Growth, body composition, hormonal and metabolic status in lambs treated long-term with growth hormone

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The effect of long-term (10 weeks) treatment with growth hormone (GH) was investigated in twin lambs, one sibling being a control and the other treated with GH (0·1 mg/kg live weight per d). The lambs were fed on a concentrate–grass cube (9:1 w/w) diet at a daily rate of 40 g fresh weight/kg live weight. The average daily live-weight gain of the GH-treated lambs was 36% greater than that of the controls (307 *v*. 225 g/d, $P < 0·01$). The carcass composition of the GH-treated lambs changed: fat content was decreased ($P < 0·01$) and protein content was increased ($P < 0·05$) when expressed relative to carcass dry matter. The absolute weights and the weights when expressed relative to fleece-free empty body of some muscles were significantly increased in GH-treated lambs. The mean retention times of both particulate- and liquid-phase components of the digesta were unchanged by GH treatment, when calculated for the rumen or for the entire gastrointestinal tract. The feed conversion ratio was significantly greater ($P < 0·01$) in GH-treated lambs compared with controls. Nitrogen retained per g N intake was also significantly increased ($P < 0·05$) by GH treatment. Plasma urea concentrations were decreased ($P < 0·05$) and glucose concentrations were increased ($P < 0·01$) in GH-treated lambs, whereas non-esterified fatty acid concentrations were unchanged. Plasma insulin and total insulin-like growth factor-1 concentrations progressively increased in GH-treated lambs as treatment time continued. They were significantly correlated after week 4 of treatment. Two types of hepatic GH-binding site were detected, with high and low affinities for GH. The capacities of both binding sites were significantly increased ($P < 0·05$) in GH-treated lambs when expressed per unit microsomal protein but, when expressed per liver, only the capacity of the high-affinity site was increased.

Growth hormone: Body composition: Hormonal and metabolic adaptation: Lamb

Growth hormone (GH) is essential for normal growth in immature animals and also has a central role in the control of nutrient partitioning between storage tissue (such as fat) and milk production or lean tissue growth (Bauman *et al.* 1982). In growing animals, therefore, GH may induce increased rates of nitrogen retention and decreased rates of fat accretion (Hart & Johnsson, 1986). Thus, exogenous GH may have potential in the improvement of nutritional efficiency for lean tissue deposition.

In ruminant animals, most studies to date have reported decreases in the carcass fat content of GH-treated animals (Johnsson *et al.* 1987), although increases in lean tissue growth rates have been more difficult to demonstrate. Short-term acute administration of GH (12–28 d) certainly increases N retention (Struempler & Burroughs, 1959; Wheatley *et al.* 1966; Eisemann *et al.* 1986*a*, 1989), but long-term treatment has not yielded consistent anabolic effects. Wagner & Veenhuizen (1978) and Johnsson *et al.* (1985) observed significant increases in carcass lean tissue content after several weeks of GH treatment, whereas Muir *et al.* (1983), Johnsson *et al.* (1987) and Sandles & Peel (1987) were unable

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to induce such changes. The reasons for these apparently paradoxical findings remain obscure, but it is important to note that growth is a complex process relying on many factors, particularly nutritional status, and that GH itself has diverse actions in vivo.

The aims of the present investigation were therefore: (1) to examine the long-term effects of exogenous GH on the growth and body composition of young growing lambs under controlled nutritional and environmental conditions, and (2) to relate these gross effects of GH to more detailed hormonal and metabolic adaptations so that the mechanism of GH action could be elucidated further.

Sections of the present work have been presented as preliminary communications (Pell *et al.* 1987; Pell & Bates, 1987).

MATERIALS AND METHODS

Animals

Ten sets of Scottish Half-bred × Suffolk twin lambs (twenty lambs in all, as eight females and twelve wethers but single-sex within twins) were weaned at 8 weeks of age and housed individually in metabolism crates from 9 weeks. The lambs were maintained on a 16 h light–8 h dark cycle and at a minimum temperature of 10°. The live weights of the lambs were determined weekly. They were offered a diet consisting of concentrate (Lamlac Start-to-Finish pellets; James Duke & Sons, Bishops Waltham, Hants) and dried grass cubes (IGAP farms) in a ratio of 9:1 by fresh weight (average dry matter (DM) content 888 g/kg, average crude protein (N × 6·25) 160 g/kg DM; average metabolizable energy (ME) 12·1 MJ/kg DM calculated from the individual ME for concentrate and grass cubes). The level of feeding was 40 g fresh weight/kg live weight per d based on retrospective weekly live weight, and the diet was given hourly using automatic feeders; any food refusals were weighed and removed daily. At 9 weeks of age, each twin was randomly allocated to either a control or a GH-treated group (0·1 mg/kg per d of recombinant bovine GH (bGH; American Cyanamid, Princeton, NJ, USA) as a subcutaneous injection in sterile carbonate-buffered (25 mmol/l, pH 9·4) saline (9 g sodium chloride/l)). All lambs were weighed and a jugular blood sample was taken weekly (before GH administration) until slaughter in pairs at approximately 21 weeks of age. Blood was collected in ice-cold heparinized tubes and centrifuged at 1500 ***g*** at 4° for 20 min; the resultant plasma was frozen at −20° until hormone and metabolite analyses were performed.

At the end of the study, two lambs (one control and one GH-treated, but from different pairs of twins) died, therefore slaughter values are presented for eight control and eight GH-treated animals.

N balance

Three successive 4 d N balances (twelve consecutive days in total) were performed on each set of twins at 16 weeks of age (Martin, 1966). Faeces were frozen daily; each 4 d collection was then thawed, pooled and mixed before subsampling for analysis. Urine was collected into sulphuric acid (initial concentration, 500 ml/l), so that the pH of the mixture was always below 2; it was stored in air-tight containers before subsampling and freezing. N contents were determined on freeze-dried faeces samples and defrosted urine samples after Kjeldahl digestion using a Kjeltec 1300 analyzer (Tecator Instruments Ltd, Bristol, Avon).

Digesta-passage-rate studies

Total mean retention times for solid- and liquid-phase components of digesta were estimated when the lambs were 18 weeks of age. The liquid-phase marker, CoEDTA, and

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the particulate-phase marker, ytterbium-labelled hay (17 mg Yb/g DM), were administered as a single dose (75 mg Co and 75 mg Yb per animal) via an oesophageal tube. Faeces, when present, were collected at hourly intervals from 12 to 72 h post dosing, and then at three-hourly intervals during the working day, plus an overnight sample, from 72 to 192 h. The marker content of faeces samples was determined by atomic absorption spectrometry and a multi-compartment first-order kinetic model was used to describe the pattern of marker excretion and to determine marker behaviour within the digestive tract. Details of marker preparation and the curve-fitting procedure are described in Dhanoa *et al.* (1985).

Hormones and metabolites

Plasma hormone concentrations were determined using radioimmunoassay: GH (Hart *et al.* 1975 as modified by Tindal *et al.* 1982); insulin (Tindal *et al.* 1978); total 3,5,3′-triiodothyronine (T_3) (Ratcliffe *et al.* 1974); insulin-like growth factor-1 (IGF-1; in acid–ethanol extracted samples; Daughaday *et al.* 1982). Concentrations of glucose (Bittner & McCleary, 1963) and urea (blood urea N; colour reagent, working solution; BDH Ltd, Poole, Dorset) were assayed using a continuous-flow autoanalyzer (Chemlab Instruments Ltd, Hornchurch, Essex). Plasma non-esterified fatty acid (NEFA) concentrations were determined using a kit method (WAKO; Alpha Laboratories, Eastleigh, Hants).

Slaughter and body composition

All lambs were stunned and slaughtered using a captive bolt followed by exsanguination. Weights of all major organs were recorded. Carcasses were divided in two bilaterally and the right side was frozen and subsequently used for dissection of nine specific muscles from the hind-quarters (Brown *et al.* 1978). All products of the dissection were retained and the half carcass was then minced before chemical analysis of a subsample (Florence & Mitchell, 1972). Carcass fat content was determined in diethyl ether extracts (Braude & Newport, 1973) and carcass protein content was calculated from N concentrations which were determined via a micro-Kjeldahl procedure using a Kjeltec apparatus (Tecator 1015 digesta and 1003 distillation unit; Tecator Instruments Ltd, Bristol, Avon).

GH binding to liver microsomes

At slaughter approximately 100 g liver were frozen quickly in liquid N_2 and stored at $-80°$. Hepatic microsomal membranes were prepared by differential centrifugation (Tsushima & Friesen, 1973). The final microsomal pellet was resuspended in 0·01 M-sodium dihydrogen phosphate buffer, pH 7·5, containing 1 mM-EDTA, 150 mM-NaCl, 25 mM-potassium chloride and 0·6 mM-merthiolate, and frozen in 1–3 ml portions at $-80°$. The microsomal preparation was not desaturated of endogenous hormones with magnesium chloride because this may induce a selective loss of protein from membranes and, therefore, an artefactual increase in hormone binding in vitro (Hayden & Smith, 1981).

The apparent dissociation constants (K_d) and the capacities of GH binding were determined for six control and six GH-treated lambs (as three sets of twin females and three sets of wethers) via Scatchard analysis (Scatchard, 1949) using self-displacement of labelled hormone, in optimum conditions for GH binding (calcium-free phosphate buffer, pH 7·5, containing bovine serum albumin (10 g/l), and incubated at 4° for 24 h). A constant amount of receptor protein (2·5 mg) was incubated with increasing amounts of ^{125}I-labelled bGH (recombinant, prepared by the Iodogen method (Salacinski *et al.* 1981), 3000 to 1200000 counts/min per tube), in the presence or absence of excess unlabelled bGH (recombinant, 1000 ng/tube). Total incubation volume was 0·5 ml and all solutions were

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made up in the 0·01 M-NaH_2PO_4 buffer described previously but also containing bovine serum albumin (10 g/l). All tubes were incubated at 4° overnight; protein-bound hormone was precipitated by a 30 min incubation at 4° with polyethylene glycol, final concentration 100 g/l. Tubes were centrifuged at 1500 ***g*** and 4° for 30 min; the supernatant fraction containing unbound hormone was removed and the radioactivity in the precipitate was measured in a gamma counter (Minaxi auto-gamma 5000; Packard, Pangbourne, Berks.). Bindability of the ^{125}I-labelled bGH to membranes (the maximum bindable fraction of the labelled hormone) and its specific radioactivity were also determined. All procedures described here were performed exactly as in Harding (1989).

The amounts (ng) of free (F) and membrane-bound (B) hormone in each of the Scatchard incubations were calculated from the specific radioactivity and the bindability of the ^{125}I-labelled bGH, and the amount of radioactivity due to ^{125}I-labelled membrane-bound hormone in each tube (counts/min). Graphs were constructed of B/F *v.* B; K_d (pmol/l) and the capacity (pmol/g microsomal protein) of the GH-binding sites were determined from the gradients and *x*-axis intercepts respectively, assuming that hormone would preferentially bind to receptors with the highest affinity.

Statistics

The significance of differences between control and GH-treated lambs were assessed in an analysis of variance, blocking for sets of twins and using initial live weight as a covariate, when appropriate. There was no significant interaction of treatment with the sex of the twins and, therefore, this is included with the error. Paired *t* tests were used to assess statistical differences in GH-binding values because all Scatchard analyses were performed in pairs using the same batch of ^{125}I-labelled bGH, so giving identical bindability and specific radioactivity.

RESULTS

Growth and body composition

Live-weight gain. Average initial live weights of the lambs were within 0·1 kg (control 22·2, GH-treated 22·1 kg; standard error of difference (SED) 1·1, *n* 10 per group) but, at slaughter, GH-treated lambs were significantly heavier than their twin controls by almost 7 kg (control 40·4, GH-treated 47·1 kg; SED 1·1, $P < 0.001$, *n* 10 per group). Over the entire study period growth was linear with respect to time and average daily live-weight gain was 0·36 greater in the GH-treated group (control, 225, GH-treated 307 g/d; SED 18, $P < 0.01$, *n* 10 per group).

Tissue weights. Table 1 summarizes fleece-free empty-body-weights (FFEBW) and tissue weights. The FFEBW was significantly greater in GH-treated lambs and GH treatment induced significant increases in the absolute weights of most 'protein-type' tissues (skin, liver, heart, lungs plus trachea, small plus large intestines). When tissue weights were normalized for FFEBW, GH treatment generally did not induce changes in tissue fractional weights However, omental fat was significantly decreased (from 1·66 to 1·21 %; SED 0·12, $P < 0.01$) and small plus large intestines were increased (from 2·52 to 2·99 %; SED 0·18, $P < 0.05$); liver and lungs plus trachea tended to increase.

Carcass composition Carcass weights, and fat and protein contents are presented in Table 2. Absolute carcass weight was significantly increased in GH-treated lambs, though not as a proportion of FFEBW. Carcass protein content, expressed as a percentage of carcass DM content, was increased by about 9 % and fat content was decreased by almost 10 % in GH-treated lambs. When total carcass weight was taken into account, the protein content was

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Table 1. *Fleece-free empty-body-weight (FFEBW) and tissue weights for control (C) and growth hormone (GH)-treated lambs**

	Wt (kg)			Statistical significance of difference: *P*
	C (*n* 8)	GH (*n* 8)	(SED)	
FFEBW	35·90	41·00	1·52	0·006
Head	2·750	3·11	0·21	> 0·1
Skin	3·10	3·94	0·27	0·017
Fleece	1·22	1·43	0·11	0·095
Liver	0·930	1·23	0·096	0·015
Spleen	0·230	0·282	0·060	> 0·1
Heart	0·225	0·284	0·019	0·018
Lungs + trachea	0·690	0·876	0·062	0·018
Stomachs, empty	1·43	1·55	0·073	> 0·1
Small + large intestines, empty	0·924	1·24	0·071	0·003
Mesenteric fat	0·196	0·135	0·054	> 0·1
Omental fat	0·595	0·506	0·041	0·063
Kidneys, cold	0·157	0·183	0·015	> 0·1
Perirenal fat, cold	0·364	0·346	0·073	> 0·1

SED, standard error of difference.
* For details, see p. 432.

Table 2. *Carcass weights and fat, protein and dry matter (DM) contents and carcass fat: carcass protein ratios in control (C) and growth hormone (GH)-treated lambs**

	C (*n* 8)	GH (*n* 8)	SED	Statistical significance of difference: *P*
Carcass				
wt (kg)	22·0	24·9	0·7	0·005
DM (g/kg)	455	433	10	0·064
Fat (g/kg DM)	548	495	15	0·009
Protein (g/kg DM)	363	395	12	0·031
Total carcass fat (kg wet wt)	5·56	5·42	0·35	> 0·1
Total carcass protein (kg wet wt)	3·69	4·24	0·14	0·006
Carcass fat: carcass protein	1·53	1·26	0·09	0·013

SED, standard error of difference.
* For details, see p. 432.

further increased in GH-treated lambs, whereas the decrease in fat content was counteracted by the heavier carcass weight. Administration of GH therefore induced a significant decrease in the carcass fat:protein ratio of about 18%.

Hind-quarters muscle weights. Seven of the nine muscles (listed in Table 3) which were dissected from the hind-quarters exhibited a significant increase in absolute weight in response to GH. Total dissected muscle weight was significantly increased by 22% in GH-treated lambs even though carcass weight was only increased by approximately 13%, thus supporting the increase in mean carcass protein content. When expressed as a percentage

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Table 3. *Weights of nine muscles dissected from the hind-quarters of control (C) and growth hormone (GH)-treated lambs**

	Wt (g)			Statistical significance of difference: P
	C (n 8)	GH (n 8)	SED	
Gluteobiceps	280·6	353·1	14·0	0·001
Semitendinosus	105·7	125·5	4·9	0·004
Vastus lateralis	136·4	164·3	4·2	< 0·001
Rectus femoris	120·1	143·1	6·3	0·008
Soleus	2·3	2·3	0·2	> 0·1
Gastrocnemius	105·6	123·5	4·9	0·008
Flexor digitorum superficialis	37·2	40·8	3·3	> 0·1
Vastus medius	47·4	61·6	1·4	< 0·001
Vastus intermedius	49·0	56·8	2·6	0·020
Total dissected	884	1072	27	< 0·001

SED, standard error of difference.
* For details, see p. 432.

of FFEBW total dissected muscle was still significantly increased (from 2·44 to 2·58 %; SED 0·05; $P < 0·05$), although only two individual muscles (gluteobiceps and vastus medius) were significantly increased.

Digesta passage rate

Total mean retention times were unchanged by GH treatment for both the liquid (control 34·6, GH-treated 34·7 h; SED 2·9, n 10 per group) and particulate phase (control 42·0, GH-treated 42·2 h; SED 3·0, n 10 per group) components of digesta. Additionally, rumen retention times were also similar for control and GH-treated lambs (liquid phase: control 17·9, GH-treated 18·5 h, SED 2·3; particulate phase: control 23·4, GH-treated 21·3 h; SED 2·1, n 10 per group), implying that the time-period spent in the absorptive region of the gastrointestinal tract was unchanged by GH. The weight of gut contents recovered at slaughter was not significantly increased in GH-treated lambs (control 6·74, GH-treated 7·11 kg wet weight; SED 0·85, n 10 per group); this was surprising since the quantity of feed consumed was proportional to live weight. However, considerably more variation was recorded for digesta weight than for other observations in the present study.

Efficiency of feed utilization and N balance

DM intake, efficiency of DM retention and the mean 4 d N balance are given in Table 4. The efficiency of DM retention was increased by 23 % in response to GH treatment; part of this will be due to the increased lean tissue content of GH-treated lambs which is more dense than fat. N retention was significantly greater in GH-treated lambs by 33 %, due to the increased feed intake of the GH-treated lambs and their reduced urinary N output, when expressed relative to N intake. Faecal N output was similar in control and GH-treated lambs when expressed relative to N intake.

Plasma hormone and metabolite concentrations

Mean plasma urea, glucose and NEFA concentrations for the pretreatment and during treatment periods are presented in Table 5. No significant differences between groups were observed before treatment commenced. However, mean urea concentrations were decreased

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Table 4. *Efficiency of food utilization and nitrogen balance for control (C) and growth hormone (GH)-treated lambs**

	C (*n* 10)	GH (*n* 10)	SED	Statistical significance of difference: *P*
Total feed intake (kg DM; weeks 9–19)	78·0	86·1	1·1	0·001
Efficiency of DM retention (kg weight gained/kg DM intake; weeks 9–19)	0·202	0·249	0·014	0·007
N intake (g/4 d)	150·3	167·9	9·1	0·088
N in faeces:				
g/4 d	38·6	43·4	4·1	> 0·1
/g N intake	0·255	0·258	0·015	> 0·1
N in urine:				
g/4 d	63·7	60·4	5·7	> 0·1
/g N intake	0·425	0·359	0·026	0·033
N retained:				
g/4 d	48·0	64·1	3·7	0·002
/g N intake	0·320	0·383	0·025	0·039

SED, standard error of difference; DM, dry matter.
* For details, see p. 432.

Table 5. *Plasma urea, glucose and non-esterified fatty acid (NEFA) concentrations before and during treatment in control (C) and growth hormone (GH)-treated lambs**

(Pretreatment concentrations (mmol/l) were not significantly different between groups being, for control and GH-treated lambs respectively: urea 5·70, 5·71; SED 0·23; glucose 4·01, 3·94; SED 0·10; NEFA 0·186, 0·178; SED 0·030)

	C (*n* 10)	GH (*n* 10)	SED	Statistical significance of difference: *P*
Urea (mmol/l)	8·29†	6·95†	0·50	0·029
Glucose (mmol/l)	3·33†	3·51†	0·05	0·006
NEFA (mmol/l)	0·072‡	0·057‡	0·014	> 0·1

SED, standard error of difference.
* For details, see p. 432.
† Means of all weekly samples.
‡ Values for week 11 of treatment only.

significantly by 19% in GH-treated lambs and mean glucose concentrations were slightly but significantly increased. Plasma NEFA concentrations are only presented for the last week of treatment but were also measured for the first and sixth weeks; they are similar for control and GH-treated lambs.

Mean plasma GH concentrations were, not surprisingly, significantly increased in GH-treated lambs (control 5·3, GH-treated 6·6 ng/ml; SED 0·5, $P < 0.05$, *n* 10 per group). Detailed GH profiles on similarly treated lambs over a 24 h period have been well-documented by Johnsson *et al.* (1985, 1987). Mean plasma total T_3 concentrations were unchanged by GH (pretreatment values: control 1·77, GH-treated 1·91 nmol/l; SED 0·41; week 11 of treatment: control 1·46, GH-treated 1·52 nmol/l; SED 0·25, *n* 10 per group).

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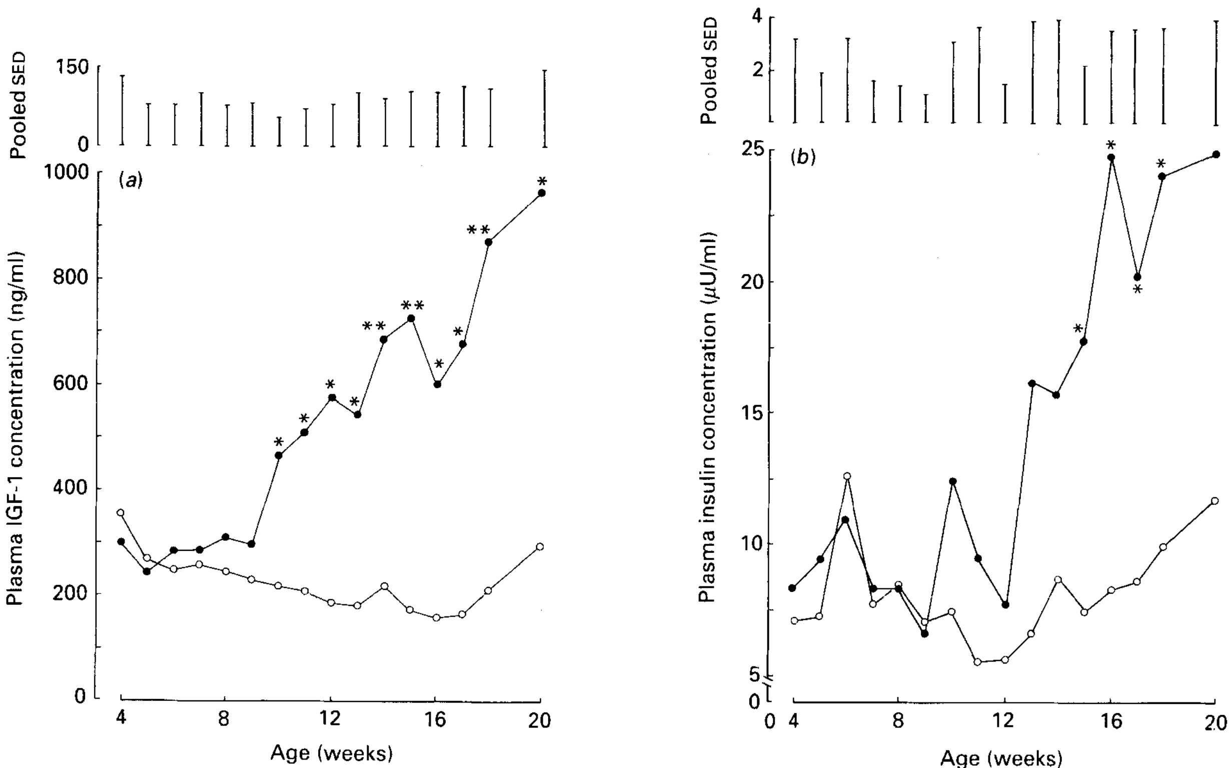

Fig. 1. Plasma (*a*) total insulin-like growth factor-1 (IGF-1) and (*b*) insulin concentrations for control (○) and growth hormone (GH)-treated (●) lambs from 4 to 20 weeks of age; treatment began at week 9. There were ten lambs per group. Mean values were significantly different from control values: $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$. The pooled weekly standard error of difference (SED) is represented by vertical bars.

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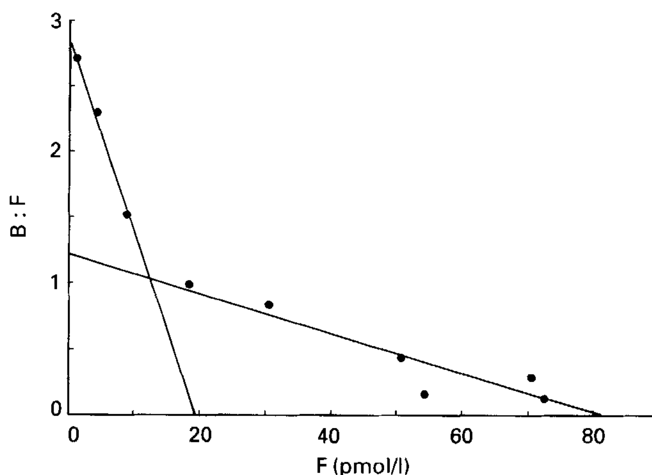

Fig. 2. The binding of ^{125}I-labelled bovine growth hormone to hepatic microsomal membranes from a control wether, expressed as bound (B): free (F) hormone concentrations in the incubation volume *v*. the free hormone concentration. Each point represents the mean of triplicate determinations.

Plasma IGF-1 and insulin concentrations are illustrated in Fig. 1. During the pretreatment period (weeks 4–9) no significant differences were observed between the two groups of lambs. Immediately after treatment began, total plasma IGF-1 concentrations increased in GH-treated lambs and continued to increase so that, during the final week of treatment, they were threefold greater than those for control lambs. Surprisingly, plasma insulin concentrations were more variable, but significant increases in insulin concentrations were observed for GH-treated lambs from 15 weeks of age (6 weeks of treatment). It is noteworthy that total plasma IGF-1 and insulin concentrations appeared to be related, and when correlation analysis was performed on all individual lambs by week, a significant correlation ($P < 0.05$ to $P < 0.01$) was found from week 4 of treatment.

Hepatic binding sites for bGH

Two classes of hepatic GH-binding sites can be identified in microsomal preparations from well-fed adult ruminants animals: high- and low-affinity sites (Gluckman *et al.* 1983; Breier *et al.* 1988). Scatchard analysis confirmed the presence of high- and low-affinity binding sites in the hepatic preparations of the present study; an example is shown in Fig. 2. The apparent K_d and capacities of the low- and high-affinity sites are given in Table 6. The apparent K_d of both sites was unchanged by GH treatment; however, receptor capacity, expressed per g microsomal protein, was significantly increased for both the low- and high-affinity sites in response to GH. Simple displacement studies additionally showed that total specific binding of ^{125}I-labelled bGH to microsomal membranes also tended to increase in GH-treated lambs (controls 23·0 (SE 5·0) %, GH-treated 29·9 (SE 5·5) %, *n* 6 per group). When yield of microsomal membrane protein and liver weight were taken into account and receptor capacity was expressed per liver, the capacity for GH binding was only significantly increased for the high-affinity site in GH-treated lambs, being 213% that for control lambs.

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Table 6. *The apparent dissociation constants* (K_d) *and capacities for high- and low-affinity hepatic growth hormone (GH)-binding sites for control and GH-treated lambs**

(Mean values with their standard errors)

	C (*n* 6)	GH (*n* 6)	Statistical significance of difference: *P*
Low-affinity binding site			
K_d (pmol/l):			
Mean	59·8	76·0	> 0·1
SE	10·5	15·3	
Capacity (pmol/g microsomal protein):			
Mean	9·42	13·9	< 0·05
SE	3·35	4·3	
Total capacity (nmol/liver):			
Mean	205·6	361·4	> 0·1
SE	65·1	111·8	
High-affinity binding site			
K_d (pmol/l):			
Mean	6·78	10·3	> 0·1
SE	2·42	4·5	
Capacity (pmol/g microsomal protein):			
Mean	3·03	6·01	< 0·05
SE	0·82	1·46	
Total capacity (nmol/liver):			
Mean	74·3	158·4	< 0·05
SE	19·5	41·5	

* For details, see p. 432.

DISCUSSION

The objective of the present investigation was to characterize metabolite and growth responses of lambs to long-term treatment with exogenous GH in controlled environmental conditions of day-length and during nutritional steady-state. Diet was, therefore, supplied continuously via automatic feeders and the quantity of feed offered (40 g per kg live weight) was estimated to be as close to *ad lib.* intake as possible whilst ensuring that the diet was always consumed. This was at least 86 and 89% of the *ad lib.* intakes for control and GH-treated lambs respectively reported by Johnsson *et al.* (1985) and avoided the palatability problems encountered by Johnsson *et al.* (1987). To date, the feed intake response of *ad lib.*-fed, GH-treated animals has been variable when compared with that of their controls, ranging from an increase (Sandles & Peel, 1987), only a marginal increase (Johnsson *et al.* 1985) to a decrease (Campbell *et al.* 1988). Season and day length may influence the rate of growth in lambs (Brinklow & Forbes, 1984), therefore lighting was maintained on a 16 h light–8 h dark cycle throughout the present study.

In the conditions of the present study, the potential anabolic actions of GH were demonstrated clearly, although published findings on the long-term anabolic effects of GH in ruminant animals are very variable. The current findings are similar to those of Johnsson *et al.* (1985) in terms of overall growth and lean tissue responses to GH. However, Johnsson *et al.* (1987) were unable to reproduce these actions of GH on N metabolism, possibly due to overall decreased feed intake, although they did achieve dose-related decreases in carcass fat content. When prepubertal heifers were treated for 21 weeks with GH (Sandles & Peel, 1987), average daily gain was increased significantly by 8·5% but, surprisingly, carcass

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composition was unchanged. In the earliest studies on long-term GH administration to lambs (Wagner & Veenhuizen, 1978), increases in daily gain and lean tissue content of 20 and 15% were observed in response to 15 mg ovine pituitary GH/d. Muir *et al.* (1983) were unable to repeat these responses using 7 mg ovine pituitary GH/d. Both these dose rates are high when compared with those of the current study (average dose 3·5 mg), even when the likely purities of the pituitary *v.* recombinant material are taken into account.

No unifying factor can account for the differences in GH responsiveness described previously and, therefore, it is relevant to try and account for the observations of the current study since GH probably has several sites of action. First, it could improve the efficiency of nutrient absorption. Second, all dietary substrates are transported to the liver via the portal vein and, therefore, GH could influence hepatic metabolism, especially since the liver is quantitatively the major site of GH binding (Wallis, 1980) and, thus, the supply of nutrients released for extrahepatic tissues could be increased or the composition could be changed. Third, the substrate requirement of target tissues, such as muscle, is likely to be different in GH-treated animals. Finally, GH must alter endocrine balance so that metabolism is co-ordinated towards lean tissue accretion and away from adipose tissue.

The nature of the increase in feed conversion efficiency can be examined in more detail. Fadlalla *et al.* (1985) reported that passive immunization against somatostatin caused a 38% decrease in apparent digesta flow, implying that the improvement in feed utilization which may occur in animals actively immunized against somatostatin (Spencer *et al.* 1983) could be due to increased efficiency of nutrient absorption. As somatostatin inhibits the release of GH from the pituitary gland, GH may be important in mediating this response. No evidence was found in the present study of any influence of exogenous GH on total or rumen retention times for digesta. When expressed relative to N intake, N excretion in the faeces was unchanged whereas N excretion in the urine of GH-treated lambs was significantly reduced, indicating that digestibility was not altered by GH but that adaptations in tissue N metabolism were post-absorptive. These conclusions are similar to those of Eisemann *et al.* (1989) for steers.

The liver has a central role in metabolic regulation since it can modulate the release of absorbed nutrients to peripheral tissues. Two of the influences controlling hepatic urea production are a decreased substrate supply (amino acids) and an inhibition of the urea cycle. If amino acid metabolism were directed towards muscle anabolism, hepatic amino acid supply from peripheral tissues could be reduced. However, Eisemann *et al.* (1989) reported little change in plasma amino acid concentrations in GH-treated steers and no evidence has been found for decreased rates of muscle protein degradation (Pell & Bates, 1987). Therefore, the liver could have an active role in the regulation of amino acid degradation by decreased urea cycle activity. Plasma glucose concentrations were increased in GH-treated lambs. This could be due to increased rates of gluconeogenesis, which would again implicate control via hepatic metabolism, decreased rates of peripheral glucose utilization, or simply to the higher feed intakes of the GH-treated lambs. Insulin sensitivity is altered in response to long-term elevated GH concentrations; for example, insulin-induced decreases in plasma glucose concentration are reduced in GH-treated lambs during an insulin-tolerance test (Hart *et al.* 1984). This decrease in insulin sensitivity is supported by the increased plasma insulin concentrations which were found in the current study and in those of others (Johnsson *et al.* 1985; Eisemann *et al.* 1986*b*, 1989). However, preliminary findings suggest an increased gluconeogenic potential by hepatocytes isolated from GH-treated lambs (C. Elcock & J. M. Pell, unpublished results) and, therefore, glucose production may be increased as well in vivo; this would be dependent on an adequate supply of gluconeogenic precursors. Glycerol, released from triacylglycerol breakdown could be a gluconeogenic substrate, although it has not yet been established

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unequivocally whether the decreased fat content often observed in GH-treated animals is a result of increased rates of lipolysis or decreased rates of lipogenesis. It is likely that this depends on the nutritional status of the animal since Peters (1986) observed an increase in plasma NEFA concentrations in GH-treated steers which were subjected to a restricted plane of nutrition, but no change in NEFA concentrations in those on an *ad lib.* intake. In the current study, no differences in NEFA concentrations were observed between control and GH-treated lambs. It must be noted though, that measurements of plasma metabolite concentrations can give no indication of the turnover of that metabolite.

Many of the anabolic actions of GH are mediated via (Salmon & Daughaday, 1957), or in conjunction with (Lindahl *et al.* 1987), IGF-1. In the present investigation, total plasma IGF-1 concentrations increased in response to GH treatment and continued to increase as the study period progressed. This implies that the lambs did not become 'GH-resistant' but maintained their sensitivity to GH. IGF-1 is present in at least three forms in blood (D'Ercole & Wilkins, 1984; Daughaday *et al.* 1987): free, and bound to large- and small-molecular-weight binding proteins (molecular weights approximately 150 kDa and 25–40 kDa respectively). The significance of these different circulating forms of IGF-1 is not yet established but it is thought that the binding proteins prolong the half-life of IGF-1 and also modulate its action (Zapf *et al.* 1986). The radioimmunoassay employed in the present study measures both bound and free IGF-1 and, therefore, their relative proportions cannot be discussed. The source of circulating IGF-1 is also of interest. Many tissues have the capacity to synthesize IGF-1 but the liver has the greatest concentration of mRNA for IGF-1 (Murphy *et al.* 1987). Therefore, IGF-1 could be secreted by the liver to its target tissues in blood and act as an endocrine hormone, or it could have an autocrine/paracrine function by acting at its tissue of synthesis, in which case plasma IGF-1 would represent 'waste' IGF-1. It is probable that both modes of action are important for optimum IGF-1 activity.

Since the liver is quantitatively the major site of GH binding, hepatic receptor affinity and capacity for GH was determined. Both high- and low-affinity receptors for GH were detected in the lambs of the present study, confirming earlier work in ruminant species (Gluckman *et al.* 1983; Breier *et al.* 1988). Breier *et al.* (1988) suggest that the high-affinity site may be a primary factor in the regulation of somatic growth in the ruminant because weight gain is correlated with the capacity of the high-affinity binding site in steers. In our studies, the capacities of both high- and low-affinity binding sites were increased in GH-treated lambs when expressed per unit microsomal protein, although when expressed per liver this increase in capacity was only statistically significant for the high-affinity site. Chung & Etherton (1986) have measured the specific binding of ^{125}I-labelled porcine GH (pGH) to liver microsomal membranes in pigs treated with different doses of pGH, and have found that exogenous pGH induces increased pGH binding which is correlated with serum IGF-1 concentrations and weight gain, indicating that hepatic GH binding has an important role in the regulation of growth rate. The apparent dissociation constants of the high- and low-affinity binding sites were similar to those of Breier *et al.* (1988) for well-fed steers and they imply that, if mean plasma GH concentrations were 5 ng/ml, the high-affinity receptor would probably be fully saturated with GH. This must focus attention on the role of receptor capacity and turnover rate as important factors in the transfer of GH 'message' to the cell.

The increases in and correlations between plasma IGF-1 and insulin concentrations imply that a complex hormonal co-operation may be required to achieve an anabolic response to exogenous GH. This concept of an 'anabolic drive' has been proposed by Millward (1989), and some evidence can be found for a positive co-ordination of GH and insulin activities; for example Wallace & Bassett (1966) suggested that optimum GH

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activity, in terms of short-term N balance, was obtained when insulin concentrations were increased. Thus, in the study reported here, nutritional, environmental and hormonal conditions were manipulated in the GH-treated lambs so that their metabolism was directed towards increased rates of growth. In addition, the body composition of the GH-treated animals was changed so that they were not only larger than their controls, but leaner.

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