Effects of insulin and insulin-like growth factors on protein and energy metabolism in tumour-bearing rats

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The effects of insulin-like growth factor-1 (IGF-I), and a more potent variant LR³-IGF-I, which binds poorly to IGF-binding proteins, were investigated in rats bearing a mammary adenocarcinoma. The effect of insulin, either alone or in combination with LR³-IGF-I, was also investigated. Peptides were infused via osmotic minipumps for 6–7 days after tumour size reached 5% of body weight. Infusion of IGFs alone at either 200 or 500 µg/day significantly decreased food intakes as well as circulating levels of insulin and glucose, and consequently failed to promote muscle protein accretion in the host. Tumour growth was increased by the IGFs, especially by LR³-IGF-I, even though these peptides did not promote growth of the adenocarcinoma in cell culture. Infusion of LR³-IGF-I, and to a lesser extent IGF-I, led to decreased rates of muscle protein synthesis and increased muscle protein breakdown, but each of these measures was closely related to the final tumour burden ($r^2 = 0.454$ and 0.810 respectively; $P < 0.01$) and possibly resulted from a decrease in substrate supply to the host tissues. Insulin infusion (100 µg/day)

increased food consumption by more than 50% and significantly decreased tumour growth. Insulin and LR³-IGF-I had a synergistic effect on host weight, which increased by 19.1 ± 1.9 , -1.1 ± 4.7 and 37.9 ± 1.5 g for insulin, LR³-IGF-I and combined treatments respectively. Carcass protein was increased by more than 10% with insulin treatment, due to increased rates of synthesis and decreased rates of muscle protein breakdown, but LR³-IGF-I had no positive effect on carcass protein accretion, either alone or in combination with insulin. Similarly, the amount of carcass fat was increased almost 2-fold by insulin treatment, whereas it was decreased by 30% by LR³-IGF-I. These changes may have arisen either from direct hormone effects on metabolism or from the indirect effects of food intake, or both. Our results suggest that IGF administration may exacerbate an insulin insufficiency associated with the tumour-bearing state and further decrease metabolic substrate supply to the host. This can be overcome by co-infusion of insulin.

INTRODUCTION

Cancer results in altered host metabolism and wasting of body tissues (cachexia), and if unchecked leads to death of the host [1–4]. The skeletal muscle, the main protein mass of the organism, is severely affected in cancer cachexia [5,6]. It has been shown that breakdown rates of muscle protein are markedly elevated in cachectic states [5,7,8], with a concomitant, though lesser, decrease in synthesis [5,9,10]. Attention has focused on hormonal therapies that may control or reverse the cachectic state, with insulin showing a number of beneficial effects [6,11–13]. Recent work in experimental animals has shown that insulin-like growth factors (IGFs), and especially certain IGF-I variants, can substantially reverse the disorders in muscle metabolism and the attendant wasting induced by diabetes, glucocorticoids and protein deficiency [14–17]. Aspects of the cachectic syndrome seen in cancer, infection and inflammation have been attributed to increased circulating levels of cytokines, and in this regard IGF-I has also been shown to maintain its protein-anabolic role in the face of high levels of tumour necrosis factor [18].

The opportunity to use IGF in the treatment of cancer cachexia raises the question of potential growth-stimulatory effects of this growth factor on the tumour. In this regard, Ng et al. [19] have recently demonstrated that infusion of IGF-I to rats bearing a methycolanthrene-induced sarcoma can preserve lean tissue mass of the host without stimulating tumour growth. However, the tumour burden was small compared with other wasting models [11,20,21].

We have previously shown that variants of IGF-I, in particular LR³-IGF-I, have more potent effects on reversal of catabolism in cachectic states [14–17], and have now examined the effect of IGF-I and LR³-IGF-I in rats bearing a mammary adenocarcinoma. Cachexia involves not only loss of muscle tissue, a site of action for IGFs, but also energy wasting through altered carbohydrate and fat metabolism, where insulin is the principal controlling hormone [11,20–22]. We have therefore also compared the effects of the IGFs and insulin on body composition, protein synthesis and aspects of carbohydrate and fat metabolism in tumour-bearing rats. The results presented in this paper show that, although the IGFs do not by themselves reverse wasting, an anabolic effect greater than that seen with insulin alone was observed with the combination of LR³-IGF-I and insulin.

MATERIALS AND METHODS

Peptides and chemicals

Recombinant human IGF-I and recombinant LR³-IGF-I, which has a 13-residue N-terminal extension and Glu-3 replaced by Arg in the IGF sequence [23], were obtained from GroPep Pty. Ltd., Adelaide, South Australia. Recombinant human insulin [Humulin; Eli Lilly (Australia) and Co., West Ryde, N.S.W., Australia] was diluted as required in a solution containing 5 mM glutamic acid. The peptides were administered to animals by using mini-osmotic pumps (model 2001; Alza, Palo Alto, CA, U.S.A.) which delivered 0.98 µl/h. The IGFs were dissolved in 0.1 M acetic acid to the concentrations required for the pumps to

deliver the allocated treatment dose. Pumps for control animals were filled with 0.1 M acetic acid.

Experiments *in vitro*

Experiments *in vitro* to ascertain the responsiveness of the tumour cells to insulin, IGF-I and LR³-IGF-I were conducted under conditions previously described [24]. The mammary adenocarcinoma cells were cultured in RPMI 1640 (Cytosystems Pty. Ltd., Castle Hill, N.S.W., Australia) with 20 mM Hepes, 24 mM NaHCO₃ and in the presence of 25 or 100 g/l foetal-calf serum (Cytosystems). Cells were inoculated at a density of 0.7×10^6 cells in 10 ml of medium in loosely capped 25 cm² culture flasks kept in an atmosphere of 5% CO₂ in air. IGFs or insulin were included at a concentration of 100 µg/l or 4.17 mg/l respectively. The concentration of IGFs was higher than that required to stimulate maximally the rate of protein synthesis in rat L6 myoblasts in culture [23]. After 3 days, growth was compared with that in cultures without growth-factor addition.

Animals and tumour

Female Dark Agouti rats (180 g body wt.) were obtained from the Animal Resource Centre, Gilles Plains, South Australia, and held in cages at 25 °C under controlled lighting (12 h-dark/12 h-light cycle). The rats were fed on a commercial chow diet (Milling Industries, Murray Bridge, South Australia) prior to implantation with a mammary adenocarcinoma. After implantation *in vivo*, the tumour grows to 15% of host body weight in 2–3 weeks [21,24]. Experiments were conducted over a 6- or 7-day period, during the time when the tumour normally grows from 5% to 15% of body wt. Six rats were included in each experimental group, but, because of limits to caging, the experiments were done in stages with either 2 (Expt. 1) or 3 (Expt. 2) animals per group. The protocol for the experiment was approved by the Animal Care and Ethics Committee of the Institute for Medical and Veterinary Science, 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. Tumours were implanted (subcutaneous injection of cell suspension in each flank) as described previously [21,24] and the rats were placed in metabolism cages, where they received a powdered starch/sucrose-based diet containing 180 g of casein and 2.5 g of methionine/kg and was free of N⁷-methylhistidine (N⁷-MH) [25]. When the tumour had grown to 5% of body weight, as assessed by caliper measurement, mini-osmotic pumps containing the appropriate hormone concentrations were implanted subcutaneously in the supra-scapular region.

Two experiments were performed. In Expt. 1 there were 5 groups treated for 6 days: vehicle; IGF-I, 200 or 500 µg/day; LR³-IGF-I, 200 or 500 µg/day. In Expt. 2 there were 4 groups treated for 7 days, with each rat receiving two pumps: vehicle+vehicle; insulin, 100 µg/day, +LR³-IGF-I vehicle; LR³-IGF-I, 200 µg/day, +insulin vehicle; insulin, 100 µg/day, +LR³-IGF-I, 200 µg/day. Food and water intake and body weights were recorded daily. Tumour dimensions were measured by calipers at the start and mid-point of peptide infusion. Urine and faeces were collected daily from 2 days before pump insertion and stored at -20 °C before analysis.

At the end of the treatment period, a solution containing 150 mM phenylalanine, 77 mM NaCl and 20 µCi of L-[ring-2,6-

³H]phenylalanine/ml was injected (20 ml/kg body wt.) into the peritoneal cavity, and the rats were returned to a cage. Then 15 min later the rats were stunned and decapitated, and trunk blood was collected into a heparinized tube. A consistent section of mixed hind-limb muscles (chiefly gastrocnemius, plantaris and biceps femoris) was rapidly excised and frozen in tongs precooled in liquid N₂. The tumour on the right flank was then rapidly exposed, and a section was excised and frozen as described for the muscle. The pelt, remaining tumour tissue and the visceral organs were then removed and weighed before being discarded. The musculo-skeletal remainder, designated as the carcass, was stored at -20 °C for later analysis.

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). The fat content of dried carcass was measured gravimetrically after chloroform/methanol (1:1, v/v) extraction. Urinary N⁷-MH was measured by continuous flow analysis [26] after initial hydrolysis and ion-exchange chromatography [27]. Muscle and tumour total RNA contents were measured as described by Munro and Fleck [28]. Rates of muscle and tumour protein synthesis were determined essentially as described by Jepson et al. [29] and expressed both as a fractional rate (K_s , % of protein pool synthesized per day) and as a rate per unit of RNA (g of protein synthesized/g of RNA per day). The rate at which a plateau of specific radioactivity was achieved in the intracellular pools of amino acids was assessed in untreated tumour-bearing (tumour size about 15% of body weight) rats killed at 1, 2, 3, 5 and 15 min after intraperitoneal injection of the [³H]phenylalanine. A plateau was reached in 2–3 min in muscle and between 3 and 5 min in the tumour. The corresponding integral of phenylalanine specific radioactivity over time for these tissues was 0.95 and 0.85 of the value calculated on the basis of instantaneous achievement of the plateau values, in general agreement with the value of 0.9 previously reported for muscle [29]. These values were used to calculate protein synthesis rates by using the equation:

$$K_s = \frac{S_B \times 1440}{I_T S_I t} \times 100$$

where S_B and S_I are the specific radioactivities for protein-bound and free intracellular phenylalanine respectively, I_T is the relative integral value for a particular tissue and t is the time of incorporation of radioactivity in minutes. The values established for I_T in tumour-bearing control rats were also used for calculating the protein-synthesis rates in treated rats.

Circulating levels of glucose, lactate, non-esterified fatty acids and triacylglycerols were measured on whole blood or plasma [21,22]. Whole-blood samples were deproteinized in 5% (w/v) HClO₄, the extracts neutralized and the metabolites determined by using semi-automated enzymic assays [21,24]. Insulin was assayed with a r.i.a. kit (ref. SB-INSI-5; CIS Bio International, Gif-sur-Yvette, France).

Muscle protein breakdown rates were assumed to be equal to the fractional excretion rate of N⁷-MH from the N⁷-MH pool in the carcass, a measure that closely reflects the rate of breakdown of myofibrillar protein [30]. The average N⁷-MH concentration was 3.01 µmol/g of carcass protein. Tumour-free body weight was calculated on the basis of a linear adjustment for tumour weight between the initial assessed weight and the final observed weight of the tumour. This extrapolation agreed reasonably with the mid-point caliper assessment of tumour weights.

Table 1 Food intake, growth and carcass composition in tumour-bearing rats receiving IGF and/or insulin treatmentValues are means $\pm$ S.E.M. for six animals in each group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus vehicle-treated control rats

Treatment	Dose (μ g/day)	Food intake (g/day)	Tumour-free body-weight gain (g)	Nitrogen balance (mg/day)	Carcass protein content† (g)	Carcass fat content† (g)	Final tumour weight† (g)	Fractional tumour weight† (g/kg body wt.)
Expt. 1 (6 days treatment)								
Vehicle	0	10.5 $\pm$ 0.5	1.8 $\pm$ 1.4	69 $\pm$ 12	21.6 $\pm$ 1.9	12.4 $\pm$ 0.8	17.9 $\pm$ 1.1	87 $\pm$ 6
IGF-I	200	10.1 $\pm$ 0.3	3.1 $\pm$ 1.4	89 $\pm$ 6	21.4 $\pm$ 1.6	11.4 $\pm$ 0.3	18.9 $\pm$ 1.3	92 $\pm$ 6
IGF-I	500	9.8 $\pm$ 0.2	5.5 $\pm$ 1.6	136 $\pm$ 5***	20.9 $\pm$ 1.1	10.0 $\pm$ 0.6*	25.6 $\pm$ 1.4***	116 $\pm$ 5***
LR ³ -IGF-I	200	9.2 $\pm$ 0.3**	2.6 $\pm$ 2.7	140 $\pm$ 7***	20.1 $\pm$ 1.2	8.8 $\pm$ 0.5***	30.8 $\pm$ 0.7***	141 $\pm$ 4***
LR ³ -IGF-I	500	8.7 $\pm$ 0.3***	8.0 $\pm$ 2.8	139 $\pm$ 8***	20.2 $\pm$ 1.9	8.9 $\pm$ 1.1***	28.7 $\pm$ 1.0***	133 $\pm$ 6***
Expt. 2 (7 days treatment)								
Vehicle	0	13.0 $\pm$ 0.2	-3.1 $\pm$ 1.9	81 $\pm$ 11	19.0 $\pm$ 0.2	8.2 $\pm$ 0.6	34.9 $\pm$ 1.7	156 $\pm$ 6
Insulin	100	19.0 $\pm$ 0.5***	19.1 $\pm$ 1.7***	110 $\pm$ 8	21.1 $\pm$ 0.2**	14.8 $\pm$ 0.9***	27.3 $\pm$ 1.6*	115 $\pm$ 6**
LR ³ -IGF-I	200	12.0 $\pm$ 0.6	-1.1 $\pm$ 4.7	142 $\pm$ 10**	18.0 $\pm$ 0.6	5.8 $\pm$ 0.5*	48.7 $\pm$ 1.3***	203 $\pm$ 6***
Insulin + LR ³ -IGF-I	100 + 200	21.3 $\pm$ 0.9***	37.9 $\pm$ 1.5***	215 $\pm$ 13***	21.3 $\pm$ 0.4***	15.3 $\pm$ 0.6***	40.5 $\pm$ 2.9*	151 $\pm$ 9

† Measured at the end of the treatment period.

Statistics

Values are presented as means, with the S.E.M. indicated. Treatment effects within each experiment were initially assessed by using two-way analysis of variance, partitioning variance due to stages and treatments. Plasma insulin data were log-transformed before analysis. Where the treatment effect was significant ($P < 0.05$), multiple comparisons among means were made by Tukey's procedure, and comparisons of treatment means with the vehicle control group were made by Dunnett's test. These tests employ the experimentwise error rate, and protect against declaring pairs of means different when they could differ by chance [31]. Dose-responses were analysed by multiple linear-regression analysis. Covariance analysis with food intake or tumour size as a covariate was performed on several variables after ensuring parallel responses between treatments. All analyses were performed with Systat 5.0 statistical software (Systat Inc., Evanston, IL, U.S.A.).

RESULTS

Experiment *in vitro*

At least three replicate studies were performed at each level of serum tested. Additions of the IGFs at 100 μ g/l or insulin at 4.17 mg/l to the culture medium containing either 25 or 100 g/l serum did not significantly stimulate growth of the tumour cells. With 100 g/l serum, relative growth was 95 ± 4 , 89 ± 6 and $88 \pm 10\%$ of controls ($n = 4$) for insulin, IGF-I and LR³-IGF-I respectively. With 25 g/l serum the equivalent values were 69 ± 5 , 86 ± 10 and 73 ± 8 ($n = 3$) respectively. Growth rate of the cells increased twofold when the fetal-calf serum was increased from 25 to 100 g/l of medium, a level which supported growth at 95% of the maximal rate achieved at 200 g/l fetal-calf serum (P. Coyle and A. M. Rofo, unpublished work).

Expt. 1

Growth-factor administration caused a small dose-related increase in the tumour-free body-weight gain ($r^2 = 0.37$; $P < 0.05$), despite an attendant decrease in food intake (Table 1), with no difference in potency between the two IGFs. Unlike body weights, it is not possible to measure directly changes in weight or composition of the carcass or organs for each individual rat, so

small treatment effects such as occurred here are more difficult to detect. Nonetheless our data show that the final carcass protein content was maintained at the expense of fat, indicating a relative sparing of lean tissue. The importance of the availability of nutrients is illustrated by the correlation between final carcass protein content and food intake ($r^2 = 0.48$, $P < 0.01$ over all groups), which accounts for the small decrease in the observed total carcass protein with growth-factor treatment. Tumour growth was enhanced by growth-factor administration, particularly by LR³-IGF-I, but the mechanism did not involve an increase in the fractional protein-synthesis rate of tumour tissue, which was not different from controls after 7 days of treatment (Table 2). Changes in nitrogen balance reflected the changes in the combined host and tumour growth but were most strongly related to the latter (Table 1).

Substrate supply to the host tissues is dependent on the levels of circulating hormones and metabolites, and also on the relative degree of competition with the tumour. Thus IGF infusion led to a significant decrease in circulating glucose and insulin levels (Table 2), and this trend closely followed the decrease in food intake associated with the dose and type of IGF (Table 1). The levels of plasma triacylglycerols were most closely related to the final tumour burden ($r^2 = 0.67$; $P < 0.001$). On the other hand, plasma lactate levels were increased only by LR³-IGF-I, indicating a specific action for this peptide (Table 2).

Muscle protein can be mobilized to provide energy, and in this regard both LR³-IGF-I, and to a lesser extent IGF-I, decreased muscle protein-synthesis rates ($P < 0.025$ for combined LR³-IGF-I versus control) and increased the rates of N⁷-MH excretion, an index of muscle protein breakdown (Table 2). These treatment effects may not be direct, as the rate of N⁷-MH excretion (Figure 1) and the fractional rate of muscle protein synthesis at the end of the treatment period were each closely related to the final tumour burden ($r^2 = 0.810$ and 0.454 respectively; $P < 0.01$). The effects of the changes in food intake on protein turnover were relatively minor and non-significant.

Expt. 2

Infusion of insulin alone increased food consumption by more than 50% and, whereas LR³-IGF-I alone decreased food intake, when combined with insulin it stimulated food intake. The gains in total body weight and tumour-free body weight also were

Table 2 Protein metabolism and plasma levels of insulin, glucose and selected metabolites related to energy metabolism in tumour-bearing rats after 6–7 days of IGF and/or insulin treatmentValues are means $\pm$ S.E.M. for six animals in each group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus vehicle-treated control rats

Treatment group	Dose ($\mu\text{g/day}$)	Muscle protein synthesis (%/day)	Muscle protein breakdown (%/day)	Tumour protein synthesis (%/day)	Plasma glucose (mmol/l)	Plasma insulin ($\mu\text{-units/ml}$)	Plasma lactate (mmol/l)	Plasma non-esterified fatty acids (mmol/l)	Plasma triacylglycerol (mmol/l)
Expt. 1 (6 days treatment)									
Vehicle	0	3.32 ± 0.28	3.17 ± 0.20	41.1 ± 2.8	7.00 ± 0.48	28 ± 4	4.69 ± 0.84	n.d.	1.09 ± 0.20
IGF-I	200	3.07 ± 0.13	3.36 ± 0.25	36.2 ± 2.9	7.11 ± 0.15	22 ± 2	3.97 ± 0.58	n.d.	0.87 ± 0.08
IGF-I	500	3.02 ± 0.15	$3.89 \pm 0.16^*$	37.0 ± 3.1	5.98 ± 0.32	$16 \pm 2^{**}$	4.39 ± 0.63	n.d.	1.19 ± 0.22
LR ³ -IGF-I	200	2.62 ± 0.20	$4.80 \pm 0.13^{***}$	42.2 ± 2.6	$5.25 \pm 0.29^{**}$	$14 \pm 2^{***}$	6.27 ± 0.56	n.d.	1.64 ± 0.19
LR ³ -IGF-I	500	2.46 ± 0.24	$4.59 \pm 0.12^{***}$	39.2 ± 2.7	$4.47 \pm 0.30^{***}$	$11 \pm 1^{***}$	6.12 ± 0.90	n.d.	1.34 ± 0.15
Expt. 2 (7 days treatment)									
Vehicle	0	2.72 ± 0.13	3.86 ± 0.28	34.8 ± 3.3	7.94 ± 0.34	16 ± 2	6.33 ± 0.47	0.84 ± 0.10	0.85 ± 0.04
Insulin	100	$3.77 \pm 0.23^{**}$	3.52 ± 0.11	46.6 ± 4.9	$3.28 \pm 0.30^{***}$	$136 \pm 19^{***}$	4.45 ± 0.56	$0.44 \pm 0.09^*$	0.81 ± 0.15
LR ³ -IGF-I	200	2.43 ± 0.09	$5.17 \pm 0.24^{***}$	40.5 ± 1.5	$6.55 \pm 0.26^*$	$9 \pm 1^*$	$10.7 \pm 0.77^{***}$	0.80 ± 0.15	$1.40 \pm 0.16^*$
Insulin + LR ³ -IGF-I	100 + 200	$3.82 \pm 0.26^{**}$	3.82 ± 0.14	43.6 ± 2.3	$3.10 \pm 0.25^{***}$	$102 \pm 25^{***}$	4.61 ± 0.58	0.61 ± 0.05	1.14 ± 0.11

Figure 1 Relationship between the rate of N³-MH excretion and relative tumour burden in groups of rats implanted with a mammary adenocarcinoma and receiving IGFs and/or insulin via continuous infusion for 6 or 7 days, commencing when tumour burden was 5% of body weight

Expt. 1: groups of 6 rats received vehicle (●), IGF-I at 200 (▼) or 500 (■) $\mu\text{g/day}$ or LR³-IGF-I at 200 (◆) or 500 (▲) $\mu\text{g/day}$. Expt. 2: groups of 6 rats received vehicle (●), insulin at 100 $\mu\text{g/day}$ (▼), LR³-IGF-I at 200 $\mu\text{g/day}$ (■) or both hormones combined (◆).

greatest with the combined infusion (Table 1; Figures 2a and 2b). LR³-IGF-I stimulated growth to a greater extent in insulin-treated rats than was the case for rats without insulin infusion, indicating a synergistic effect of the two hormones for this response.

Tumour growth was suppressed by insulin treatment and enhanced as previously in rats receiving LR³-IGF-I. Although

Figure 2 Growth rate (a), food intake (b) and N³-MH excretion (c) by groups of 6 rats implanted with a mammary adenocarcinoma and receiving vehicle, insulin and/or LR³-IGF-I

Treatments and symbols are given in the legend to Figure 1 (Expt. 2). Values are means $\pm$ S.E.M.

rats receiving the combined insulin and LR³-IGF-I infusion had significantly larger tumours than untreated rats, the relative tumour burden (g/kg body wt.) for these groups was not significantly different (Table 1).

Figure 3 Distribution of retained nitrogen in the tissues of groups of 6 rats implanted with a mammary adenocarcinoma and receiving vehicle, insulin and/or LR³-IGF-I

Treatments are given in the legend to Figure 1 (Expt. 2) legend. Bars show the amount of total nitrogen gain (□) apportioned to tumour (▨) and host (■) and, within the host, nitrogen gain apportioned to carcass (▩) and non-carcass (▧) tissues. Values (mg/day) are means ± S.E.M. Total nitrogen retention, final tumour nitrogen and final carcass nitrogen were directly measured. Initial carcass nitrogen was calculated as 15.2 g/kg initial host wt. (carcass = 500 g/kg host wt. and 190 g/kg of protein) and initial tumour nitrogen was calculated as 1.01 g/kg initial body wt. (tumour = 50 g/kg body wt. and 125 g/kg of protein). Distribution of nitrogen between defined tissue groups was calculated by difference. Because of the nature of the calculations, no statistical analysis has been performed, but S.E.M. values are shown as a guide to the variability of the calculated data.

Nitrogen balance was increased up to 2.5-fold by hormone infusion, with an apparent synergistic response in incremental nitrogen retention when both hormones were infused simultaneously. The carcass protein component of total nitrogen retention was less responsive, but increased by more than 10% with insulin treatment, LR³-IGF-I having no effect either alone or in combination with insulin (Table 1). Although the tumour-free weights of rats receiving the combined treatment were about 17 g higher than for insulin-treated rats when killed (Figure 2a), this was not reflected in carcass protein or fat content. Since treatment effects were relatively large in this experiment, we have been able to estimate reliably the patterns of nitrogen gain by the tumour and the host (both carcass and non-carcass components). Calculations were based on our measurements of nitrogen retention and the final nitrogen contents of carcass and tumour, as well as estimates of initial carcass and tumour nitrogen content (Figure 3). Insulin treatment stimulated nitrogen deposition to the carcass, whereas LR³-IGF-I treatment stimulated nitrogen accretion by the non-carcass component of the host. Whereas insulin treatment caused a shift of nitrogen deposition from tumour to carcass, with LR³-IGF-I the shift was from carcass to non-carcass tissues. The combined hormones are synergistic for nitrogen retention, largely because their individual anabolic effects prevail and nitrogen accretion is thus increased in both carcass and non-carcass tissues.

The anabolic effect of insulin was due both to an increase of 35–40% in muscle protein-synthesis rates and a consistent decrease of more than 10% in average muscle protein-breakdown

rates ($P < 0.02$) over the treatment period, as judged by N⁷-MH excretion (Table 2; Figure 2c). Insulin treatment increased both the ratio of RNA to protein in the muscle (1.15-fold; $P < 0.05$) and the rate of protein synthesis per unit of RNA (1.25-fold; $P < 0.01$) compared with control rats (results not shown). Despite large variations in the growth of the tumour, protein-synthesis rates in the tumour tissue at the completion of the study were unaffected by treatment. The fat content of the carcass was also substantially increased by almost 2-fold in insulin-treated rats, which also closely reflected the changes in food intake for each group.

Circulating insulin and metabolite changes elicited by LR³-IGF-I were consistent with those observed in Expt. 1, that is a decrease in blood glucose and insulin and an elevation of lactate and triacylglycerol levels compared with the control group. These effects were countered by co-infusion of insulin (Table 2). The high prevailing plasma insulin levels in insulin-infused rats resulted in a relative hypoglycaemia, which was not affected by the presence of LR³-IGF-I (Table 2). Lactate and non-esterified fatty acid levels were decreased by insulin treatment ($P < 0.01$, insulin versus non-insulin-treated), but triacylglycerol levels were unaffected. Apart from glucose levels, the metabolite concentrations during the combined insulin and LR³-IGF-I infusions were intermediate to those found when either peptide was infused alone.

DISCUSSION

Our results from both experiments show that infusion of IGFs alone to rats with mammary tumour burdens of 5–15% was not associated with improved muscle protein accretion; neither muscle protein synthesis nor carcass protein content was increased. Furthermore, the IGFs decreased food intake and increased tumour growth. These effects were unexpected, in view of (i) our experiments *in vitro*, which showed that the IGFs tested did not promote growth of the mammary adenocarcinoma under optimal cell-culture conditions, and (ii) our previous studies *in vivo*, in which IGF treatment was never seen to decrease food intakes [14–17]. The increasing energy and amino acid requirements for the growing tumour were reflected in the strong relationship between the tumour size and the rate of N⁷-MH excretion, an index of the rate of muscle protein breakdown. Thus the tumour burden was the primary determinant of the metabolic state of the host in rats treated with IGFs alone. These data do not support the published evidence for an anti-catabolic effect of IGFs in cancer cachexia, where it was shown that infusion of IGF-I preserved gastrocnemius muscle mass in the tumour-bearing rat [19]. In that study, rats inoculated with a methylcholanthrene-induced sarcoma showed loss of muscle protein relative to controls, and treatment with IGF-I ameliorated this catabolic effect. Furthermore, this was achieved without stimulating tumour growth, although the tumour burden (2–3%) was small compared with our wasting models [21] and those of others [11,20]. It seems likely that the methylcholanthrene-induced sarcoma was secreting catabolic factors such as tumour necrosis factor (TNF- α , cachectin) and that IGF-I countered their effects [18]. The mammary adenocarcinoma used here does not lead to detectable changes in plasma TNF levels (A. M. Rofo and P. Coyle, unpublished work).

One interpretation for the lack of an anabolic effect of the IGFs on muscle tissue in our study is that the IGFs may have directly inhibited insulin release [32] and thus exacerbated an insulin insufficiency associated with the tumour-bearing state [22]. Studies with animal tumour models, including those from our laboratory [20–22], point to altered pancreatic function in

the tumour-bearing host which results in a relative lack of insulin. The altered insulin release and, in some models, anorexia, lead to a decrease in the supply of intracellular substrates, which is thought to contribute to the metabolic changes seen in host tissues, including increased gluconeogenesis, muscle proteolysis and adipose-tissue lipolysis. Thus the tumour, which is non-insulin-sensitive [21,22], gains an increased substrate supply at the expense of the host and grows more rapidly. A second interpretation is that the IGFs may have directly stimulated tumour growth *in vivo*, an action which also would lower blood glucose and insulin. However, this contradicts our findings *in vitro* and also does not explain the observed anorexia. We have never observed anorexia in these tumour-bearing rats when tumours are less than 15% of body weight, and this is consistent with other rat/tumour models where food intake usually only declines with large tumour burdens (i.e. > 15–20% of body wt.) [20]. This action of the IGFs to decrease food intake could either be direct, by an unknown mechanism, or secondary to its pancreatic effects on insulin release. The effectiveness of insulin replacement in completely reversing the anorectic effect of LR³-IGF-I favours the latter explanation.

In considering this lack of insulin and the central anabolic role of this hormone, it has been suggested that insulin may have a therapeutic role in managing cancer cachexia [33,34]. Thus studies in rats indicate that insulin administration (subcutaneous injection) may be useful in reversing or controlling host wasting without increasing tumour growth [6,11,12]. Pre-operative insulin treatment of rats has been shown to decrease mortality following tumour resection [13], and the anorexia and subsequent loss in body weight associated with chemotherapy is also reversed by insulin treatment [35]. In other experimental systems, insulin has been shown to reverse some of the catabolic effects of TNF- α [36] and to have beneficial effects when used in combination with parenteral nutrition and anti-tumour therapy [37].

In our second experiment, insulin treatment led to an increase in food intake and in body weight of the host, while the tumour grew more slowly. These results suggest that administering insulin by constant infusion markedly alters host/tumour substrate competition in favour of the host. Co-infusion of LR³-IGF-I and insulin increased host weight gain to a greater extent than insulin alone, despite the fact that host weight decreased in the presence of LR³-IGF-I alone. Both the stimulation of food intake by insulin and the apparent differences in tissue specificities of the two hormones (Figure 3) may be important factors in this response. Carcass protein content and indices of muscle protein turnover confirmed an anabolic response in muscle tissue to insulin infusion with, or without, concurrent LR³-IGF-I. On the other hand, LR³-IGF-I appeared to enhance protein deposition only in non-carcass tissues of the rat. These separate effects of insulin and LR³-IGF-I were maintained in the combined treatment and contributed substantially to their synergism.

The response of tumour growth to the treatments did not parallel that of the host, which supports the view that its growth was controlled largely by available nutrient supply, in competition with the host, rather than directly by the infused hormones. In this rat/tumour model, we have previously found that tumour size and host weight are directly related to food intake (up to *ad libitum*) such that the tumour burden remains constant (C. S. Bourgeois and A. M. Rofe, unpublished work). Therefore the decrease in tumour burden seen in association with increased food intake in insulin-treated rats, and conversely, the increase in tumour burden seen in association with decreased food intake in IGF-treated rats, points to mechanisms not directly related to food intake. In the study reported here, we did not include a matched-fed control for the insulin-treated group.

Rats continuously infused with insulin cannot safely be denied free access to food, as this can lead to fatal hypoglycaemia. Although increased nutritional intake can be maintained or increased by enteral or parenteral feeding, these procedures were outside the scope of this study. However, others have shown that, in tumour-bearing rodents which have normal nutrient intakes maintained via total parenteral nutrition, insulin injection can have anabolic effects without tumour progression [37]. Thus, elevated food intake is not essential for the anabolic effects of insulin, although it appears to potentiate the response. Conversely, there is now a body of evidence indicating that cancer cachexia is associated with insulin resistance and/or a poor insulin-secretory response to glucose [1,22], which limits the anabolic response to increased nutrient intake in the absence of measures to increase insulin concentrations. In the clinical setting, parenteral feeding alone has generally been found to have little beneficial effect in reversing host wasting (see [37]).

IGFs can reverse muscle wasting characterized by decreased synthesis and increased degradation rates via a co-ordinate stimulation of synthesis and inhibition of degradation rates towards normal [16,18]. The failure of IGFs to stimulate protein synthesis in our experiment may be due to the lack of substrate supply, due to the presence of the tumour, rather than a direct block to the synthetic process. This hypothesis is supported by the significant increase in protein synthesis following insulin treatment and the attendant increase in food intake. Recent reports have shown that maintenance of nutrient intake by total parenteral nutrition substantially enhanced the ability of insulin, and the anabolic agent Clenbuterol, to decrease the wasting of gastrocnemius muscle in tumour-bearing rats [37,38]. IGF infusion in such a model may reveal some of the mechanisms in the interaction between IGF levels and substrate supply in tumour bearing rats.

Rates of protein breakdown at the end of the treatment period were strongly correlated with final tumour weight in both experiments. However, in Expt. 2, covariance analysis showed that, after allowing for tumour size, the N⁷-MH excretion in the combined-treatment group was significantly lower than that for the LR³-IGF-I treated group ($P < 0.05$). Of note, the insulin-treated groups showed an immediate decrease in N⁷-MH excretion relative to the tumour-free body weight, the rates for both groups being 20% lower than controls on days 2, 3 and 4 of treatment (Figure 2c). By contrast, the LR³-IGF-I-treated group showed rates of N⁷-MH excretion between 5 and 10% higher than controls during this same period. Possible contributions of N⁷-MH from non-skeletal tissues, particularly the gut [30], must be considered. Empty gut weight was not affected by insulin treatment alone (34.3 ± 0.9 and 35.3 ± 1.0 g/kg for control and insulin-treated groups), but was increased by LR³-IGF-I either alone (45.0 ± 1.0 g/kg) or in combination with insulin (47.1 ± 0.5 g/kg), in line with previous reports [39,40]. Whereas N⁷-MH excretion was increased during LR³-IGF-I treatment alone, this was not the case in rats receiving both insulin and LR³-IGF-I, which suggests that the gut mass was not an important element in the N⁷-MH response. Thus insulin, but not IGFs, may have an independent effect to suppress rates of muscle protein breakdown, which would further contribute to its anabolic effect on the host.

Although insulin infusion significantly stimulated protein metabolism and increased carcass protein content, it caused a much more marked increase in carcass fat deposition. IGF infusion has the reverse effect, and these data highlight the inability of IGFs to stimulate fat deposition, due largely to the lack of type 1 receptors in that tissue [41]. The relative changes in circulating metabolite levels (shown in Table 2) and par-

tioning of nutrients from the tumour to the host with either insulin or IGF infusion reflects this essential difference. Interpretation of these data is difficult because of the hypophagic effect of LR³-IGF-I and the hyperphagic effect of insulin. Further studies with controlled intakes and other IGF variants, which may not affect intake in this way, are warranted.

In conclusion, IGF-I, or the more potent variant LR³-IGF-I, does not enhance the ability of the host to compete with a non-malignant mammary tumour and decrease muscle wasting. However, insulin does enhance nutrient uptake and muscle growth by the host at the relative expense of the tumour, but especially so in combination with LR³-IGF-I. The synergistic enhancement of the host by insulin and LR³-IGF-I may arise from the combination of their specific anabolic effects on different components of lean tissue. The clinical use of such a combined therapy may need to incorporate enteral or parenteral nutrition to maximize anabolic effects, depending on the patients' ability to increase food intake voluntarily.

Note added in proof (received 31 May 1994)

In a recent paper [42], IGF-I, but not insulin, was shown to decrease the loss of host tissues in rats bearing an implanted dimethylhydrazine-induced tumour. Circulating cytokine levels were not reported.

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