three doses of the three IGF-I peptides are given below in the format of dose–response curves for the complete 7-day treatment period. The changes in body weight in the dexamethasone-only (-19.0 ± 3.0 g) and low IGF-I dose (-21.2 ± 2.5 g) groups were similar, but were increased significantly to -7.5 ± 3.2 g ($P < 0.05$) with 278 μg of IGF-I/day and to $+6.2 \pm 0.6$ g ($P < 0.001$) with 695 μg of IGF-I/day (Fig. 3). Des(1–3)IGF-I and LR^{3}IGF-I produced similar dose–response curves that were displaced to the left of the IGF-I curve (Fig. 3), with 111 μg doses of des(1–3)IGF-I or LR^{3}IGF-I/day showing essentially the same effect as 278 μg of IGF-I/day. Statistical comparisons between the three log/linear dose–response regressions indicated that IGF-I was significantly less potent ($P < 0.01$) than the two variants.

Nitrogen retention expressed as the cumulative retention over the 7-day period (Fig. 4) gave a similar pattern to that obtained

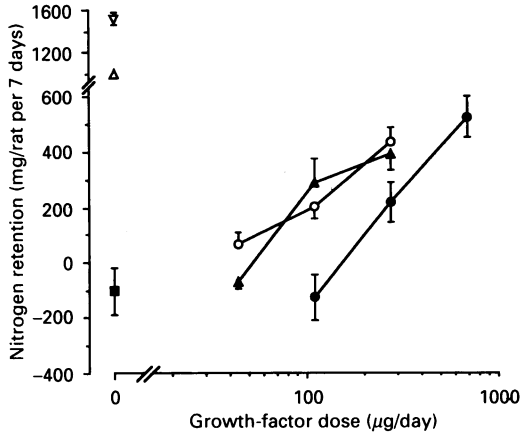

Fig. 4. Cumulative nitrogen retention over the 7-day treatment period

Nitrogen retention is expressed in mg per rat over 7 days. Other details are given in the legend to Fig. 3.

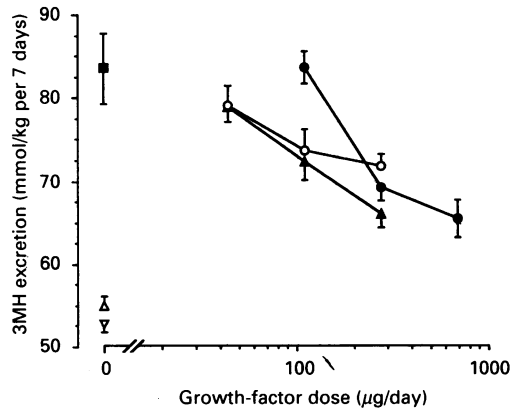

Fig. 5. Cumulative 3MH excretion over the 7-day treatment period

3MH excretion is expressed as μmol/kg body wt. over 7 days. Other details are given in the legend to Fig. 3.

with body weight. Both IGF variants were more potent than IGF-I, with the 111 μg/day dose of des(1–3)IGF-I and LR³IGF-I producing retentions of 283±86 mg and 196±40 mg respectively, substantially greater than with the same dose of IGF-I, −132±83 mg, and approximately equal to the 210±73 mg obtained with a 2.5-fold higher amount of IGF-I. These effects were not associated with differences in nitrogen intake. Thus the three IGF peptides administered at doses of 111 μg/day were associated with average intakes of 2.4 g of nitrogen/kg body wt. per day over the 7-day period.

Muscle protein breakdown has been assessed by the urinary excretion of 3MH. The cumulative excretion of 3MH over the 7-day treatment period was 83.5±4.2 μmol/kg for the dexamethasone-only group (Fig. 5). This value was unchanged at the lowest IGF-I dose and was decreased progressively to 69.0±1.7 and 65.1±2.2 μmol/kg in animals treated with 278 and 695 μg of IGF-I/day. Similar patterns were obtained with des(1–3)IGF-I and LR³IGF-I, but treatment with 111 μg of these variants/day led to lower excretion rates of 72.1±2.2 and 73.4±2.6 respectively, compared with 83.4±1.9 μmol/kg for IGF-I at the same dose.

Even at the highest dose of IGF-I, the cumulative excretion of 3MH over the 7-day period (65.1±2.2 μmol/kg) remained significantly ($P < 0.001$) higher than the 54.9±1.2 μmol/kg

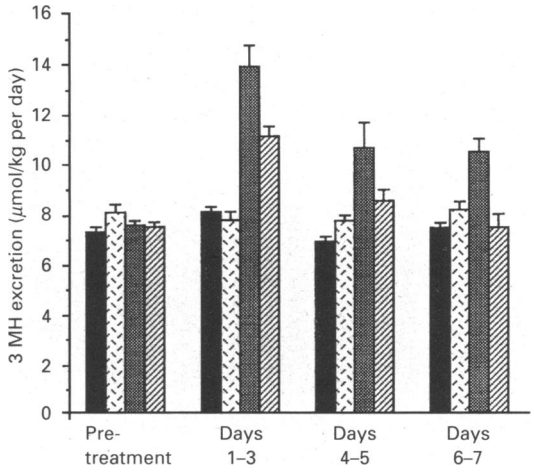

Fig. 6. 3MH excretion in reference animals and in those treated with 695 μg of IGF-I/day

3MH is expressed as μmol/kg body wt. per day in control animals fed *ad libitum* (■), dexamethasone-only animals (▨), control animals pair-fed with reference to the dexamethasone-only group (■) and dexamethasone-treated animals also receiving 695 μg of IGF-I/day (▨). Data have been calculated from urine pools on the three pre-treatment days as well as on days 1–3, 4–5 and 6–7 of treatment. Values are means±S.E.M. for six animals in each group.

Table 1. Muscle protein synthesis measured on day 7 of treatment

Values are means±S.E.M. for six animals in each group: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ versus dexamethasone-treated animals not receiving an IGF peptide.

Treatment group	RNA muscle (mg/g)	Protein synthesis (K_s, %/day)	Protein synthesis (g of protein/day per g of RNA)
Control, fed *ad libitum*	1.91±0.09‡	11.92±0.48‡	12.40±0.15‡
Pair-fed control	1.78±0.01‡	10.58±0.34‡	11.73±0.37‡
Dexamethasone	1.08±0.06	4.61±0.16	8.88±0.52
+IGF-I, 111 μg/day	1.19±0.04*	4.75±0.38	8.24±0.62
+IGF-I, 278 μg/day	1.17±0.05*	5.10±0.55	9.03±0.84
+IGF-I, 695 μg/day	1.23±0.05†	5.59±0.62*	9.00±0.78
+des(1–3)IGF-I, 44 μg/day	1.14±0.03	5.50±0.51	9.71±0.81
+des(1–3)IGF-I, 111 μg/day	1.15±0.05	5.56±0.39	10.26±0.71
+des(1–3)IGF-I, 278 μg/day	1.16±0.04	6.38±0.39†	10.88±0.40*
+LR³IGF-I, 44 μg/day	1.10±0.09	5.05±0.40	9.63±0.69
+LR³IGF-I, 111 μg/day	1.17±0.05*	5.28±0.32	9.14±0.48
+LR³IGF-I, 278 μg/day	1.22±0.06†	6.13±0.33†	10.24±0.62

measured for the pair-fed control group. However, this 7-day total masked a progressive decline from a partial response early in the treatment period to a complete restoration of the average daily control rate during the last 2 treatment days (Fig. 6). The time course shown for the highest IGF-I dose in Fig. 6 was also evident with the IGF variants; with doses of 278 μg/day the days 6–7 excretion rates were 8.4±0.4 μmol/kg per day with des(1–3)IGF-I and 8.2±0.6 μmol/kg per day with LR³IGF-I,

Table 2. Organ and tissue weights

Values are means ± S.E.M. for six animals in each group: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ versus dexamethasone-treated rats not receiving an IGF peptide.

Treatment group	Weight (g/kg body wt.)					
	Carcass	Thymus	Spleen	Gut	Liver	Kidneys
Control, fed *ad libitum*	446±7‡	2.10±0.22‡	3.10±0.19‡	34.5±1.1†	52.4±0.9‡	8.86±0.27‡
Pair-fed control	452±6‡	2.24±0.12‡	2.69±0.08‡	38.1±0.7	39.5±1.0‡	8.89±0.12‡
Dexamethasone	399±6	0.48±0.07	1.19±0.05	39.3±1.9	71.9±2.4	11.13±0.24
+IGF-I, 111 μg/day	397±5	0.62±0.09	1.69±0.05‡	44.5±2.1†	72.4±2.6	12.85±0.38‡
+IGF-I, 278 μg/day	393±6	0.48±0.06	1.84±0.05‡	49.5±2.5‡	66.5±3.2	12.11±0.42†
+IGF-I, 695 μg/day	394±4	0.53±0.06	2.08±0.10‡	51.8±1.4‡	60.4±2.4†	11.90±0.27*
+des(1–3)IGF-I, 44 μg/day	398±4	0.52±0.05	1.67±0.06‡	45.5±2.3†	71.8±2.4	13.11±0.26‡
+des(1–3)IGF-I, 111 μg/day	391±6	0.53±0.06	1.88±0.08‡	49.2±2.7‡	70.0±2.0	12.69±0.29‡
+des(1–3)IGF-I, 278 μg/day	405±4	0.57±0.06	1.91±0.05‡	53.3±1.9‡	60.6±2.5†	12.38±0.22†
+LR^{3}IGF-I, 44 μg/day	397±10	0.56±0.03	1.69±0.06‡	46.2±2.0‡	69.4±2.0	11.99±0.21*
+LR^{3}IGF-I, 111 μg/day	390±7	0.55±0.09	1.93±0.06‡	53.0±2.2‡	66.0±1.7	12.41±0.18†
+LR^{3}IGF-I, 278 μg/day	386±7	0.61±0.04	2.07±0.06‡	54.9±2.3‡	62.4±1.7†	11.98±0.27*

Table 3. Carcass composition analysis

Values are means ± S.E.M. for six animals in each group: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ versus dexamethasone-treated rats not receiving an IGF peptide. Weight is in grams, with protein calculated as nitrogen × 6.25.

Treatment group	Composition (g)				
	Total	Water	Protein	Fat	Residue
Control, fed *ad libitum*	86.8±3.4‡	60.6±2.2‡	16.7±0.6‡	5.8±0.5‡	3.8±0.3†
Pair-fed control	74.7±1.1‡	53.1±0.9‡	15.2±0.4‡	3.5±0.2	2.9±0.1
Dexamethasone	51.9±1.5	34.6±1.1	10.3±0.3	4.0±0.2	3.0±0.2
+IGF-I, 111 μg/day	51.7±1.0	33.7±0.6	10.3±0.3	4.5±0.4	3.3±0.2
+IGF-I, 278 μg/day	55.7±1.2	37.4±0.6*	11.1±0.3*	4.0±0.3	3.3±0.2
+IGF-I, 695 μg/day	61.9±1.3‡	42.0±0.9‡	12.2±0.3‡	4.3±0.3	3.5±0.2*
+des(1–3)IGF-I, 44 μg/day	53.7±1.5	35.7±1.1	10.6±0.2	4.3±0.2	3.2±0.2
+des(1–3)IGF-I, 111 μg/day	56.1±2.0*	37.6±1.2*	11.0±0.3	4.1±0.4	3.5±0.3*
+des(1–3)IGF-I, 278 μg/day	60.9±1.0‡	41.8±0.7‡	11.9±0.2‡	3.8±0.2	3.3±0.2
+LR^{3}IGF-I, 44 μg/day	52.3±0.7	34.8±0.7	10.5±0.2	3.8±0.4	3.2±0.2
+LR^{3}IGF-I, 111 μg/day	54.5±1.5	36.6±1.0	10.7±0.2	3.8±0.3	3.4±0.2
+LR^{3}IGF-I, 278 μg/day	57.9±1.4†	38.9±0.9†	11.3±0.3†	4.0±0.5	3.6±0.2*

similar to the 8.1±0.3 μmol/kg per day observed with the pair-fed group.

Unlike the quantification of muscle protein breakdown, muscle protein synthesis could only be measured as the animals were killed. The synthesis rate, expressed as the percentage of the protein mass synthesized per day (K_s, %/day), was decreased in the dexamethasone-only group to 44% of the value obtained in the pair-fed control animals (Table 1). This fall in K_s was significantly less ($P < 0.05$) in the group receiving dexamethasone plus 695 μg of IGF-I/day, and to an even greater extent ($P < 0.01$) with dexamethasone plus 278 μg of des(1–3)IGF-I or LR^{3}IGF-I/day. However, even the highest K_s obtained was only 60% of that measured in pair-fed controls.

The RNA content of muscle from the dexamethasone-only group was 60% of that from the pair-fed controls even though protein content remained unaffected by treatment (203.6±2.0 mg/g; results not shown). This decreased RNA content was only marginally increased even at the highest doses of IGF-I or IGF variants (Table 1). Accordingly, muscle protein synthesis expressed as g of protein synthesized/day per g of RNA was decreased to a relatively smaller extent after dexamethasone treatment. This rate was partially restored by the medium and high doses of des(1–3)IGF-I as well as by the high dose of LR^{3}IGF-I (Table 1).

The fractional weights (g/kg body wt.) of several organs were altered by dexamethasone (Table 2). The carcass weight was decreased from 452±6 g/kg in the pair-fed controls to 399±6 g/kg in the dexamethasone-only group. No dose of IGF produced any significant restoration of the fractional carcass weight. A similar situation occurred with the thymus, which was decreased by 80% in weight by dexamethasone. The spleen weight was also decreased markedly after dexamethasone treatment, but with this organ a dose-dependent increase was produced by each IGF peptide (Table 2). Very substantial increases in the total gut weight occurred in animals given IGF-I, des(1–3)IGF-I and especially LR^{3}IGF-I. Liver and kidney weights were increased by dexamethasone. The liver weights were decreased somewhat in a dose-dependent manner by each IGF peptide, whereas kidney weights were further increased, especially at the lowest doses of IGF-I and des(1–3)IGF-I. Higher doses of the IGF peptides appeared to decrease kidney weights (Table 2). Of the other tissues measured, no effects of any IGF peptide were found with the pelt, lungs or adrenals. The heart weight was increased by dexamethasone and partly restored

Table 4. IGF-I measurements in plasma obtained on day 7

Plasma was obtained from blood collected when the animals were killed on day 7 of the experiment. After molecular-sieve chromatography under acid conditions to remove binding proteins, the IGF was measured by using either a polyclonal or a monoclonal anti-human IGF-I antibody with human IGF-I as reference. Values are means ± S.E.M. of six animals in each group.

Treatment group	IGF-I (μg/l) as measured with: Polyclonal antibody	Monoclonal antibody
Control, fed *ad libitum*	823 ± 76	80 ± 18
Pair-fed control	546 ± 49	91 ± 15
Dexamethasone	378 ± 35	106 ± 11
+ IGF-I, 111 μg/day	924 ± 50	776 ± 79
+ IGF-I, 278 μg/day	1123 ± 73	1081 ± 75
+ IGF-I, 695 μg/day	1544 ± 70	1657 ± 160
+ des(1–3)IGF-I, 44 μg/day	485 ± 35	322 ± 23
+ des(1–3)IGF-I, 111 μg/day	597 ± 44	521 ± 75
+ des(1–3)IGF-I, 278 μg/day	606 ± 27	796 ± 46
+ LR^3IGF-I, 44 μg/day	404 ± 34	140 ± 42
+ LR^3IGF-I, 111 μg/day	452 ± 40	150 ± 40
+ LR^3IGF-I, 278 μg/day	446 ± 40	198 ± 38

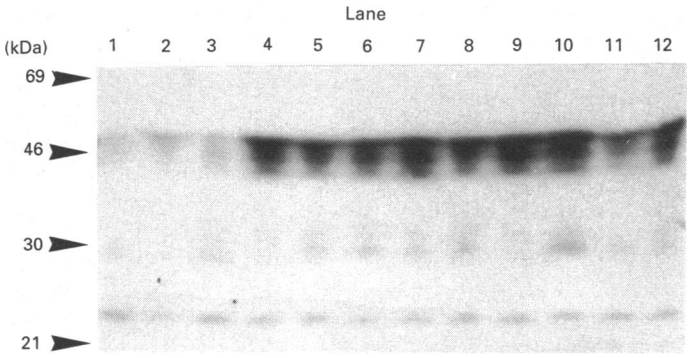

Fig. 7. IGF-binding proteins measured in plasma collected on day 7

Binding proteins in plasma pools from each group of animals were analysed by the ligand-blotting technique, using ^{125}I-labelled IGF-I as the probe. The molecular masses of protein standards are shown. The lanes represent 5 μl of plasma obtained from the following treatment groups: 1, controls fed *ad libitum*; 2, dexamethasone only; 3, pair-fed controls; 4, des(1–3)IGF-I at 44 μg/day; 5, LR^3IGF-I at 44 μg/day; 6, IGF-I at 111 μg/day; 7, des(1–3)IGF-I at 111 μg/day; 8, LR^3IGF-I at 111 μg/day; 9, IGF-I at 278 μg/day; 10, des(1–3)IGF-I at 278 μg/day; 11, LR^3IGF-I at 278 μg/day; 12, IGF-I at 695 μg/day.

to normal by the highest doses of IGF-I, des(1–3)IGF-I or LR^3IGF-I (results not shown).

Total carcass weights, carcass water and protein were markedly decreased in the dexamethasone-only animals as compared with the two control groups (Table 3). Dexamethasone treatment did not lower carcass fat or residue (largely minerals and carbohydrate) from the values obtained in the pair-fed animals. However, fat and residue were higher in the controls fed *ad libitum* than in the dexamethasone-only group. The largest doses of each IGF peptide produced highly significant ($P < 0.01$ or $P < 0.001$) increases in carcass water, protein and total weight. In contrast with these responses, no significant effects of the IGF peptides were observed with fat, which remained at approx. 4 g per carcass even under those conditions where the total carcass weight was 20% higher.

The circulating IGF-I levels achieved on the last treatment day are shown in Table 4. IGF-I measured with the polyclonal antibody was decreased by dexamethasone and increased above this value 2.4-, 3.0- and 4.1-fold by the increasing doses of exogenous IGF-I. Small increases occurred with des(1–3)IGF-I and essentially none with LR^3IGF-I, consistent with the immunological cross-reactivities of these variants in the polyclonal assay. Similar results were found with the monoclonal-antibody assay when allowances were made for the higher des(1–3)IGF-I cross-reactivity and the very weak cross-reactivity with rat IGF-I (Baxter *et al.*, 1982).

The circulating IGF-binding proteins were assessed by the ligand-blot procedure (Hossenlopp *et al.*, 1986). At the autoradiograph exposure used, binding proteins were only barely detected in controls fed *ad libitum*, pair-fed and dexamethasone-only groups (Fig. 7). However, the administration of all three IGF peptides led to substantial induction of the IGF-binding-protein (IGFBP)-3 triplet, with the effects somewhat less with LR^3IGF-I.

DISCUSSION

Subcutaneous administration of IGF-I to normal (Hizuka *et al.*, 1986; Fagin *et al.*, 1990), hypophysectomized (Schoenle *et al.*, 1985), nitrogen-restricted (Tomas *et al.*, 1991*a*), diabetic (Scheiwiller *et al.*, 1986; Tomas *et al.*, 1991*b*) or partially gut-resected (Lemmey *et al.*, 1991) rats leads either to an increased weight gain or to a reversal of the weight loss. In those investigations where nitrogen balance was also measured, it too was improved. The present experiment demonstrates a similar growth response in animals made catabolic with dexamethasone, confirming the preliminary reports by Fagin *et al.* (1989, 1990) and further establishing the generality of IGF-I as a growth promotant.

The activities of des(1–3)IGF-I and LR^3IGF-I are striking. Both variants are approx. 2.5-fold more potent than IGF-I at improving weight gain and nitrogen retention when the comparisons are made at the midpoints of the dose–response plots (Figs. 3 and 4) and somewhat more active at lower doses. The variants differ from IGF-I by binding poorly to IGF-binding proteins (Forbes *et al.*, 1988; Walton *et al.*, 1990), a property that may increase their delivery from the blood to tissue sites of action. Certainly the pharmacokinetics of des(1–3)IGF-I indicate a faster removal rate of this variant from the blood (Ballard *et al.*, 1991). Notwithstanding its very low binding affinities for several IGF-binding proteins, the high potency of LR^3IGF-I is surprising, because this variant has a 3-fold lower affinity than IGF-I or des(1–3)IGF-I for the type 1 IGF receptor (Walton *et al.*, 1990). We presume that the low receptor affinity of LR^3IGF-I must be counteracted by an even more rapid transfer from blood to tissues or higher local free concentrations than found for des(1–3)IGF-I. It is important to note that the higher potencies *in vivo* of des(1–3)IGF-I and LR^3IGF-I argue strongly against the generality of the concept that binding proteins promote the bioactivity of IGF peptides.

IGF-binding proteins, especially the IGFBP-3 triplet, are induced in rats by the administration of IGF-I (Zapf *et al.*, 1989; Tomas *et al.*, 1991*a*,*b*). Dexamethasone also leads to an increase in IGFBP-1 and IGFBP-3 (Luo *et al.*, 1990; Luo & Murphy, 1990). The combination of both treatments in the present

investigation produced a very substantial effect, as shown by the ligand blots in Fig. 7. The animals treated only with dexamethasone have low plasma IGF-I levels but undiminished binding-protein concentrations, indicative of an excess IGF-binding capacity. This situation may explain why low doses of IGF-I given to dexamethasone-treated animals did not increase growth rates, whereas the administration of des(1–3)IGF-I or LR^3IGF-I was effective.

In normal rats the eviscerated carcass accounts for about 45% of the body weight and a similar percentage of the nitrogen content. Dexamethasone treatment decreases this proportion, along with the weights of the total carcass, water and nitrogen (Tables 2 and 3). The highest doses of IGF-I, des(1–3)IGF-I and LR^3IGF-I led to 10–18% increases in carcass protein above the dexamethasone-only group. These results are consistent with the decreased rate of muscle protein breakdown as measured by 3MH excretion throughout the IGF treatment period as well as the higher rates of muscle protein synthesis on day 7. We should also consider that the fall in 3MH excretion produced by the IGF peptides in part may reflect a decrease in the amount of this amino acid released from non-skeletal-muscle sources of actin or myosin. In this context it has been argued that intestinal smooth muscle contributes significantly to urinary 3MH in the rat (Millward & Bates, 1983). However, a fall in gut-derived 3MH in IGF-treated rats will be compensated, at least in part, by the concomitant increase in gut mass.

The effects of IGF peptides on 3MH excretion differ substantially between dexamethasone-treated animals and diabetic animals. Since 3MH excretion is not decreased at all by IGF treatment of diabetic rats (Tomas *et al.*, 1991*b*), notwithstanding a powerful growth response. In contrast with IGF, insulin treatment of the diabetic animals decreased 3MH excretion to normal. It is possible that insulin might have contributed to the fall in 3MH excretion in dexamethasone-treated animals between days 1–3 and days 6–7 (Fig. 6), because insulin levels are known to increase after glucocorticoid administration (Tomas, 1982). We note, however, that none of the IGF treatments affected plasma glucose or glucose excretion in these mildly hyperglycaemic dexamethasone-treated rats (results not shown). The effects on protein synthesis also differ from those reported for diabetic rats (Tomas *et al.*, 1991*b*), where there were no changes in translational efficiency as measured by synthesis per unit of RNA. In diabetic animals, IGF treatment increased protein synthesis by restoring the RNA content to normal. Dexamethasone, on the other hand, decreased the capacity for protein synthesis as measured by RNA content, as well as the translational efficiency (Table 1). Both were partially increased by the higher doses of IGF peptides.

In a number of experimental conditions the administration of IGF-I leads to increases in spleen and thymus weights (Guler *et al.*, 1988; Binz *et al.*, 1990; Lemmey *et al.*, 1991). A similar effect on the spleen occurred here, since the decrease in spleen weight in the dexamethasone-treated rats was partly prevented by IGF treatment (Table 2). However, the thymus weight was not increased. We therefore infer that the immunosuppressive effects of dexamethasone that lead to thymus atrophy cannot be prevented by IGF-I.

Although exogenous glucocorticoids may not fully mimic the response to trauma or stress, the results presented here certainly suggest that the administration of IGF-I to cases of polytrauma will lead to an amelioration of the attendant catabolism and, hence, improved recovery. Moreover, IGF variants that bind poorly to the circulating binding proteins are likely to be significantly more potent than IGF-I.

REFERENCES

Ballard, F. J., Read, L. C., Francis, G. L., Bagley, C. J. & Wallace, J. C. (1986) Biochem. J. **233**, 223–230

Ballard, F. J., Knowles, S. E., Walton, P. E., Edson, K., Owens, P. C., Mohler, M. A. & Ferraiolo, B. L. (1991) J. Endocrinol. **128**, 197–204

Baxter, R. C., Axiak, S. & Raison, R. L. (1982) J. Clin. Endocrinol. Metab. **54**, 474–476

Binz, K., Joller, P., Froesch, P., Binz, H., Zapf, J. & Froesch, E. R. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 3690–3694

Clemmons, D. R., Underwood, L. E., Dickerson, R. N., Brown, R. O., Hak, L. J., MacPhee, R. D. & Heizer, W. D. (1985) Am. J. Clin. Metab. **41**, 191–198

Coates, C. L., Burwell, R. G., Carlin, S. A., Cason, J. S., Littlejohn, S., Selby, C. & Swanwell, A. J. (1981) Burns **7**, 425–433

Fagin, K. D., Kaplan, D. L., Hseih, P., Tsai, L. B., Rohde, M., Morris, C. F. & Miller, J. A. (1989) Endocr. Soc. Annu. Meet. 71st, abstr. 1666

Fagin, K. D., Kaplan, D. L., Huscher, L. L., Hsieh, P., Rosenfeld, R., Tsai, L. B., Mueller, W. & Miller, J. A. (1990) Endocr. Soc. Annu. Meet. 72nd abstr. 913

Forbes, B., Szabo, L., Baxter, R. C., Ballard, F. J. & Wallace, J. C. (1988) Biochem. Biophys. Res. Commun. **157**, 196–202

Francis, G. L., Upton, F. M., Ballard, F. J., McNeil, K. A. & Wallace, J. C. (1988) Biochem. J. **251**, 95–103

Francis, G. L., McNeil, K. A., Wallace, J. C., Ballard, F. J. & Owens, P. C. (1989) Endocrinology (Baltimore) **124**, 1173–1183

Frayn, K. N., Price, D. A., Maycock, P. F. & Carroll, S. M. (1984) Clin. Endocrinol. **20**, 179–187

Goldberg, A. L. (1969) J. Biol. Chem. **244**, 3223–3229

Guler, H.-P., Zapf, J., Scheiwiller, E. & Froesch, E. R. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 4889–4893

Hizuka, N., Takano, K., Shizume, K., Asakawa, K., Miyakawa, M., Tanaka, I. & Horikawa, R. (1986) Eur. J. Pharmacol. **125**, 143–146

Hossenlopp, P., Seurin, D., Segovia-Quinson, B., Hardouin, S. & Binoux, M. (1986) Anal. Biochem. **154**, 138–143

Lemmey, A. B., Martin, A. A., Read, L. C., Tomas, F. M., Owens, P. C. & Ballard, F. J. (1991) Am. J. Physiol. **260**, E213–E219

Luo, J. & Murphy, L. J. (1990) Mol. Cell. Endocrinol. **74**, 213–219

Luo, J., Reid, R. E. & Murphy, L. J. (1990) Endocrinology (Baltimore) **127**, 1456–1462

Millward, D. J. & Bates, P. C. (1983) Biochem. J. **214**, 607–615

Mossier, H. D., Jr., Spencer, E. M., Dearden, L. C. & Jansons, R. A. (1987) Pediatr. Res. **22**, 92–95

Odedra, B. R. & Millward, D. J. (1982) Biochem. J. **204**, 663–672

Scheiwiller, E., Guler, H.-P., Merryweather, J., Scandella, C., Maerki, W., Zapf, J. & Froesch, E. R. (1986) Nature (London) **323**, 169–171

Schoenle, E., Zapf, J., Hauri, C., Steiner, T. & Froesch, E. R. (1985) Acta Endocrinol. (Copenhagen) **108**, 167–174

Tomas, F. M. (1982) Biochem. J. **208**, 593–601

Tomas, F. M., Munro, H. N. & Young, V. R. (1979) Biochem. J. **178**, 139–146

Tomas, F. M., Murray, A. J. & Jones, L. M. (1984*a*) Biochem. J. **220**, 469–479

Tomas, F. M., Murray, A. J. & Jones, L. M. (1984*b*) Br. J. Nutr. **51**, 323–337

Tomas, F. M., Knowles, S. E., Owens, P. C., Read, L. C., Chandler, C. S., Gargosky, S. E. & Ballard, F. J. (1991*a*) J. Endocrinol. **128**, 97–105

Tomas, F. M., Knowles, S. E., Owens, P. C., Read, L. C., Chandler, C. S., Gargosky, S. E. & Ballard, F. J. (1991*b*) Biochem. J. **276**, 547–554

Unterman, T. G. & Phillips, L. S. (1985) J. Clin. Endocrinol. Metab. **61**, 618–626

Walton, P. E., Francis, G. L., Ross, M., Brazier, J. A., Wallace, J. C. & Ballard, F. J. (1990) J. Cell Biol. **111**, abstr. 2645

Zapf, J., Hauri, C., Waldvagel, M., Futo, E., Häsler, H., Binz, K., Guler, H. P., Schmid, C. & Froesch, E. R. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 3813–3817

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