Aust. J. Biol. Sci., 1987, **40**, 295–306

Effects of Exogenous Growth Hormone on Milk Production and Nutrient Uptake by Muscle and Mammary Tissues of Dairy Cows in Mid-lactation

G. H. McDowell,[A] *J. M. Gooden,*[A] *D. Leenanuruksa,*[A] *M. Jois*[A,B] *and A. W. English*[C]

[A] Dairy Research Unit, Department of Animal Husbandry, University of Sydney, Camden, N.S.W. 2570.

[B] Present address: Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland, Canada, A1B 3X9.

[C] Department of Veterinary Clinical Studies, University of Sydney, Camden, N.S.W. 2570.

Abstract

Responses to exogenous growth hormone were measured in lactating dairy cows surgically prepared to allow measurement of nutrient exchanges across mammary and hind-limb muscle tissues. Cows were injected daily with either saline or growth hormone, at a dose of 0·1 mg/kg liveweight, over periods of 6 days.

During administration of growth hormone milk yield, milk fat content and yields of milk fat protein and lactose increased. Arterial plasma concentrations of glucose and non-esterified fatty acids were increased, uptake of glucose by leg muscle tissue decreased, lactate release from leg muscle tended to increase, mammary uptake of non-esterified fatty acids increased, blood flow to leg muscle tended to increase and blood flow to mammary tissue increased during injection of growth hormone.

The results show that growth hormone affects supply to and utilization of key nutrients by tissues, resulting in the supply to the mammary gland of additional precursors for milk synthesis.

Introduction

Exogenous growth hormone (GH) increases milk production in dairy cows with maximum responses occurring after attainment of peak yields (see reviews by McCutcheon and Bauman 1985; McDowell 1985; Johnsson and Hart 1986). In most short-term studies, where GH has been administered over several days to a few weeks, increased milk production has occurred in the absence of a change in feed intake. Further, in studies of this type, exogenous GH has not altered digestibilities of dry matter, energy or nitrogen (Peel *et al.* 1981; Tyrrell *et al.* 1982). Thus it appears that GH alters the partition of nutrients such that nutrient supply to the mammary gland increases, perhaps at the expense of maintenance and/or growth of other tissues (see reviews by Bauman and Currie 1980; Bines and Hart 1982; see also McDowell *et al.* 1987).

There are now several data indicating that exogenous GH affects nutrient partition and/or utilization. Peel *et al.* (1982) reported decreased glucose oxidation and increased oxidation of non-esterified fatty acids (NEFA) in the whole body of GH-treated cows. Similarly, McDowell *et al.* (1983, 1987) found that exogenous GH altered whole-body irreversible losses of key metabolites (glucose, NEFA and urea) in lactating cows. There are, however, no direct demonstrations of effects of exogenous GH on nutrient utilization in individual tissues of lactating cows.

We report here results of studies on the effects of exogenous GH in lactating cows which had been surgically prepared to allow measurement of nutrient uptake across the tissues of the hind limb (predominantly muscle) and the mammary gland. Preliminary data have been reported elsewhere (McDowell *et al.* 1984).

0004-9417/87/030295$02.00

Materials and Methods

Cows and Rations

Three multiparous Friesian cows (third or fourth lactation), which had been lactating for 10–12 weeks when the experiment commenced, were housed indoors in tie-stalls. The cows had been maintained in the stalls for 4 weeks before the experiment and 'trained' such that experimental procedures could be performed without obvious disturbance. Milking occurred at *c.* 0700 and *c.* 1600 hours each day using a portable machine. All cows remained free of mastitis and in good health throughout the experiment.

The cows were fed a good quality mixed ration (10·4 MJ metabolizable energy per kilogram dry matter; assessed by measurement of *in vitro* digestibility—Tilley and Terry 1963) containing 60 : 40 (w : w, air dry), concentrate : roughage, in sufficient quantities to meet requirements for metabolizable energy for maintenance plus milk production (Anon. 1975) at the commencement of the experiment. Daily feed allowances for each cow remained constant. All cows consumed all offered food throughout the 3 weeks of the experiment.

The concentrate portion of the ration (50 : 50, w : w air dry, rolled barley : commercial concentrate pellets) was fed continuously with a belt feeder whereas the daily allocation of roughage (50 : 50, w : w air dry, chopped lucerne hay : chopped pasture hay) was offered in equal portions at intervals of 6 h. In addition, each cow was offered 800 g/day (air dry) long pasture hay (6·6 MJ metabolizable energy per kilogram air dry) in four equal portions at intervals of 6 h. Water was available *ad libitum*.

Surgical Preparation

Before the experiment commenced, polyvinyl chloride catheters (1·0 mm internal diameter by 2·0 mm external diameter; Dural Plastics, N.S.W.) were inserted under general anaesthesia (sedation with intravenous xylazine—Rompun, Bayer Australia—followed by intravenous chloral hydrate) into an external jugular vein, one femoral artery via a medial saphenous artery, the contra-lateral deep femoral vein via the lateral saphenous vein and in a medial subcutaneous mammary vein. The catheter in the deep femoral vein was inserted, via the lateral saphenous vein, so the tip was located adjacent to the pelvic symphysis (*c.* 70 cm from the junction of the caudal and cranial branches of the lateral saphenous vein). This catheter was located in a position to allow collection of blood draining predominantly muscle tissue (see Bell *et al.* 1976), whereas the catheter in the mammary vein allowed collection of mixed mammary venous blood. Catheters were filled with sterile heparinized saline ($2{\cdot}5 \times 10^5$ i.u. heparin, 9·0 g NaCl per litre) containing streptomycin (2 g/l) and serviced daily by flushing with minimum volumes of heparinized saline to maintain patency.

Cows were denied food for *c.* 18 h before surgery. Food intakes and milk yields returned to the levels before surgery by 7–8 days and the experiment commenced 5–6 days later.

Experimental Procedures

The experiment was conducted over 3 consecutive weeks during winter (June). During the first and third weeks (periods 1 and 3), cows received a subcutaneous injection (5 ml) of sterile saline (9 g NaCl per litre, pH adjusted to 10·0 with 1 M NaOH) each day for 6 days. In the second week (period 2), cows were injected subcutaneously each day for 6 days with 0·1 mg/kg liveweight GH dissolved in 5 ml sterile saline (pH 10·0). Injections were given at 0900 hours.

Liveweights were measured on the last day of each week. Milk yield was recorded at each milking and representative subsamples of milk from each day retained for subsequent analyses after addition of potassium bichromate as preservative.

On the second day of each period blood samples were collected from the arterial catheter at intervals commencing 1 h before and continuing throughout the 24 h after injection to allow measurement of diurnal changes in metabolites and hormones.

On either the third or fourth day of each period blood samples were collected for measurement of arterio-venous (A – V) differences of metabolites across leg muscle and mammary tissues. Three sets of blood samples (20 ml) were withdrawn simultaneously from the catheters in the femoral artery, the deep femoral vein and the subcutaneous mammary vein at intervals of *c.* 20 min commencing *c.* 2 h after the daily injection of saline or GH. At each sampling 3–5 ml blood were withdrawn from catheters and discarded prior to collection of samples for analyses. Tissue blood flows were then measured, during the third hour after injections using a modification of the procedure described by Oddy *et al.* (1981). Briefly, a continuous infusion (1 ml/min) of tritiated water (TOH) was administered via the jugular catheter over 1 h. Initially, 1 ml of TOH at a concentration of $1{\cdot}85 \times 10^7$ Bq/ml was infused for

exactly 1 min. Thereafter, for the remaining 59 min, an initial 4 ml of TOH at this concentration was diluted exponentially with TOH at a concentration of $3 \cdot 7 \times 10^5$ Bq/ml. During the 1 h of infusion of TOH, blood was withdrawn continuously at a rate of 1 ml/min, over 12 consecutive intervals of 5 min, from the catheters in the femoral artery, the deep femoral vein and the subcutaneous mammary vein. Clotting of the withdrawn blood was prevented by introduction of heparin ($7 \cdot 5 \times 10^5$ i.u./l in saline) into the collection tubing, *c.* 1m from the tip in the blood vessel, at the rate of 0·04 ml/min. Aliquots of collected blood were deproteinized with 0·6 M perchloric acid prior to measurement of TOH concentration using a scintillation counter. Blood flow was then determined using the relationships outlined by Oddy *et al.* (1981).

Chemical Analyses

Contents of milk fat, protein and lactose were measured with a Milkoscan 104 (A/S N. Foss Electric, Denmark) at the laboratories of the New South Wales Dairy Corporation, Sydney. Analyses were conducted at the end of each period of the experiment. Blood and plasma metabolites were measured in samples which were kept chilled and processed within 30 min of collection pending subsequent analyses after storage at −20°C.

Blood lactate [L-(+)-lactate] was measured by a semi-automated procedure developed from the method described by Gutman and Wahlefeld (1974). An aliquot of blood (0·5 ml) was deproteinized with ice-cold 0·5 M perchloric acid (1·0 ml) and the supernatant stored pending analysis. A further aliquot of blood (1·0 ml) was stored frozen for subsequent measurement of D-3-hydroxybutyrate, by the auto-analyser method of Zivan and Snarr (1973) after deproteinization with 0·15 M $Ba(OH)_2 . 8H_20$–5% (w/v) $ZnSO_4 . 7H_20$ (Somogyi 1945).

Acetate was isolated from whole blood (3·0 ml) as the sodium salt by steam distillation, after addition of isobutyric acid as internal standard (Annison 1954). Concentrations of acetate were determined by gas–liquid chromatography using a Varian 370 gas chromatograph (Varian, California) and a computing integrator (SP4100, Spectra-Physics, California). Distilled samples were acidified with 6·5 M formic acid immediately before 3 μl were injected into the gas–liquid chromatograph. A pre-column insert filled with small glass beads, replaced daily, prevented deterioration of the column which was packed with Chromasorb 101 (Varian, California). Temperatures of injector, detector and column were respectively 200, 250 and 145°C and a nitrogen gas flow of 25 ml/min was maintained.

Plasma glucose was measured after deproteinization of plasma (0·2 ml) with cold 0·33 M perchloric acid (1·8 ml). Glucose in the protein-free supernatants was assayed subsequently by the method of Bernt and Lachenicht (1974). The NEFA in plasma were extracted into heptane using reagents described by Dole (1956). Subsequently, plasma NEFA were determined by the procedure of Kelley (1965).

Hormone Assays

Concentrations of GH and insulin were measured by the talc radioimmunoassays originally described by Wallace and Bassett (1970) and Rosselin *et al.* (1966) respectively. Details of the assays have been described previously (Gow *et al.* 1981). Sensitivities of the assays, as measured by the procedure of Burger *et al.* (1972), were 1·0 μg/l and 2·4 mU/l for GH and insulin respectively. To overcome variability between assays for a particular hormone all samples which were to be compared were measured in the one assay. Intra-assay coefficients of variation were less than 15%.

Growth Hormone

Growth hormone for injection was extracted from bovine pituitary glands by the salt precipitation method of Ellis (1961) and the preparation was assayed for its biological activity (1·4 U/mg) and contamination with prolactin (<5%) by Dr I. C. Hart.

Statistical Analyses

The significance of differences between mean values for parameters measured during the periods of the experiment were assessed using paired *t*-tests.

Results

Liveweight, Milk and Milk Constituents

Mean values for liveweight, milk yield, yields and contents of milk constituents are shown in Table 1. There were no significant ($P > 0 \cdot 05$) changes in liveweight over the experiment.

Milk yield, both actual and 4% fat-corrected, increased significantly ($P < 0{\cdot}05$) during period 2 when GH was injected. Yields during the control periods were not significantly different ($P > 0{\cdot}05$).

The content of fat in milk increased significantly ($P < 0{\cdot}05$) during treatment with GH, but contents of protein and lactose were not significantly ($P > 0{\cdot}05$) affected by GH. Yields of each milk component were significantly increased ($P < 0{\cdot}05$) during treatment with GH and values for the control periods were not significantly different ($P > 0{\cdot}05$).

Table 1. Mean values ($n = 3$) for liveweight, milk yield and yields and contents of milk constituents during injections of saline (periods 1 and 3) or GH (period 2)
Liveweight was recorded at the end of each treatment period. For other parameters, the mean values shown represent the mean of mean values for the 6 days of each period for each cow. For a particular parameter, values with different superscripts differ significantly ($P < 0{\cdot}05$)

	Period 1	Period 2	Period 3
Liveweight (kg)	510	510	518
Milk yield – actual (kg/day)	$21{\cdot}4^{a}$	$24{\cdot}1$	$21{\cdot}9^{a}$
– 4% FCM[A]	$22{\cdot}3^{a}$	$26{\cdot}1^{b}$	$23{\cdot}3^{ab}$
Milk fat – content (g/kg)	$42{\cdot}9^{a}$	$45{\cdot}4^{b}$	$44{\cdot}2^{ab}$
– yield (g/day)	917^{a}	1095^{b}	969^{ab}
Milk protein – content (g/kg)	$29{\cdot}8$	$29{\cdot}7$	$29{\cdot}6$
– yield (g/day)	637^{a}	715	647^{a}
Milk lactose – content (g/kg)	$49{\cdot}0$	$48{\cdot}7$	$49{\cdot}4$
– yield (g/day)	1048^{a}	1174^{b}	1083^{ab}

[A] 4% fat corrected milk yield.

Plasma Hormones

Concentrations of GH and insulin throughout the second day of each treatment period are shown in Fig. 1 and concentrations during the third hour after injections on days 3 or 4 of each period are shown in Table 2.

No significant differences ($P > 0{\cdot}05$) were measured for concentrations of insulin throughout the second day of each treatment period and concentrations were relatively stable between *c.* 20–30 mU/l. However, analyses for insulin in samples collected on days 3 or 4 of each period showed that concentrations were lower during treatment with GH than during control periods when saline was injected.

Plasma GH was significantly increased ($P < 0{\cdot}05$) during GH treatment. By 2 h after injection, concentrations had increased from *c.* 5 to *c.* 12 μg/l and remained at this level for 5–6 h before gradually decreasing throughout the remaining hours before the next injection. The peak concentrations measured during injection of GH were three to four times higher than concentrations during control periods when concentrations were stable at *c.* 3·5–4·0 μg/l (see Fig. 1).

Plasma and Blood Metabolites

Concentrations of metabolites in arterial blood throughout the second day of each treatment period are shown in Fig. 2, whereas concentrations on days 3 or 4 of each period are shown in Table 2. Also shown in Table 2 are A – V differences and extractions [(A – V)/A × 100] of metabolites across leg muscle and mammary tissue during days 3 or 4 of each period.

Plasma glucose

During the control periods plasma glucose concentrations remained essentially constant

throughout the day at *c.* 3·5 mM. Treatment with GH appeared to increase plasma concentrations some 5–6 h after injection to *c.* 4·0 mM but differences were not significant ($P > 0{\cdot}05$). By day 3 or 4 after commencement of GH treatment plasma glucose had increased and was significantly higher ($P < 0{\cdot}05$) during treatment with GH than during the second control period.

The A – V difference and extraction for glucose across leg muscle were significantly ($P < 0{\cdot}05$) reduced by GH but there was no effect of GH on A – V difference or extraction of glucose across mammary tissue.

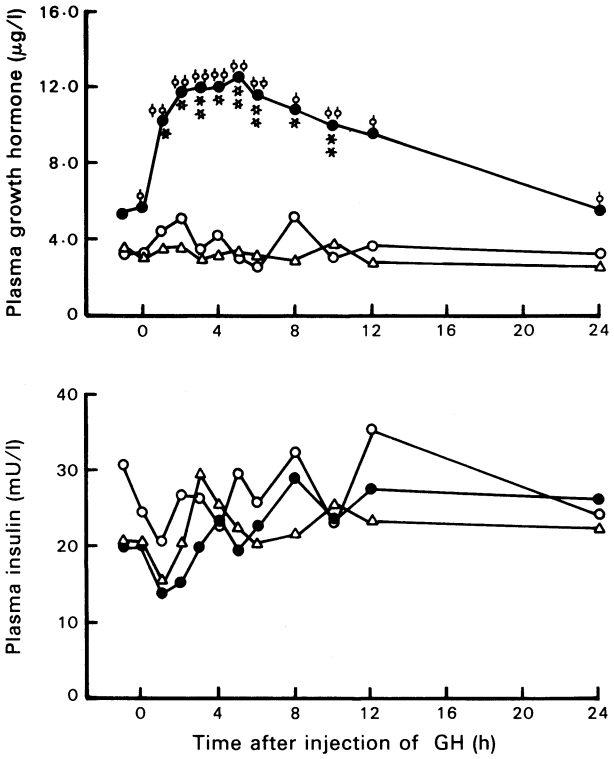

Fig. 1. Mean concentrations ($n = 3$) of plasma hormones throughout the second day of periods when cows were injected with saline (○, period 1; △, period 3) or GH (●, period 2). At particular times of the day values for individual periods which differ significantly are indicated thus – saline period 1 *v.* GH period 2: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$; saline period 3 *v.* GH period 2: $\phi P < 0{\cdot}05$, $\phi\phi P < 0{\cdot}01$.

Plasma NEFA

Plasma NEFA were significantly higher during treatment with GH than during the control periods. In each period, plasma concentrations throughout the day were essentially constant. Concentrations on day 2 of the periods when saline was administered were significantly higher ($P < 0{\cdot}05$) for period 3 than period 1. By days 3 or 4 differences for the control periods were not significant ($P > 0{\cdot}05$).

The A – V differences of NEFA across leg muscle tissue were not significantly affected by GH but extraction across leg muscle was lower during treatment with GH than during control periods. The A – V difference and extraction of NEFA across mammary tissue were significantly increased during treatment with GH.

Blood acetate

Concentrations of blood acetate were essentially constant during day 2 of each period and differences between periods were not significant. Similarly, on days 3 or 4 differences

Table 2. Mean values (n = 3) for plasma hormones, tissue blood flow, plasma and/or blood concentrations of metabolites, arterio-venous differences (A − V) for metabolites and proportion of metabolite extracted [(A − V)/A × 100] from blood and/or plasma during injections of saline (periods 1 and 3) or GH (period 2)
Measurements were made on the third or fourth day of each treatment period. For each metabolite and hormone, the value for each animal was the mean of three measurements made during the third hour after injections. For a particular parameter, values with different superscripts differ significantly: a,b, $P < 0{\cdot}05$; x,y, $P < 0{\cdot}1$. Positive values indicate uptake, negative values indicate release

	Period 1	Period 2	Period 3
Plasma hormones			
Growth hormone (μg/l)	$4{\cdot}2^{a}$	$13{\cdot}1$	$3{\cdot}0^{a}$
Insulin (mU/l)	$25{\cdot}6^{xy}$	$18{\cdot}1^{x}$	$26{\cdot}1^{y}$
Tissue blood flow (ml/kg/min)			
Leg muscle	111	134	103
Mammary gland	349^{a}	449	318^{a}
Plasma glucose (mM)			
Arterial	$3{\cdot}47^{ab}$	$3{\cdot}69^{b}$	$3{\cdot}51^{a}$
A − V leg muscle	$0{\cdot}17^{a}$	$0{\cdot}02^{b}$	$0{\cdot}07^{ab}$
A − V mammary gland	$0{\cdot}71$	$0{\cdot}76$	$0{\cdot}52$
Glucose extraction (%)			
Leg muscle	$4{\cdot}8^{a}$	$0{\cdot}75^{ab}$	$1{\cdot}84^{b}$
Mammary gland	$20{\cdot}10$	$22{\cdot}70$	$15{\cdot}49$
Plasma NEFA (μM)			
Arterial	193^{a}	316	222^{a}
A − V leg muscle	$28{\cdot}3$	$30{\cdot}3$	$41{\cdot}5$
A − V mammary gland	$36{\cdot}0^{a}$	113	$52{\cdot}7^{a}$
NEFA extraction (%)			
Leg muscle	$14{\cdot}94^{ab,x}$	$9{\cdot}55^{a}$	$19{\cdot}20^{b,x}$
Mammary gland	$18{\cdot}66^{ab,x}$	$35{\cdot}91^{a}$	$23{\cdot}67^{b,x}$
Blood acetate (mM)			
Arterial	$1{\cdot}67$	$1{\cdot}73$	$1{\cdot}59$
A − V leg muscle	$0{\cdot}44^{a}$	$0{\cdot}38^{ab}$	$0{\cdot}52^{b}$
A − V mammary gland	$0{\cdot}95$	$1{\cdot}03$	$0{\cdot}90$
Acetate extraction (%)			
Leg muscle	$27{\cdot}07$	$21{\cdot}60$	$33{\cdot}69$
Mammary gland	$57{\cdot}32$	$59{\cdot}35$	$55{\cdot}82$
Blood 3-hydroxy-butyrate (mM)			
Arterial	$1{\cdot}10$	$0{\cdot}99$	$0{\cdot}87$
A − V leg muscle	$0{\cdot}20$	$0{\cdot}12$	$0{\cdot}18$
A − V mammary gland	$0{\cdot}28$	$0{\cdot}32$	$0{\cdot}31$
3-Hydroxybutyrate extraction (%)			
Leg muscle	$19{\cdot}77$	$12{\cdot}94$	$24{\cdot}96$
Mammary gland	$27{\cdot}72$	$35{\cdot}20$	$37{\cdot}35$
Blood lactate (mM)			
Arterial	$0{\cdot}71$	$0{\cdot}75$	$0{\cdot}70$
A − V leg muscle	$-0{\cdot}01^{a}$	$-0{\cdot}08^{ab}$	$-0{\cdot}05^{b}$
A − V mammary gland	$0{\cdot}08$	$-0{\cdot}05$	$-0{\cdot}06$
Lactate extraction (%)			
Leg muscle	$-1{\cdot}33^{a}$	$-10{\cdot}86^{ab}$	$-6{\cdot}44^{b}$
Mammary gland	$11{\cdot}47$	$-6{\cdot}60$	$-6{\cdot}45$

between values for the periods were not significant. Extraction and A – V differences for acetate across leg muscle and mammary tissue were not affected by treatment with GH.

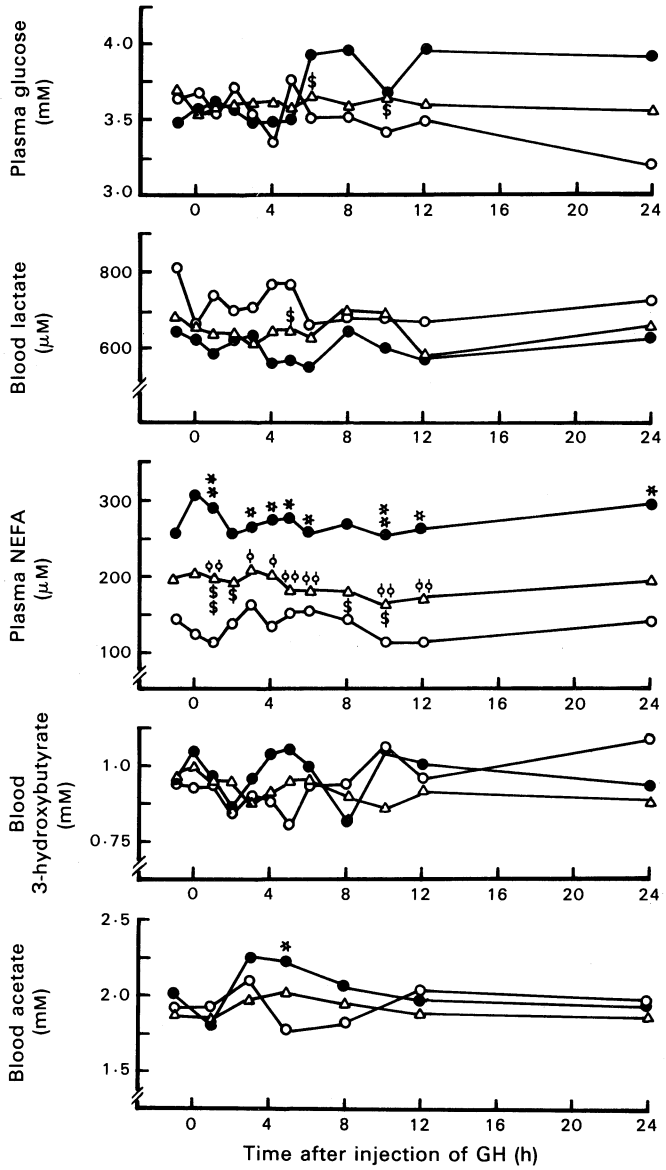

Fig. 2. Mean concentrations (n = 3) of metabolites in blood or plasma throughout the second day of periods when cows were injected with saline (○, period 1; △, period 3) or GH (●, period 2). At particular times of the day, values for individual periods which differ significantly are indicated thus—saline period 1 v. GH period 2: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$; saline period 3 v. GH period 2: $\phi P < 0{\cdot}05$, $\phi\phi P < 0{\cdot}01$; saline period 1 v. saline period 3: $\$P < 0{\cdot}05$, $\$\$P < 0{\cdot}01$.

Blood 3-hydroxybutyrate

Concentrations of 3-hydroxybutyrate in arterial blood were similar (*c.* 1 mM) during each period of the experiment and remained essentially constant throughout day 2 in each period. Extractions and A – V differences across leg muscle and mammary tissue were not affected by GH.

Blood lactate

Growth hormone did not affect blood lactate concentrations and concentrations during day 2 of each period were reasonably stable. Significant differences for A – V differences and extractions of lactate across leg muscle tissue were measured between periods. In general there was a tendency for lactate output to increase during GH treatment. Injection with GH had no effects on A – V differences or extraction of lactate across mammary tissue.

Tissue Blood Flow

During treatment with GH blood flow to both leg muscle and mammary tissue increased. The relationship between milk yield (Y kg/d) and mammary blood flow (F, ml kg^{-1} min^{-1}) was

$$Y = 9{\cdot}831 + 0{\cdot}032F \qquad (r = 0{\cdot}897,\ P < 0{\cdot}01)\,.$$

The procedure used for measurement of blood flow did not provide measurements of absolute blood flow (viz. ml/min). However, it is possible to obtain an approximate estimate of absolute blood flow by assuming that the mass of mammary tissue is related to milk yield in the ratio of 1:1, as shown by G. M. Hough and K. R. King (personal communication) for lactating ewes.

Table 3. Relationships between mammary blood flow and milk yield for individual cows during treatment with saline (periods 1 and 3) or GH (period 2)

	Period	Cow 1	Cow 2	Cow 3	Mean
Udder mass (kg)[A]		22·6	20·4	21·9	21·6
Mammary blood flow (ml/min)[B]	1	8459	7289	6912	7553
	2	10163	8595	10411	9723
	3	–	7297	8935	8116
Milk yield (ml/min)[B]	1	15·21	13·75	14·58	14·51
	2	17·57	15·90	17·08	16·85
	3	16·11	14·51	15·83	15·48
Ratio of blood flow to milk yield	1	556	530	474	520
	2	578	541	610	576
	3	–	503	564	534

[A] Assuming 1:1 ratio between milk yield and mass of udder tissue, no change between treatment periods. Value is based on average milk yields for periods 1 and 3.
[B] Derived using values measured for individual periods for each cow.

The data presented in Table 3 were derived by first assuming the above relationship and secondly that there was no increase in the mass of mammary tissue during GH treatment. As shown in Table 3 the ratio of mammary blood flow (ml/min) : milk yield (ml/min) was *c.* 520 : 1 during the control periods and *c.* 580 : 1 during treatment with GH.

Discussion

It is clear from Fig. 1 that plasma concentrations of GH were low and stable at *c.* 4 μg/l throughout the second day of each control period. This observation is consistent with the cows being fed adequately and continuously, as others have shown raised plasma GH in underfed animals (Trenkle 1971; Bassett 1974; Gow *et al.* 1981) and marked fluctuations in plasma GH associated with intermittent feeding (Tindal *et al.* 1982). During treatment with GH, plasma concentrations of the hormone were increased significantly for much of each day. Within 1 h of injection plasma concentrations had increased to 10 μg/l and remained at or above this value for some 9 h before gradually decreasing.

Plasma concentrations of insulin were similar during the second day of each treatment period at values which were indicative of the cows being adequately fed. There was no evidence for an increase in plasma insulin following treatment with GH as observed under some situations (Bines *et al.* 1980; McDowell *et al.* 1987; Leenanuruksa and McDowell, unpublished observations). Indeed plasma insulin was decreased during days 3 or 4 of treatment with GH (see Table 2) and this may have been due to the marked galactopoietic effect of GH and a corresponding increase in glucose utilization for lactose synthesis (see below). Alternatively, the decreased insulin measured on day 3 or 4 of treatment with GH may be attributable to 'anti-insulin' effects exerted by GH (Hart 1983; Lennanuruksa and McDowell 1986). It is of interest that the reduced concentrations of plasma insulin were observed at a time when there was a tendency for concentrations of plasma glucose to increase (see Fig. 2, Table 2). This would be consistent with GH exerting diabetogenic effects.

In common with results of numerous previous studies (see reviews by McCutcheon and Bauman 1985; McDowell 1985; Johnsson and Hart 1986) GH exerted marked galactopoietic effects. Milk yield, milk fat content and yields of fat, protein and lactose were increased during treatment with GH.

The increased production of milk and milk constituents occurred in the absence of any change in feed intake. There was no measurable decrease in liveweight during treatment with GH, as has been recorded in other similar studies (for example McDowell *et al.* 1987). However, the precision with which changes in liveweight can be measured is low, particularly over short periods.

In spite of the failure to measure changes in liveweight, there was evidence for altered partition and/or utilization of key nutrients by muscle and mammary tissues during treatment with GH. The most marked changes were measured for plasma glucose and NEFA for which significant changes in A – V differences across muscle and mammary tissue were measured. Thus A – V difference for glucose across leg muscle tissue decreased and A – V difference for NEFA across mammary tissue increased.

For other metabolites, although changes for A – V differences across muscle and mammary were less clear, there were trends. The A – V difference for acetate across leg muscle was lower during treatment with GH than during the second control period. Similarly, A – V difference for 3-hydroxybutyrate was lower during treatment with GH than saline (both periods) and the release of lactate from leg muscle was lower during treatment with saline than GH.

Overall, the data obtained in the present study provide the first direct evidence that exogenous GH alters the partition of nutrients in the lactating cow as has been proposed previously (see Bauman and Currie 1980; Bines and Hart 1982). Further, evidence for altered tissue utilization of nutrients was obtained.

It was clear that exogenous GH was lipolytic – plasma concentrations of NEFA were *c.* 50% higher during treatment with GH than saline. Further, evidence was obtained for a diabetogenic effect of GH as plasma glucose tended to be higher during treatment with GH than saline. In these respects the data obtained in our study are consistent with observations recorded by Hart (1983).

It seems reasonable to assert that leg muscle tissue is representative of skeletal muscle tissue throughout the body. Given that this is the case, the decreased uptake of glucose and increased output of lactate across leg muscle tissue during GH treatment would be indicative of altered glucose metabolism by muscle tissue throughout the body. The tendency for plasma glucose concentrations to increase during GH treatment is consistent with this.

The marked increase in mammary A – V difference for NEFA is of particular interest. It can be calculated that the increased uptake of NEFA (A – V difference × blood flow) across the mammary gland was more than sufficient to account for the additional secretion of milk fat – even if all the additional fatty acids in milk fat were derived from plasma NEFA. Results of preliminary studies at our laboratory indicate increased oxidation of fatty

acids by tissues, including the mammary gland, during treatment with GH (McDowell *et al.* 1985). This would enable sparing of glucose oxidation resulting in greater availability of glucose for lactose synthesis in the mammary gland.

In other preliminary studies at our laboratory a reduction in plasma concentrations of very low density lipoproteins has been measured during GH treatment (Niumsup *et al.* 1985). This would require increased utilization of plasma NEFA for milk fat synthesis during GH treatment, and may in part explain the increased uptake of NEFA measured in the present study.

During treatment with GH, blood flow to the mammary gland increased significantly and blood flow to leg muscle tissue tended to increase. Increased mammary blood flow has been recorded previously in lactating goats (Hart *et al.* 1980; Mepham *et al.* 1984) and cows (McNamara *et al.* 1983; Davis and Collier 1985) during treatment with GH. It is suggested that the increased blood flow to the mammary gland, and apparent increase in blood flow to the leg muscle tissue, reflected increased cardiac output during treatment with GH (see Mepham *et al.* 1984). Although cardiac output was not measured in the present study there are indications that chronic administration of GH increases heart size in sheep (Johnsson *et al.* 1985).

The calculated ratio of mammary blood flow : milk yield during the saline control periods (*c.* 520 : 1) is similar to values reported previously for lactating ruminants (King 1983; Linzell 1974). During treatment with GH the ratio was calculated as *c.* 580 : 1. This would result in an increased supply of nutrients to the mammary gland in the absence of changes in plasma concentrations or extractions by the tissue. Indeed, mammary extractions of glucose, acetate and 3-hydroxybutyrate were similar during treatment with saline and GH (see Table 2).

In conclusion, the results of the present study provide insights into the physiological action of exogenous GH in lactating dairy cows. Direct evidence was obtained for effects of GH on the partition and/or utilization of nutrients in the body. Moreover, evidence was obtained that GH affects the supply of key nutrients for mammary metabolism by altering plasma concentrations and blood flow to the mammary gland.

Acknowledgments

The skilled technical assistance of Miss S. L. Baker and Mrs K. A. van der Jagt and the patient care of cows provided by Mr K. McKean and Mrs D. F. Horsley are acknowledged with thanks. Financial support for the work was provided by the Australian Dairying Research Committee. D.L. and M.J. were supported by scholarships of the Faculty of Agriculture of the University of Sydney. Dr I. C. Hart, formerly of the National Institute for Research in Dairying, Shinfield, U.K. (currently, American Cyanamid Co., Princeton, U.S.A.), kindly assessed the biological activity and purity of the GH preparation used. Mr L. McPhee from Homebush Abattoir Corporation, Homebush Bay, N.S.W., kindly supplied bovine pituitary glands. Mr C. Chen, N.S.W. Dairy Corporation Laboratories, Sydney, arranged for assays of milk constituents.

References

Annison, E. F. (1954). Studies on the volatile fatty acids of sheep blood with special reference to formic acid. *Biochem. J.* **58**, 670–80.

Anon. (1975). 'Energy Allowances and Feeding Systems for Ruminants.' Technical Bulletin 33. (H.M.S.O.: London.)

Bassett, J. M. (1974). Diurnal patterns of plasma insulin, growth hormone, corticosteroid and metabolite concentrations in fed and fasted sheep. *Aust. J. Biol. Sci.* **27**, 167–81.

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.

Bell, A. W., Hilditch, T. E., Horton, P. W., and Thompson, G. E. (1976). The distribution of blood flow between individual muscles and non-muscular tissues in the hind limb of the young ox (*Bos taurus*): values at thermoneutrality and during exposure to cold. *J. Physiol. (Lond.)* **257**, 229–43.

Bernt, E., and Lachenicht, R. (1974). Determination in blood, serum, or plasma with automatic analysers (GOD-PERID^{R} Method). In 'Methods of Enzymatic Analysis'. (Ed. H. U. Bergmeyer.) 2nd End. Vol. 3. pp. 1215–22. (Academic Press: New York and London.)

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.

Bines, J. A., Hart, I. C., and Morant, S. V. (1980). Endocrine control of energy metabolism in the cow: the effect on milk yield and levels of some blood constituents of injecting growth hormone and growth hormone fragments. *Brit. J. Nutr.* **43**, 179–88.

Burger, H. G., Lee, V. W. K., and Rennie, G. C. (1972). A generalised computer programme for the treatment of data from competitive protein binding assays, including radioimmunoassays. *J. Lab. Clin. Med.* **80**, 302–12.

Davis, S. R., and Collier, R. J. (1985). Mammary blood flow and regulation of substrate supply for milk synthesis. *J. Dairy Sci.* **68**, 1041–58.

Dole, V. P. (1956). A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35**, 150–4.

Ellis, S. (1961). Studies on the serial extraction of pituitary proteins. *Endocrinology* **69**, 554–70.

Gow, C. B., McDowell, G. H., and Annison, E. F. (1981). Control of gluconeogenesis in lactating sheep. *Aust. J. Biol. Sci.* **34**, 469–78.

Gutman, I., and Wahlefeld, A. W. (1974). L-(+)-Lactate. Determination with lactate dehydrogenase and NAD. In 'Methods of Enzymatic Analysis'. (Ed. H. U. Bergmeyer.) 2nd Edn. Vol. 3. pp. 1464–6. (Academic Press: New York and London.)

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

Hart, I. C., Lawrence, S. E., and Mepham, T. B. (1980). Effect 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.

Kelley, F. (1965). Improved method for microtitration of fatty acids. *Analyt. Chem.* **37**, 1078–9.

King, K. R. (1983). Lipid metabolism in the lactating ewe. M.Sc.Agr. Thesis, University of Sydney.

Leenanuruksa, D., and McDowell, G. H. (1986). Use of the alloxan diabetic sheep to show the diabetogenic effect of growth hormone. *Proc. Endocrinol. Soc. Aust.* **29**, Abstr. 69.

Linzell, J. L. (1974). Mammary blood flow and methods of identifying and measuring precursors of milk. In 'Lactation: A Comprehensive Treatise. Volume I. The Mammary Gland/Development and Maintenance'. (Eds B. L. Larsen and V. R. Smith.) pp. 143–225. (Academic Press: New York.)

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.)

McDowell, 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.)

McDowell, G. H., Gooden, J. M., van der Walt, J. G., Smithard, R., Leenanuruksa, D., and Niumsup, P. (1985). Metabolism of glucose and free fatty acids in lactating ewes treated with growth hormone. *Proc. Nutr. Soc. Aust.* **10**, 155.

McDowell, G. H., Hart, I. C., Bines, J. A., and Lindsay, D. B. (1983). Effects of exogenous growth hormones in lactating cows. *Proc. Nutr. Soc. Aust.* **8**, 165.

McDowell, G. H., Hart, I. C., Bines, J. A., Lindsay, D. B., and Kirby, A. C. (1987). Effects of pituitary-derived bovine growth hormone on production parameters and biokinetics of key metabolites in lactating dairy cows at peak and mid lactation. *Aust. J. Biol. Sci.* **40**, 191–202.

McDowell, G. H., Leenanuruksa, D., Gooden, J. M., Jois, M., and Annison, E. F. (1984). Effects of growth hormone on muscle and mammary metabolism in cows. *Proc. Nutr. Soc. Aust.* **9**, 164.

McNamara, J. P., Davis, S. R., and Collier, R. J. (1983). Effect of growth hormone and thyroxine on plasma lipids, mammary lipid uptake and milk fat in Jersey cows. *J. Dairy Sci.* **66** (Suppl. 1), 232–3.

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

Niumsup, P., McDowell, G. H., Leenanuruksa, D., and Gooden, J. M. (1985). Plasma triglyceride metabolism in lactating ewes and growing calves treated with growth hormone. *Proc. Nutr. Soc. Aust.* **10**, 154.

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.

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

Peel, C. J., Steinhour, W. D., Bauman, D. E., Tyrrell, H. F., Brown, A. C. G., Reynolds, C. J., and Haaland, C. 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.

Rosselin, G., Asson, R., Yalow, R. S., and Berson, S. A. (1966). Separation of antibody-bound and unbound peptide hormones labelled with iodine-131 by talcum powder and precipitated silica. *Nature (Lond.)* **212**, 355–7.

Somogyi, M. (1945). Determination of blood sugar. *J. Biol. Chem.* **160**, 69–73.

Tilley, J. M. A., and Terry, R. A. (1963). A two-stage technique for the *in vitro* digestion of forage crops. *J. Brit. Grassl. Soc.* **18**, 104–11.

Tindal, J. S., Blake, L. A., Simmonds, A. D., Hart, I. C., and Mizuno, H. (1982). Control of growth hormone release in goats: effect of vagal cooling, feeding and artificial distension of the rumen. *Horm. Metab. Res.* **14**, 425–9.

Trenkle, A. (1971). Effects of diet upon levels of plasma growth hormone in sheep. *J. Anim. Sci.* **32**, 111–14.

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. Sundstol.) pp. 46–9. (Agricultural University of Norway: Aas.)

Wallace, A. L. C., and Bassett, J. M. (1970). Plasma growth hormone concentration in sheep measured by radioimmunoassay. *J. Endrocrinol.* **47**, 21–36.

Zivan, J. A., and Snarr, J. F. (1973). An automated colorimetric method for the measurement of 3-hydroxybutyrate concentrations. *Analyt. Biochem.* **52**, 456–61.

Manuscript received 13 January 1987, accepted 13 April 1987