Responses of Lactating Ewes to Exogenous Growth Hormone: Short- and Long-term Effects on Productivity and Tissue Utilization of Key Metabolites

L. D. Sandles,A,B Y. X. Sun,A A. G. C. D'Cruz,A G. H. McDowell,A,C and J. M. GoodenA

A Dairy Research Unit, Department of Animal Husbandry, University of Sydney, Camden, N.S.W. 2570.
B While at CSIRO Division of Human Nutrition, Kintore Avenue, Adelaide, S.A., 5000.
Present address: Department of Animal Science, Cornell University, Ithaca, New York 14853, U.S.A.
C Address all correspondence to Dr G. H. McDowell.

Abstract

Responses to daily injections of bovine growth hormone (GH, 0·15 mg kg⁻¹ liveweight), beginning on day 10 of lactation, were measured in lactating ewes. Milk yields of GH-treated ewes increased soon after commencement of injections and continued to increase for some 25 days before reaching plateau levels. By comparison, yields of ewes injected with excipient (controls) decreased over the experiment. There was a tendency for contents of milk fat to be higher and milk protein to be lower for GH-treated than for control ewes during the first 15–20 days after injections were started.

At the beginning and over the first 15–20 days of the experiment feed intakes of both groups of ewes were similar, but thereafter intakes of GH-treated ewes gradually increased to reach plateau levels some 200–300 g day⁻¹ higher than for control ewes by about day 35. Liveweights of both groups of ewes decreased during the first 2 weeks of treatment then increased, with GH-treated ewes losing, then gaining, more weight than control ewes. The efficiency of food utilization for milk production was higher for GH-treated than control ewes throughout the experiment but digestibility of food organic matter was not different during the eighth week of the experiment. At the end of the experiment, body composition, assessed by dilution of tritiated water, was similar for both groups of ewes. Differences in milk production were not sustained after withdrawal of GH injections.

Measurements of tissue uptake of key metabolites were made on days 3 and 45 of GH treatment. On day 3, GH lowered uptake of glucose and non-esterified fatty acids by leg muscle tissue and increased mammary uptake of non-esterified fatty acids. By day 45 there were no apparent differences of tissue uptake of key metabolites.

The results indicate that there is a biphasic response to exogenous GH in the lactating ruminant. It appears that initially GH affects nutrient partition thereby increasing supplies to the mammary gland of key nutrients for milk synthesis. In the longer term, GH increases feed intake, which provides sufficient nutrients to sustain increased milk production and also liveweight gain.

Extra keywords: Milk yield and composition, feed intake and digestibility, liveweight and body composition, arterial metabolites and hormones, arterio-venous differences, leg muscle and mammary tissue, non-esterified fatty acid kinetics, tissue blood flow.

Introduction

There are now several reports that exogenous bovine growth hormone (GH) increases milk production in dairy cattle (see McCutcheon and Bauman 1985; McDowell 1985; Johnsson and Hart 1986), sheep (Dracy and Jordan 1954; Jordan and Shaffhausen 1954; Hart et al. 1985, McDowell et al. 1988) and goats (Hart et al. 1980; Mepham et al. 1984) when administered over short periods of several days. In such short-term studies it has been shown 0004-9417/88/030357$03.00
that nutrient utilization in the body as a whole (Peel et al. 1982; McDowell et al. 1987b) and in muscle and mammary tissues (McDowell et al. 1987a, 1988) is altered by GH so as to increase the supply of nutrients for milk synthesis as was suggested originally by Bauman and Currie (1980) and Bines and Hart (1982).

To date, there have been few reports of effects of long-term administration of GH in the lactating ruminant. In one study, Bauman et al. (1985) noted a sustained increase in milk production of high-producing dairy cows treated for several months with GH. Although the increased productivity apparently was due, at least in part, to effects of GH in promoting food intake, the cows were offered rations that differed according to milk yield, thereby confounding effects of GH on feed intake. In a more recent study with pasture-fed cows, Peel et al. (1985) measured a sustained increase in milk production, which was associated with increased feed intake starting several weeks after commencement of GH injections. No measurements have been made of whole-body or tissue utilization of nutrients in long-term studies.

The objects of the present studies were to measure long-term effects of exogenous GH on productivity, feed intake and digestibility and on whole-body and tissue utilization of key nutrients in lactating sheep.

Materials and Methods

Sheep

Eight primiparous cross-bred (Border Leicester × Merino) ewes were separated permanently from their lambs within 24 h of parturition. Ewes were kept in metabolism cages in a semi-enclosed shed lit artificially with fluorescent lighting. All were free of obvious abnormalities of the mammary glands and could be milked by machine (at c. 0800 and 1600 hours daily) with minimum restraint. None of the ewes had been trained for milking so at each milking 0·2 i.u. oxytocin (Syntocinon, Sandoz Pty Ltd, Basel, Switzerland) was administered via a jugular vein catheter (see below) to ensure complete milk removal. Milk yields were recorded daily and representative sub-samples of total daily milk were kept at 4°C after addition of formalin as preservative (10%, w/v; 3 drops/10 ml milk) pending weekly analyses for major milk constituents. Ewes were fed individually and to appetite a mixture (50:50, w:w air dry) of good quality lucerne chaff : rolled barley grain. The daily feed allocation was offered in approximately equal portions at milking times (except during measurement of tissue metabolism) and the daily feed intake was recorded. Starting 1 week before measurement of tissue metabolism, feed was provided using continuous belt feeders.

On day 10 post-partum ewes were allocated to treatment groups (viz. control and GH-treated) by restricted randomization such that ewes were matched for liveweight and milk production and at the start of the experiment mean values were similar for both groups (see Figs 1 and 4).

Surgical Preparation

Three ewes from each group were surgically prepared to allow measurement of key metabolites and hormones in arterial blood and arterio-venous differences for metabolites across muscle tissue of the hind limb (A-LV) and mammary tissue (A-MV). Indwelling catheters had been fitted in both jugular veins of each ewe on the day of parturition.

The procedures used for insertion of catheters [polyvinyl chloride; Dural Plastics, Sydney] and catheter sizes were as described previously (Oddy et al. 1984; Teleni and Annison 1986; McDowell et al. 1988). Arterial catheters were inserted 8–10 days before, and venous catheters 3–4 days before, measurements of tissue metabolism at peak and during mid-to-late lactation (days 3 and 45 of the experiment, respectively). Leg and mammary vein catheters were removed after collection of samples on day 3 and new catheters were inserted in mammary and contra-lateral leg veins before sampling was performed on day 45. Jugular vein and arterial catheters were maintained throughout the experiment.

Catheters were kept patent by being periodically flushed with minimum volumes of heparinized saline (2 × 10^3 i.u. heparin and 9·0 g NaCl per litre). In the event that catheter patency was not maintained, flexible wire guides (Cook Incorporated, Bloomington, Indiana, U.S.A.) were used to clear blockages and/or to replace venous catheters at least 2 h before collection of samples. At sampling times, 3–5 ml of blood were withdrawn and discarded prior to collection of samples for analyses.
Experimental Procedures

Commencing day 10 post-partum (viz. day 0) ewes were given daily subcutaneous injections (c. 5 ml) of either excipient (pH 9·5 bicarbonate buffer) or 0·15 mg kg⁻¹ liveweight of GH dissolved in excipient.

Measurements were made of arterial concentrations, A-LV and A-MV for metabolites, arterial concentrations of hormones, whole-body irreversible loss of non-esterified fatty acids (NEFA) and tissue blood flow on days 3 and 45 after injections were begun. Blood flow was measured over 50 min (see below) and blood collected for this purpose provided integrated samples for measurement of hormones and metabolites. Samples for measurement of irreversible loss of NEFA were collected immediately before and after collection of samples for measurement of blood flow.

Milk composition was monitored daily during weeks 1 and 7 (viz. days 1–7 and days 42–48) of the experiment and in other weeks on a single day mid-week. Arterial blood samples collected on this day were used for measurement of 'weekly values' for plasma hormones. During week 8 after injections began, digestibility of organic matter was assessed between days 48 and 52, then on day 53 body water content was measured as a means of estimating body composition. Immediately after measurement of body water content, daily injections of GH or excipient ceased.

Growth Hormone

The preparation of GH was extracted from bovine pituitary glands, assayed for impurities and assessed for its biological activity (1·6 U mg⁻¹) as outlined previously by Sandles and Peel (1987).

Analytical Procedures

Milk composition

The content of milk fat was measured by the 'Babcock' procedure, and milk protein was determined as total nitrogen, measured by the 'Kjeldahl' procedure, times the factor 6·38 (see Davis 1959). Lactose was measured as described by Teles et al. (1978).

Metabolites and hormones

Plasma glucose and NEFA, blood acetate, lactate and 3-hydroxybutyrate and plasma insulin and growth hormone were measured using the procedures outlined by McDowell et al. (1987a, 1988).

Whole-body irreversible loss of NEFA was measured by assuming that palmitate was a representative plasma fatty acid.

Radio-labelled palmitate (1-14C-palmitate, Amersham International plc, Amersham, U.K.) was infused, via a jugular catheter, at the rate of c. 3·7 × 10⁴ Bq min⁻¹ for 240 min. Samples collected during the last 60 min of infusion were used to measure irreversible loss of NEFA as previously described by McDowell et al. (1987b, 1988).

Tissue blood flow

Blood flow was measured by assessing the dilution of tritiated water (Amersham International plc) infused via a jugular vein catheter. The procedure originally described by Oddy et al. (1981) and modified by McDowell et al. (1988) was used.

Body composition

The procedures described by Searle (1970) were used to measure body water content and so, indirectly, body composition. Briefly, ewes were denied access to feed and water for 24 h, liveweights were measured and body water content assessed by measuring dilution of a known dose (c. 7·4 × 10⁶ Bq) of tritiated water (Amersham International plc). A period of 6 h was allowed between infusion of tritiated water via one jugular catheter and collection of blood via the other jugular catheter.

Statistical Analysis

The significance of differences between mean values for parameters measured were assessed using the student t-test and correlation coefficients between measured parameters determined as outlined by Steel and Torrie (1960).
Results

Milk Yield and Composition

At the start of the experiment milk yields of control and GH-treated ewes were similar and it appeared that yields had peaked by this time. It is clear from Fig. 1 that milk yields of control ewes decreased gradually from the start of injections, whereas yields of GH-treated ewes increased gradually over the first 25 days of the experiment. Thereafter, yields of GH-treated ewes remained at plateau levels, whereas yields of control ewes continued to decrease. The data summarized in Table 1 show that yields of GH-treated ewes tended to be higher at day 3 and were significantly higher \(P < 0.10\) by day 45 than yields of control ewes.

![Graph](image)

**Fig. 1.** Mean \(n = 4\) milk yields of control (○——○) and GH-treated (●——●) ewes throughout the experiment. (a) Actual daily yields. Times of insertion of arterial catheters (C), measurements of tissue metabolism (M) and cessation of injections (CI) are indicated. (b) Average daily yields throughout each week of the experiment. (c) Average daily yields of milk, corrected to a fat content at 40 g kg\(^{-1}\) (FCM), throughout each week of the experiment.

Insertion of catheters, particularly prior to the second sampling period, was followed by abrupt and marked reduction in milk yields in both control and GH-treated ewes. By the time samples were collected on day 45 of the experiment, yields had recovered in part and had stabilized at values that were lower than before catheterization. However, differences
between the yields of the two groups were still marked. Soon after cessation of injections, at the end of the eighth week of the experiment, yields of GH-treated ewes decreased, to be similar to those of control ewes.

Table 1. Milk and milk fat yields, arterial plasma growth hormone (GH) and insulin (INS), blood flow to leg muscle (L) and mammary (M) tissues, arterial concentrations (A) and arterio–venous concentration differences across leg muscle (A–LV) and mammary (A–MV) tissue of key metabolites and whole body irreversible loss (IL) of NEFA in control and GH-treated ewes on days 3 or 45 of the experiment

Values are means ± s.e.m. for four ewes except for blood flows and NEFA–IL, which are means for three ewes. For individual parameters at each time, values that differ significantly are indicated:

*P < 0·1; **P < 0·05; ***P < 0·01.

|                      | Day 3       | Day 45      |
|----------------------|-------------|-------------|
|                      | Control     | GH-treated  | Control     | GH-treated  |
| Milk yield (g d⁻¹)   | 1202±194·9  | 1421±69·2   | 858±37·8    | 1266±188·2* |
| Milk fat (g d⁻¹)     | 65·7±9·89   | 83·4±1·12   | 47·2±4·11   | 67·6±7·52** |
| Plasma GH (µg l⁻¹)   | 1·3±0·75    | 9·0±0·55*** | 0·6±0·17    | 7·0±0·82*** |
| Plasma INS (mU l⁻¹)  | 24·2±2·81   | 47·8±9·79*  | 31·4±9·50   | 92·8±26·45* |
| Tissue blood flow    | L           | M           |             |             |
| (ml kg⁻¹ min⁻¹)      | 94±10·3     | 373±58·8    | 123±14·4    | 371±69·0    | 368±19·5 |
|                      | A-L         | A-LV        | A-M       |             |             |
| Plasma glucose       | 3·41±0·440  | 3·97±0·881  | 3·56±0·177 | 3·54±0·109  |             |
|                      | 0·19±0·025  | 0·06±0·025***| 0·09±0·052 | 0·20±0·57  |             |
|                      | 0·72±0·091  | 0·84±0·116  | 0·78±0·108 | 0·57±0·082  |             |
|                      | 257±28·6    | 343±37·4    | 156±5·64   | 178±23·1    |             |
| Plasma NEFA (µM)     | 42±14·4     | -5±13·7***  | 30±21·4    | 25±12·3     |             |
|                      | 71±8·7      | 135±25·2**  | 55±13·9    | 64±18·5     |             |
| Blood acetate (mm)   | 1·22±0·115  | 1·41±0·208  | 1·18±0·082 | 1·16±0·110  |             |
|                      | 0·38±0·020  | 0·69±0·065  | 0·36±0·109 | 0·34±0·137  |             |
| Blood lactate (mm)   | 0·81        | 0·80±0·267  | 0·50±0·279 | 0·67±0·096  |             |
|                      | 0·85±0·039  | 0·88±0·108  | 0·64±0·025 | 0·60±0·043  |             |
| Blood 3-hydroxybutyrate (mm) | 0·01±0·028  | -0·01±0·045 | 0·03±0·025 | 0·00±0·007 |             |
|                      | 0·04±0·035  | 0·10±0·026  | 0·07±0·018 | 0·04±0·015  |             |
| Blood 3-hydroxybutyrate (mm) | 0·46±0·063  | 0·53±0·088  | 0·56±0·064 | 0·43±0·110  |             |
|                      | 0·11±0·033  | 0·07±0·022  | 0·09±0·025 | 0·07±0·009  |             |
|                      | 0·21±0·43   | 0·25±0·025  | 0·22±0·018 | 0·18±0·049  |             |
| NEFA–IL (µmol min⁻¹) | 657±72·3    | 805±119·9   | 605±65·1   | 1338±317·8**|             |

During the first 3 weeks after injections began, the content of milk fat was higher for GH-treated than for control ewes (mean values 60·8 v. 53·0 g kg⁻¹, P < 0·05), but thereafter contents of milk fat were similar (c. 54 g kg⁻¹) for both groups of ewes. As shown in Table 1, yields of milk fat tended to be higher at day 3 and were significantly higher at day 45 for GH-treated than for control ewes. The marked increase in fat content of milk during the first weeks of the experiment led to the large increase in yield of fat-corrected milk (see Fig. 1).

There was a tendency for milk protein content to be lower for GH-treated than for control ewes during the first 3 weeks of injections (mean values 48·6 v. 50·9 g kg⁻¹, P > 0·05). Thereafter, contents of milk protein were similar for both groups. The content of milk lactose increased gradually over the experiment and there were no apparent differences between GH-treated and control ewes.

**Feed Intake**

Mean daily feed intakes are depicted in Fig. 2. Over the first 15–20 days of the experiment feed intakes of control and GH-treated ewes were similar. The intakes of GH-treated ewes...
then gradually increased over the next 15 days to reach levels 200–300 g day$^{-1}$ higher than for control ewes. At the time of insertion of catheters for the second sampling, feed intake of both groups decreased dramatically then increased by similar amounts.

Feed intake, expressed in terms of liveweight, was not significantly affected ($P > 0.05$) by GH injections. Even so, there was a tendency for feed intake per unit liveweight to be lower for GH-treated than for control ewes during the first 2 weeks or so of injections (see Fig. 3). The efficiency of utilization of food for milk production was increased by GH (see Fig. 3) and this increased efficiency was maintained throughout the period of GH injections.

![Graph](image)

Fig. 2. Mean ($n = 4$) daily feed intakes of control (●—●) and GH-treated (●—●) ewes throughout the experiment. (a) Actual daily intakes. Times of insertion of arterial catheters (C), measurements of tissue metabolism (M) and cessation of injections (Cl) are indicated. (b) Average daily feed intakes throughout each week of the experiment.

Apparent digestibility of feed organic matter during the eighth week of the experiment was similar for GH-treated and control ewes—709 ± 29.5 v. 707 ± 9.4 g kg$^{-1}$ (mean ± s.e.) for GH-treated and control ewes respectively.
Liveweight and Body Composition

Liveweight decreased in both groups of ewes during the first 2 weeks of injections. Thereafter, there were steady increases in liveweight in both groups, with GH-treated ewes gaining weight at a faster rate than control ewes. The effects of the reduced feed intake, following insertion of catheters for the second sampling period, were apparent (Fig. 4).

![Liveweight and Body Composition](image)

Fig. 3. Mean (n = 4) values throughout each week of the experiment for feed intake per unit liveweight (FI/LW) and efficiency of utilization of food for milk production (FCE) in control (○---○) and GH-treated (●---●) ewes.

Data for body composition, measured 8 weeks after starting injections, are summarized in Table 2. There was a tendency for GH-treated ewes to have higher 'empty liveweights' than control ewes but mean values were not significantly different (P > 0·10). Similarly, body composition of the two groups of ewes was not significantly different (P > 0·10).

|                          | Control         | GH-treated     |
|--------------------------|-----------------|----------------|
| Empty liveweight (kg)    | 44·8 ± 3·62     | 47·2 ± 1·40    |
| Body water space (l)     | 30·6 ± 2·43     | 31·0 ± 0·90    |
| Fat-free body tissue (kg)| 37·4 ± 3·00     | 37·8 ± 1·10    |
|                          | (g kg⁻¹)        |                |
| Body protein (kg)        | 835 ± 60·8      | 803 ± 8·5      |
|                          | (g kg⁻¹)        |                |
| Body fat tissue (kg)     | 6·5 ± 0·47      | 6·9 ± 0·20     |
|                          | (g kg⁻¹)        |                |
|                          | 175 ± 51·7      | 213 ± 6·3      |

Table 2. Empty liveweight, body water space and derived values for body composition of control and GH-treated ewes 8 weeks after injections began

Values shown are means ± s.e. for four ewes.

Plasma Hormones

Concentrations of GH and insulin on days 3 and 45 of the experiment are shown in Table 1. Concentrations of GH were significantly higher in GH-treated than in control ewes at both times; concentrations of insulin tended to be higher in GH-treated than in control
Fig. 4. Mean \((n = 4)\) liveweights of control \((\circ \cdots \circ)\) and GH-treated \((\bullet \cdots \bullet)\) ewes measured once each week throughout the experiment.

Table 3. Correlation coefficients for relationships between parameters measured on day 3 of the experiment

The parameters were milk yield \((MY, \, g \, d^{-1})\), milk fat \((MF, \, g \, kg^{-1})\), arterial plasma concentrations of growth hormone \((GH, \, \mu g \, l^{-1})\), glucose \((GLUC, \, mm)\) and non-esterified fatty acids \((NEFA, \, \mu M)\), arterial blood concentrations of 3-hydroxybutyrate \((3OH, \, mm)\), lactate \((LACT, \, mm)\) and acetate \((ACET, \, mm)\), whole body irreversible loss of NEFA \((ILFA, \, \mu mol \, min^{-1})\), food intake \((FI, \, g \, d^{-1})\) and mammary blood flow \((MBF, \, ml \, kg^{-1} \, tissue \, min^{-1})\). Significant coefficients are indicated thus: \(*P < 0.10; \**P < 0.05; \***P < 0.01\)

|     | MY  | MF  | GH  | GLUC | NEFA | ILFA | 3OH | ACET | LACT | MBF |
|-----|-----|-----|-----|------|------|------|-----|------|------|-----|
| GH  | 0.69* | 0.69* | | | | | | | | |
| GLUC | 0.95*** | 0.63 | 0.60 | | | | | | | |
| NEFA | 0.63 | 0.72* | 0.64 | 0.65 | | | | | | |
| ILFA | 0.43 | 0.52 | 0.27 | 0.49 | 0.91*** | | | | | |
| 3OH | 0.47 | 0.63 | 0.05 | 0.46 | 0.69* | 0.82** | | | | |
| ACET | 0.02 | 0.77** | 0.53 | 0.14 | 0.09 | 0.36 | 0.24 | | | |
| LACT | 0.01 | 0.07 | 0.22 | 0.09 | 0.35 | 0.45 | 0.56 | 0.34 | | |
| MBF  | 0.11 | 0.22 | 0.30 | 0.27 | 0.68 | 0.60 | 0.53 | 0.18 | 0.51 | |
| FI   | 0.38 | 0.52 | 0.02 | 0.20 | 0.31 | 0.49 | 0.45 | 0.43 | 0.46 | 0.42 |

Table 4. Correlation coefficients for relationships between parameters measured on day 45 of the experiment

Details of parameters measured as for Table 3. Significant coefficients are indicated thus: \(*P < 0.10; \**P < 0.05; \***P < 0.01\)

|     | MY  | MF  | GH  | GLUC | NEFA | ILFA | 3OH | ACET | LACT | MBF |
|-----|-----|-----|-----|------|------|------|-----|------|------|-----|
| GH  | 0.85** | 0.25 | | | | | | | | |
| GLUC | 0.01 | 0.19 | 0.08 | | | | | | | |
| NEFA | 0.76** | 0.52 | 0.59 | 0.04 | | | | | | |
| ILFA | 0.99*** | 0.51 | 0.82** | 0.13 | 0.75* | | | | | |
| 3OH | 0.86** | 0.39 | 0.55 | 0.05 | 0.63 | 0.86** | | | | |
| ACET | 0.40 | 0.86** | 0.01 | 0.38 | 0.36 | 0.43 | 0.33 | | | |
| LACT | 0.14 | 0.28 | 0.18 | 0.18 | 0.65 | 0.14 | 0.36 | 0.23 | | |
| MBF  | 0.15 | 0.08 | 0.01 | 0.06 | 0.49 | 0.24 | 0.08 | 0.39 | 0.40 | |
| FI   | 0.64 | 0.56 | 0.26 | 0.45 | 0.79** | 0.68 | 0.64 | 0.70* | 0.60 | 0.74* |
ewes \((P < 0.1)\). There was a suggestion that plasma GH decreased and insulin increased in control ewes between day 3 and day 45.

**Plasma and Blood Metabolites**

Arterial concentrations and arterio-venous differences for metabolites on days 3 and 45 are shown in Table 1.

On day 3 of injections arterial concentrations of plasma glucose and NEFA tended to be higher for GH-treated than for control ewes, but differences were not significant \((P > 0.10)\). At this time there were significant decreases in values for A-LV for glucose \((P < 0.01)\) and NEFA \((P < 0.05)\), a significant increase \((P < 0.05)\) in A-MV for NEFA and a tendency for whole-body irreversible loss of NEFA to be higher in GH-treated than in control ewes. No differences were observed for arterial concentrations or arterio-venous differences for blood acetate, lactate and 3-hydroxybutyrate.

By day 45 of the experiment arterial concentrations and arterio-venous differences for all metabolites were similar in GH-treated and in control ewes. Whole-body irreversible loss of NEFA was significantly higher \((P < 0.05)\) for GH-treated than for control ewes at this time.

**Tissue Blood Flow**

Values for blood flow to muscle and mammary tissues are summarized in Table 1. Although differences between mean values were not significant \((P > 0.10)\) there was a consistent tendency for tissue blood flow to be higher for GH-treated than for control ewes on day 3 of the experiment. By day 45 of the study, mean values for blood flow to both tissues, expressed in terms of tissue mass, were similar with no suggestion of differences associated with GH treatment.

**Relationships Between Parameters Measured**

Correlation coefficients between parameters measured on days 3 and 45 of injections are shown in Tables 3 and 4 respectively.

On day 3 of the experiment significant correlations were measured between milk yield and both plasma GH and plasma glucose. Milk fat content was significantly correlated with plasma GH, plasma NEFA and blood acetate. Plasma NEFA concentration was significantly correlated with irreversible loss of NEFA and blood 3-hydroxybutyrate. Finally, blood concentration of 3-hydroxybutyrate was significantly correlated with irreversible loss of NEFA.

At day 45 of the experiment significant correlations were measured between milk yield and plasma GH, plasma NEFA, irreversible loss of NEFA and blood 3-hydroxybutyrate. Milk fat content and blood acetate were correlated significantly as were plasma GH and irreversible loss of NEFA, plasma NEFA and irreversible loss of NEFA, and blood 3-hydroxybutyrate and irreversible loss of NEFA. Food intake was significantly correlated with plasma NEFA, blood acetate and mammary blood flow.

**Discussion**

It is clear that exogenous GH was galactopoietic, with milk yield increasing soon after injections began. In this respect the present results are consistent with results of previous short-term studies with sheep (Dracy and Jordan 1954; Jordan and Shaffhausen 1954; Hart et al. 1985; McDowell et al. 1988), goats (Hart et al. 1983; Mepham et al. 1984) and dairy cattle (see McCutcheon and Bauman 1985; McDowell 1985; Johnsson and Hart 1986). Similarly, the present results with sheep are in conformity with results of previous long-term studies with dairy cows (Bauman et al. 1985; Peel et al. 1985; Chalupa et al. 1987). Thus GH-treated ewes had significantly higher milk yields than did control ewes throughout the experiment and differences increased with time after commencing injections.
During the first week of the experiment, at which time milk yields were at or near peak (as shown by yields of control ewes), the galactopoietic response to exogenous GH was relatively small. Similar responses have been observed in previous studies with lactating sheep (G. H. McDowell and I. C. Hart, unpublished data) and dairy cows (McDowell et al. 1987b). The relatively small response to exogenous GH at or near peak lactation may have been due to the energy status of the ewes. At peak lactation the ewes would have been in negative energy balance (see Cowan et al. 1979; Vernon et al. 1981) with the mammary gland having a high priority for available nutrients. It is clear that liveweight of ewes in both groups decreased during the first 2 weeks of the experiment (see Fig. 4).

Changes in plasma/blood metabolites and plasma hormones measured for the control ewes between day 3 and day 45 of the experiment follow the pattern previously reported by Vernon et al. (1981). Thus for control ewes, plasma GH was higher, plasma insulin lower, plasma glucose lower and plasma NEFA higher on day 3 than on day 45.

Although the increase in actual milk yield during the first week of injection of GH was small, there was a marked increase in yield of fat-corrected milk. This was due principally to an increase in fat content of milk in response to exogenous GH. The increase in milk fat, together with the trend for decreased milk protein, has been considered to be characteristic of animals treated with GH while in negative energy and protein balance (Peel et al. 1983; McCutcheon and Bauman 1985).

Initially, exogenous GH increased milk yield without affecting feed intake (see Fig. 2). After some 15–20 days, feed intake of GH-treated ewes gradually increased and there was a concomitant gradual increase in milk yield and liveweight of GH-treated ewes. By 15–20 days of GH injections, milk yield and feed intake had stabilized and at this time the higher feed intake of the GH-treated ewes would have provided more than sufficient additional nutrients to account for differences in milk production.

During the first week of the experiment (viz. day 3), GH affected the partition/utilization of nutrients in leg muscle and mammary tissues. The consistent tendency for tissue blood flow to increase would have increased supplies of nutrients to the tissues even in the absence of changes in arterial concentration of nutrients. Arterial concentrations of both glucose and NEFA tended to increase in response to GH, A–LV for glucose and NEFA decreased and A–MV for NEFA increased. These changes are in conformity with the previous reports that exogenous GH exerts lipolytic and diabetogenic effects (see Hart 1983) and alters nutrient partition such that supplies of key nutrients to the mammary gland increase (McDowell et al. 1987a, 1988). Interestingly, plasma insulin was higher in GH-treated than in control ewes at both stages of the experiment (see Table 1). This observation, together with the higher plasma glucose for GH-treated ewes on day 3 of the experiment, confirms the diabetogenic effect of GH.

The observation that exogenous GH did not act as a potent lipolytic agent and merely tended to increase irreversible loss of NEFA at peak lactation (viz. day 3 of the experiment) is in agreement with previous results. In this connection, McDowell et al. (1987b) found that exogenous GH did not exert lipolytic effects in cows at the peak of lactation. In the present study, plasma concentration of NEFA in control ewes was higher on day 3 than on day 45 of the experiment, suggesting an elevated rate of lipolysis in the absence of exogenous GH. This observation is consistent with data presented by Vernon et al. (1981) and would correlate with mobilization of body fat depots in early lactation as reported by Cowan et al. (1979).

The higher irreversible loss of NEFA for GH-treated than for control ewes on day 45 of GH injections possibly reflected differences in nutrient intake between GH-treated and control ewes at this time. Indeed, food intake and irreversible loss of NEFA were significantly correlated at this stage of the experiment (see Table 4). It is apparent that feed intakes of both groups of ewes were very variable around the time measurements were made. However, on day 45 when irreversible losses of NEFA were measured feed intakes of ewes in both groups were similar (see Fig. 2), raising the possibility that the higher irreversible loss of NEFA for GH-treated ewes was due to lipolytic effects of GH.
Although oxidation of fatty acids was not measured there were indications that exogenous GH increased the rate of oxidation of fatty acids as reported previously by Kronfeld (1965) and McDowell et al. (1988). Blood concentrations of 3-hydroxybutyrate were not affected appreciably by GH at either stage of the experiment but were significantly correlated with irreversible loss of NEFA at both stages of the experiment and with plasma NEFA during the first week of the experiment (see Tables 3 and 4). Plasma concentrations of NEFA and irreversible loss of NEFA tended to be higher at day 3 for GH-treated than for control ewes and at day 45 irreversible loss of NEFA was increased significantly in GH-treated ewes.

By day 45 of the experiment there were no differences between GH-treated and control ewes for arterial concentrations or arterio–venous differences of metabolites across leg muscle and mammary tissues. Similarly, tissue blood flow, expressed in terms of unit tissue mass, was not different for GH-treated and control ewes at this time. This is perhaps surprising in view of reports that exogenous GH increases heart size (Johnsson et al. 1985, A. S. Zainur and R. C. Kellaway, personal communication) and cardiac output (Mepham et al. 1984). It is considered likely that cardiac output was increased in the present study as tissue weights would have been greater for GH-treated than for control ewes. Indeed, liveweights of GH-treated ewes were considerably greater than those of control ewes during the latter part of the study (see Fig. 4).

It is of interest that milk fat content was correlated significantly with plasma NEFA on day 3 but not day 45 of GH treatment. Recently, McDowell et al. (1988) found that treatment of lactating ewes with GH decreased arterial concentrations and mammary arterio–venous differences of very low density lipoproteins (VLDL), resulting in NEFA being relatively more important as precursors of milk fat, at least in the early stages of GH administration. The present results suggest that by day 45 of GH treatment, plasma NEFA were not important precursors of milk fat. At both stages of the experiment milk fat content was correlated significantly with blood acetate (see Tables 3 and 4), which is in conformity with acetate being important as a key substrate for de novo synthesis of the shorter-chain fatty acids of milk triglyceride in the ruminant (see Annison 1983).

A feature of results of numerous short-term (see Johnsson and Hart 1986) and long-term studies (Bauman et al. 1985; Peel et al. 1985; Chalupa et al. 1987) has been improved efficiency of food utilization for milk synthesis, which occurs during GH treatment. In the present study, improved efficiency of food utilization was maintained throughout the experiment (see Fig. 4). It is presumed that the maintenance of improved efficiency of utilization of food for milk synthesis is due to dilution of maintenance energy requirements in view of previous evidence that the partial efficiency of milk synthesis is not affected by exogenous GH (Peel et al. 1981; Tyrrell et al. 1982). Certainly, there was no evidence that digestibility of organic matter was affected by GH treatment in the present study, a finding reported previously by Tyrrell et al. (1982).

The patterns of change in liveweight throughout the experiment were similar for both groups of ewes, with changes being greater for GH-treated than for control ewes (see Fig. 4). Thus during the first 2 weeks of the experiment, GH-treated ewes lost more live-weight than did control ewes. Thereafter when liveweight increased, presumably reflecting changes from negative to positive energy balance, the increases were greater for GH-treated than for control ewes. It seems likely that, initially, the increased liveweight of GH-treated ewes was due to increased gut fill and/or an increased body water content. Subsequently, the increases in liveweight of GH-treated ewes were likely to be due to increased tissue gain.

Increased liveweight gains have been reported in non-lactating sheep (Wagner and Veenhuizen 1978; Johnsson et al. 1985) and cattle (Eisemann et al. 1986; Sandles and Peel 1987) injected with GH. In each of these studies increased liveweight gain was associated with decreased carcass fat.

In the present study, there was no difference between body composition, assessed by dilution of tritiated water, of GH-treated and control ewes at the end of the experiment. Failure to detect any differences in body composition between the two groups of ewes may have been due to imprecision of the technique used. Alternatively, the higher feed intakes
of the GH-treated ewes allowed accretion of body fat to the same extent as in control ewes. Indeed, feed intakes were such that the GH-treated ewes were well in positive energy balance, despite increased milk yields.

It is of interest that milk yield of the GH-treated ewes decreased rapidly after withdrawal of GH injections (see Fig. 1). This rapid decrease in milk yield may have been confounded by the fact that food was withheld for 30 h to allow measurements of body composition and further confounded by the decrease in feed intake of ewes following insertion of catheters for blood sampling at the latter stage of the experiment. None-the-less, the results raise the possibility that maintenance of high plasma GH is necessary to sustain the production increases. In this connection, Peel et al. (1985) and Sandles and Peel (1987) reported that increased milk production from dairy cows and increased growth of dairy heifers were not sustained after withdrawal of GH treatment.

In conclusion, the results of the present studies indicate that exogenous GH increases milk production initially by invoking changes that result in alteration of nutrient partition/utilization to supply additional nutrients for milk synthesis without requiring increased feed intake. Ultimately, the homeostatic mechanisms of the body over-ride short-term homeorhetic effects of GH leading to increased feed intake and as a result milk production. The maintenance of increased feed intake and milk yield apparently requires maintenance of high plasma GH.

Acknowledgments

Skilled technical support for these studies was conscientiously provided by Mrs S. L. Catt and Mrs K. A. van der Jagt. Assistance with maintenance of the animals was provided by Mr N. K. J. Catt and Mr K. M. McKeans. Financial support for the work was provided by the Australian Dairy Research Council, Rural Credits Development Fund of the Reserve Bank of Australia and the Dairy Husbandry Research Foundation.

References

Annison, E. F. (1983). Metabolite utilization by the ruminant mammary gland. In 'Biochemistry of Lactation'. (Ed. T. B. Mepham.) pp. 399-466. (Elsevier: Amsterdam and New York.)

Bauman, D. E., and Currie, W. B. (1980). Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63, 1514-29.

Bauman, D. E., Eppard, P. J., de Geeter, M. J., and Lanza, G. M. (1985). Responses of high-producing dairy cows to long-term treatment with pituitary somatotropin and recombinant somatotropin. J. Dairy Sci. 68, 1352-62.

Bines, J. A., and Hart, I. C. (1982). Metabolic limits to milk production, especially roles of growth hormone and insulin. J. Dairy Sci. 65, 1357-89.

Chalupa, W., Marsh, W. E., and Galligan, D. T. (1987). Bovine somatotropin: lactational responses and impacts on feeding programs. Proc. Maryland Nutr. Conf. Fd Manuf. pp. 48-57.

Cowan, R. T., Robinson, J. J., Greenhalgh, J. F. D., and McHattie, I. (1979). Body composition changes in lactating ewes estimated by serial slaughter and deuterium dilution. Anim. Prod. 29, 81-90.

Davis, J. G. (1959). 'Milk Testing. The Laboratory Control of Milk.' 2nd Edn. (Dairy Industries Limited: London.)

Dracy, A. E., and Jordan, R. M. (1954). The effect of bovine and porcine somatotropin upon lactating ewes. J. Anim. Sci. 13, 1018-19.

Eisemann, J. H., Tyrrell, H. F., Hammond, A. C., Reynolds, P. J., Bauman, D. E., Haaland, G. L., McMurty, J. P., and Varga, G. A. (1986). Effects of bovine growth hormone administration on metabolism of growing Hereford heifers: dietary digestibility, energy and nitrogen balance. J. Nutr. 116, 157-63.

Hart, I. C. (1983). Endocrine control of nutrient partition in lactating ruminants. Proc. Nutr. Soc. 42, 181-94.

Hart, I. C., Chadwick, P. M. E., James, S., and Simmonds, A. D. (1985). Effect of intravenous bovine growth hormone or human pancreatic growth hormone-releasing factor on milk production and plasma hormones and metabolites in sheep. J. Endocrinol. 105, 189-96.
Hart, I. C., Lawrence, S. E., and Mepham, T. B. (1980). Effects of exogenous growth hormone on mammary blood flow and milk yield in lactating goats. J. Physiol. (Lond.) 308, 46-7P.

Johnsson, I. D., and Hart, I. C. (1986). Manipulation of milk yield with growth hormone. In 'Recent Advances in Animal Nutrition, 1986'. (Eds W. Haresign and D. J. A. Cole.) pp. 105–23. (Butterworths: London.)

Johnsson, I. D., Hart, I. C., and Butler-Hogg, B. W. (1985). The effects of exogenous growth hormone and bromocriptine on growth, body development, fleece weight and plasma concentrations of growth hormone, insulin and prolactin in female lambs. Anim. Prod. 41, 207–17.

Jordan, R. M., and Shaffhausen, D. D. (1954). Effect of somatotropin on milk yield of ewes. J. Anim. Sci. 13, 706–10.

Kronfeld, D. S. (1965). Growth hormone-induced ketosis in the cow. J. Dairy Sci. 48, 342–6.

McCUTCHEON, S. N., and Bauman, D. E. (1985). The application of hormones produced by recombinant DNA technology to increasing animal productivity. In 'Biotechnology and Recombinant DNA Technology in the Animal Production Industries. Reviews in Rural Science' 6. (Eds R. A. Leng, J. S. F. Barker, D. B. Adams and K. J. Hutchinson.) pp. 131–40. (University of New England: Armidale.)

Mc Dowell, G. H. (1985). Exogenous growth hormones in animal production. In 'Recent Advances in Animal Nutrition in Australia in 1985'. (Ed. R. B. Cumming.) Paper 35. (University of New England: Armidale.)

Meph am, T. B., Lawrence, S. E., Peters, A. R., and Hart, I. C. (1984). Effects of exogenous growth hormone on mammary function in lactating goats. Horm. Metab. Res. 16, 248–59.

Oddy, V. H., Brown, B. W., and Jones, A. W. (1981). Measurement of organ blood flow using tritiated water. I. Hind limb muscle blood flow in conscious ewes. Aust. J. Biol. Sci. 34, 419–25.

Oddy, V. H., Gooden, J. M., and Annison, E. F. (1984). Partitioning of nutrients in merino ewes. I. Contribution of skeletal muscle, the pregnant uterus and the lactating mammary gland to total energy expenditure. Aust. J. Biol. Sci. 37, 375–88.

Peel, C. J., Bauman, D. E., Gorewit, R. C., and Sniffen, C. J. (1981). Effect of exogenous growth hormone on lactational performance in high yielding dairy cows. J. Nutr. 111, 1662–71.

Peel, C. J., Fromk, T. J., Bauman, D. E., and Gorewit, R. C. (1983). Effect on exogenous growth hormone in early and late lactation on lactational performance of dairy cows. J. Dairy Sci. 66, 776–82.

Peel, C. J., Sandles, L. D., Quelch, K. J., and Herington, A. C. (1985). The effects of long-term administration of bovine growth hormone on the lactational performance of identical-twin dairy cows. Anim. Prod. 41, 135–42.

Peel, C. J., Steinhour, W. D., Bauman, D. E., Tyrrell, H. F., Brown, A. C. G., Reynolds, P. J., and Haaland, G. L. (1982). Administration of bovine growth hormone to high yielding Holstein cows II. Influence on irreversible loss and oxidation rate of free fatty acids and glucose. J. Dairy Sci. 65 (Suppl. 1), 120.

Sandles, L. D., and Peel, C. J. (1987) Growth and carcass composition of pre-pubertal dairy heifers treated with bovine growth hormone. Anim. Prod. 44, 21–7.

Searle, T. W. (1970). Body composition in lambs and young sheep and its prediction in vivo from tritiated water space and body weight. J. Agric. Sci. (Camb.) 74, 357–62.

Steel, R. G. D., and Torrie, J. H. (1960). 'Principles and Procedures of Statistics with Special Reference to the Biological Sciences.' (McGraw-Hill Book Company, Inc.: New York, Toronto, London.)

Teleni, E., and Annison, E. F. (1986). Development of a sheep hind-limb muscle preparation for metabolic studies. Aust. J. Biol. Sci. 39, 271–81.

Teles, F. F., Young, C. K., and Stull, J. W. (1978). A method for rapid determination of lactose. J. Dairy Sci. 61, 506-8.
Tyrrell, H. F., Brown, A. C. G., Reynolds, P. J., Haaland, G. L., Peel, C. J., Bauman, D. E., and Steinhour, W. D. (1982). Effect of growth hormone on utilization of energy by lactating Holstein cows. In 'Energy Metabolism in Farm Animals'. EAAP Publication No. 29. (Eds A. Ekern and F. Sundstøl.) pp. 46–9. (Agricultural University of Norway: Aas.)

Vernon, R. G., Clegg, R. A., and Flint, D. J. (1981). Metabolism of sheep adipose tissue during pregnancy and lactation. Adaptation and regulation. Biochem. J. 200, 307–14.

Wagner, J. F., and Veenhuizen, E. L. (1978). Growth performance, carcass composition and plasma hormone levels in wether lambs when treated with growth hormone and thyroprotein. J. Anim. Sci. 47 (Suppl. 1), 397.

Manuscript received 21 September 1987, revised 19 February 1988, accepted 29 March 1988