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Short-term Effects of Exogenous Growth Hormone: Effects on Milk Production and Utilization of Nutrients in Muscle and Mammary Tissues of Lactating Ewes

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Abstract

Exogenous bovine growth hormone at a dose of 0·1 mg kg^{-1} liveweight increased yields of milk and milk constituents and milk fat content when injected over 5 days into ewes in mid-lactation. These changes in milk production were associated with changes in the supply to, and utilization of, nutrients by leg muscle and mammary tissues.

Arterial concentrations of glucose and non-esterified fatty acids increased significantly, concentrations of lactate and 3-hydroxybutyrate tended to increase, and concentrations of triglycerides associated with very low-density lipoproteins decreased significantly. Growth hormone increased mammary uptake of non-esterified fatty acids, decreased mammary uptake of very low-density lipoproteins and tended to reduce the release of lactate from leg muscle. Oxidation of non-esterified fatty acids in the whole body and mammary tissue was increased by growth hormone and there was a tendency for reduction of glucose oxidation in mammary tissues.

During injection of growth hormone, blood flow to leg muscle and mammary tissues increased as did the calculated ratio of blood flow : milk yield. These changes in blood flow, together with changes in arterial concentrations and tissue utilizations of key metabolites, were sufficient to account for the synthesis of extra milk and milk constituents.

Extra keywords: milk yield, milk components, arterio-venous differences of nutrients across leg muscle and mammary tissue, oxidation of glucose and non-esterified fatty acids, plasma triglycerides, blood flow.

Introduction

Although the galactopoietic effects of growth hormone (GH) in ruminants are now well recognized (see McCutcheon and Bauman 1985; Johnsson and Hart 1986), the mechanisms by which the hormone exerts these effects are ill-defined. There is now evidence to show that exogenous GH does not exert direct effects on the mammary gland *in vivo* (McDowell *et al.* 1987*c*) or *in vitro* (Skarda *et al.* 1982; Gertler *et al.* 1983), at least when the hormone is administered over periods of a few days.

The observation that exogenous GH has no effect on digestibilities of dry matter, energy or nitrogen (Peel *et al.* 1981; Tyrrell *et al.* 1982) raises the possibility that GH affects the partition of nutrients, thereby increasing the supply to the mammary gland of key nutrients for milk biosynthesis as suggested by Bauman and Currie (1980) and Bines and Hart (1982). Data

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supporting this contention have been presented by Peel *et al.* (1982) and McDowell *et al.* (1987*b*), who showed that exogenous GH affects whole-body irreversible losses of key metabolites, in particular glucose and non-esterified fatty acids (NEFA), in lactating cows. More recently, McDowell *et al.* (1987*a*) reported that GH exerts direct effects on the partition/utilization of nutrients between skeletal muscle and mammary tissues of cows.

The present studies were conducted to measure effects of exogenous GH on nutrient utilization in the body as a whole, and in muscle and mammary tissues in particular, of lactating ewes. Preliminary data have been presented elsewhere (McDowell *et al.* 1985; Niumsup *et al.* 1985).

Materials and Methods

Sheep

Six multiparous crossbred ewes (Border Leicester × Merino), with no obvious abnormalities of the mammary glands, were separated permanently from their lambs on the day of parturition and kept in metabolism cages. A good-quality ration containing rolled barley:chopped lucerne (50:50 air dry; 9·6 MJ metabolizable energy and 218 g crude protein per kg dry matter) was fed continuously using belt feeders and in sufficient amounts to satisfy requirements for metabolizable energy for maintenance plus milk production (Anon. 1975). Water was available *ad libitum.*

All ewes were accustomed to handling and could be bled from catheters (see below) and milked by hand without restraint and obvious distress. Milking occurred at *c.* 0830 and 1630 each day.

Surgical Preparation

Approximately 10 days before measurements were made, polyvinyl chloride catheters (0·86 mm internal diameter by 1·27 mm external diameter; Dural Plastics, Dural, New South Wales) were inserted into a deep femoral artery (A). Three to five days before experiments, additional polyvinyl chloride catheters (1·00 mm internal diameter by 1·5 mm external diameter) were inserted in both external jugular veins, a medial subcutaneous mammary vein (MV) and a deep femoral vein (LV), via the lateral saphenous vein as outlined previously (Oddy *et al.* 1981; Teleni and Annison 1986). Catheter tips were located so as to collect blood draining predominantly leg muscle and mammary tissues, respectively. The patency of catheters was maintained by flushing with small quantities of sterile heparinized saline (9·0 g NaCl and 2×10^5 i.u. heparin per litre) twice daily. If it was not possible to sample from a venous catheter, the catheter was replaced using a flexible wire guide (Cook Inc., Bloomington, IN, U.S.A.) at least 2 h before blood sampling commenced.

Growth Hormone

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

Experimental Procedures

The experiment commenced when ewes had been lactating for *c.* 30 days. Experience has shown that the galactopoietic effects of GH are relatively greater in mid to late than in early lactation (G. H. McDowell and I. C Hart, unpublished observations).

Ewes were given daily subcutaneous injections of sterile saline (9·0 g NaCl l^{-1}, pH adjusted to 10·0 with 1 M NaOH) for 5 days, then for a further 5 days subcutaneous injections of GH dissolved in 5 ml sterile saline adjusted to pH 10·0, at the rate of 0·1 mg GH kg^{-1} liveweight. Milk yields were recorded daily and a representative subsample of total milk from each day was retained at 4°C pending subsequent analyses after addition of formalin (100 ml l^{-1}; 4 drops/10 ml milk).

On the fifth day of each period of 5 days, metabolite biokinetics and tissue blood flows were measured, and blood samples collected for measurement of hormones and metabolites. Briefly, three ewes were given primed/continuous intravenous infusions, via a jugular catheter, of [U-^{14}C]glucose ($92·5 \times 10^4$ Bq priming dose then *c.* $1·55 \times 10^4$ Bq min^{-1}) and the other ewes were given continuous intravenous infusions of [U-^{14}C] palmitic acid at the rate of *c.* $3·7 \times 10^4$ Bq min^{-1}. The isotopically-labelled metabolites were obtained from Amersham International plc (Amersham, U.K.) and infusions were administered over 4 h.

Blood samples were collected simultaneously from the catheters in A, MV and LV at *c.* 150, 170 and 240 min after commencement of infusions for measurement of blood gases and metabolite biokinetics (see below).

Between 180 and 230 min after commencement of infusions, blood flows in LV and MV were measured by dilution of tritiated water (see below). During measurement of tissue blood flows, samples of blood were withdrawn continuously at the rate of 1 ml min^{-1} from A, LV and MV. After aliquots of these samples were withdrawn for measurement of blood flows, the samples were pooled to allow measurement of metabolite concentrations and arterio-venous differences (viz. A–LV, A–MV) for metabolites.

Chemical Analyses

Contents of fat and protein in milk were measured as described by Fulkerson and McDowell (1974), and milk lactose was measured as described by Teles *et al.* (1978). Blood samples were collected into chilled tubes and processed within 30 min of collection before storage of either plasma or protein-free supernatant at –20°C pending analyses. Plasma concentrations of glucose and NEFA and blood concentrations of lactate and 3-hydroxybutyrate were measured as described by McDowell *et al.* (1987*a*).

Concentrations and specific radioactivities of blood CO_2 were measured using the procedures described by Oddy *et al.* (1984). Blood pCO_2 and pH were measured, within 30 min of collection of blood into chilled and sealed syringes, using a Blood Gas Analyzer (Corning Medical and Scientific, Medfield, Mass.)

The whole-body irreversible losses of glucose and NEFA were measured as outlined by McDowell *et al.* (1987*b*). The proportion of total plasma NEFA which was palmitate was measured using a Varian Model 370 Gas Chromatograph and the procedures outlined by Christie (1976). It was assumed that irreversible loss of palmitate was related directly to the proportion of total plasma NEFA as palmitate.

The proportions of CO_2 derived from oxidation of glucose and NEFA were measured using the relationships outlined by Pethick *et al.* (1983).

Plasma Triglycerides

Plasma concentrations of very low-density lipoproteins plus chylomicrons (VLDL, δ <1·006) and low-density lipoproteins (LDL, δ <1·040) were separated by ultacentrifugation using modifications of the methods described by Raphael *et al.* (1973) and Redgrave *et al.* (1975). Briefly, the density of plasma was adjusted to δ = 1·210 by addition of solid KBr. Volumes of 4 ml of plasma, with densities adjusted, were placed in ultracentrifuge tubes (volume 13·5 ml) and overlain by successive additions of 3 ml aliquots of NaCl solutions with densities of 1·063, 1·040 and 1·006 respectively.

Tubes were then centrifuged for 24 h at 20°C in a Beckman Model L2·50 Ultracentrifuge (Beckman Instruments Inc., Palo Alto, Calif.) using a SW41 Rotor at a speed of 41 000 rpm (average RCF = 201 125 *g*). A tube slicer was used to collect the VLDL and LDL fractions from which the lipid was extracted by the method of Folch *et al.* (1957). Triglycerides were separated using thin-layer chromatography, and concentrations of component fatty acids were measured using the Varian Model 370 Gas Chromatograph following the procedures of Christie (1976).

The VLDL-fraction isolated as described above from plasma of adult ruminants fed conventional roughage-based diets contains trivial amounts of chylomicrons (Leat *et al.* 1976; Gooden *et al.* 1979).

Measurement of Blood Flow

Rates of blood flow in leg muscle and mammary tissues were measured using modifications of the procedure outlined by Oddy *et al.* (1984). Briefly, a continuous infusion (1 ml min^{-1}) of tritiated water (TOH) was administered via a jugular venous catheter over 50 min. Initially, 1 ml TOH with a radioactive concentration of $1{\cdot}85 \times 10^7$ Bq ml^{-1} was infused over exactly 1 min. Thereafter, for the remaining 49 min, an initial 4 ml TOH at the above concentration was diluted exponentially with TOH at a concentration of $1{\cdot}85 \times 10^5$ Bq ml^{-1}. Blood was withdrawn continuously from A, LV and MV over ten consecutive intervals of 5 min.

Hormone Assays

Concentrations of GH and insulin in plasma were measured by talc radioimmunoassays, details of which have been provided previously by McDowell *et al.* (1987*a*). All samples to be compared were assayed in single assays which had intra-assay coefficients of variation of less than 15%. The sensitivities of the assays were 1·0 μg l^{-1} and 2·4 mU l^{-1} for GH and insulin, respectively.

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

Milk Yields and Contents and Yields of Milk Constituents

Mean values for milk yields and the contents and yields of major milk constituents are shown in Table 1. Milk yield and yield of each major milk constituent was increased significantly by treatment with GH. Whereas milk fat content increased signifcantly during GH treatment, the content of milk lactose was unaffected and that of milk protein was significantly decreased.

Table 1. Yields of milk and milk constituents, and concentrations of milk constituents during injections of saline or growth hormone (GH)

Values are means ± s.e.m. for six ewes, and the individual values used for computation of the mean values shown were the mean daily values for each period of 5 days. Values for individual parameters which differ significantly are indicated: $^{***}P < 0{\cdot}01$; $^{****}P < 0{\cdot}001$; ns, no significant difference

Parameter		Injected with saline	Injected with GH	Significance
Milk yield	(g day^{-1})	1434 ± 71·8	1647 ± 56·6	***
Milk fat	(g kg^{-1})	68·2 ± 6·04	79·5 ± 6·74	***
	(g day^{-1})	96·3 ± 7·05	132·3 ± 8·64	****
Milk lactose	(g kg^{-1})	55·0 ± 0·08	53·8 ± 0·69	ns
	(g day^{-1})	78·7 ± 3·36	90·2 ± 3·43	***
Milk protein	(g kg^{-1})	58·0 ± 1·28	54·4 ± 0·72	***
	(g day^{-1})	83·0 ± 3·08	91·1 ± 2·93	***

Plasma Hormones and Metabolites

It is clear from the data in Table 2 that concentrations of GH and insulin were significantly higher during injection of GH than of saline.

Although the arterio-venous differences A–LV and A–MV for blood lactate and blood 3-hydroxybutyrate were not affected significantly by GH, arterial concentrations of both metabolites tended to increase, and release of lactate from leg muscle tended to decrease. There were significant effects of GH on arterial plasma glucose, arterial plasma NEFA and A–MV for NEFA. In each case, values increased during treatment with GH (see Table 2).

Table 2. Arterial concentrations (A) of hormones and metabolites, together with arterio-venous differences for metabolites across leg-muscle (A–LV) and mammary (A–MV) tissues during treatment with saline or growth hormone (GH)

Positive values represent uptake, negative values release. Values are means ± s.e.m. for samples collected on day 5 of each treatment period from the six ewes except for plasma NEFA which are means ± s.e.m. for three ewes only. For individual parameters, values which differ significantly are indicated: $^{*}P < 0{\cdot}1$; $^{**}P < 0{\cdot}05$; $^{***}P < 0{\cdot}01$; $^{****}P < 0{\cdot}001$; ns, no significant difference

Parameter	Sample	Injected with saline	Injected with GH	Significance
Plasma GH (μg l^{-1})	A	0·5 ± 0·21	6·0 ± 0·64	****
Plasma insulin (mU l^{-1})	A	30·3 ± 3·79	64·7 ± 12·38	**
Plasma glucose (mM)	A	3·35 ± 0·110	3·73 ± 0·239	*
	A–LV	0·36 ± 0·037	0·44 ± 0·054	ns
	A–MV	0·83 ± 0·053	0·94 ± 0·093	ns
Plasma NEFA (μM)	A	389 ± 33·5	594 ± 54·1	**
	A–LV	37 ± 15·9	87 ± 19·4	ns
	A–MV	89 ± 33·4	195 ± 31·8	**
Blood lactate (μM)	A	785 ± 71·2	839 ± 56·3	ns
	A–LV	−79 ± 33·7	−46 ± 50·1	ns
	A–MV	34 ± 39·7	46 ± 50·5	ns
Blood 3-hydroxybutyrate (μM)	A	712 ± 71·4	872 ± 123·0	ns
	A–LV	128 ± 41·4	179 ± 40·6	ns
	A–MV	278 ± 50·7	323 ± 63·8	ns

During treatment with GH, arterial concentrations of total plasma triglycerides and VLDL decreased significantly. Similarly, A–LV and A–MV for VLDL decreased significantly during GH injection (Table 3). In spite of these effects of GH on concentrations of triglycerides, particularly VLDL-triglyceride, there were no effects of GH on proportions of fatty acids comprising the triglycerides (Table 4). Similarly, GH did not affect proportions of fatty acids comprising plasma NEFA (Table 4).

Table 3. Arterial plasma (A) concentrations and arterio-venous differences across leg-muscle (A–LV) and mammary (A–MV) tissues for triglycerides associated with very low-density lipoproteins plus chylomicrons (VLDL-TG) and low-density lipoproteins (LDL-TG) as well as total-TG for ewes during treatment with saline or growth hormone (GH)

Values are means ± s.e.m. for samples collected on day 5 of each period from the three ewes in which NEFA kinetics were measured. Values which differ significantly are indicated: $^{*}P < 0{\cdot}1$; $^{**}P < 0{\cdot}05$; $^{***}P < 0{\cdot}01$; ns, no significant difference

Parameter	Sample	Injected with saline	Injected with GH	Significance
Total-TG (mg 100 ml^{-1})	A	10·6 ± 0·12	7·2 ± 0·33	***
	A–LV	2·0 ± 0·70	1·1 ± 0·60	**
	A–MV	4·7 ± 1·00	2·2 ± 0·74	**
VLDL-TG (mg 100 ml^{-1})	A	7·7 ± 0·18	3·6 ± 0·07	***
	A–LV	1·7 ± 0·65	0·6 ± 0·28	*
	A–MV	4·3 ± 0·85	1·4 ± 0·34	**
LDL-TG (mg 100 ml^{-1})	A	1·3 ± 0·16	1·7 ± 0·27	**
	A–LV	0·1 ± 0·06	0·3 ± 0·23	ns
	A–MV	0·2 ± 0·05	0·5 ± 0·27	ns

Utilizations of Glucose and NEFA

Values for whole-body irreversible losses of glucose and palmitate and proportions of CO_2 derived from oxidation of glucose and palmitate are presented in Table 5. Injection of GH did not significantly affect whole-body or tissue utilization of glucose. Even so, there was a suggestion that oxidation of glucose by mammary tissue was decreased by GH. Marked effects of GH on utilization of palmitate were observed. Thus, whole-body irreversible loss of palmitate and oxidation of palmitate in the whole body and in the mammary gland increased significantly during injections of GH.

Table 4. Percentage (by wt) of individual fatty acids comprising the lipid of arterial plasma triglycerides (total-TG), arterial plasma very-low-density-plus-chylomicron triglycerides (VLDL-TG) and arterial plasma NEFA during injections of saline or growth hormone (GH)

Values are means ± s.e.m. for samples collected on day 5 of each period from the three ewes in which NEFA kinetics were measured. In no case were differences for individual fatty acids significantly affected by GH

Fatty acid	Total-TG saline	Total-TG GH	VLDL-TG saline	VLDL-TG GH	NEFA saline	NEFA GH
C14:0	1·3 ± 0·19	2·5 ± 1·30	3·0 ± 1·25	3·4 ± 1·32	1·2 ± 0·12	1·7 ± 0·37
C16:0	32·2 ± 1·74	28·3 ± 4·74	30·3 ± 4·10	32·2 ± 1·41	27·5 ± 1·41	26·7 ± 0·43
C16:1	0·6 ± 0·57	0·2 ± 0·16	4·2 ± 2·27	4·3 ± 0·15	2·5 ± 0·17	2·3 ± 0·09
C18:0	19·4 ± 9·51	21·6 ± 2·75	22·0 ± 12·51	24·6 ± 4·79	26·3 ± 0·84	29·1 ± 0·55
C18:1	39·4 ± 8·46	32·1 ± 1·40	29·8 ± 8·78	27·8 ± 1·24	31·3 ± 0·99	30·3 ± 1·20
C18:2	5·0 ± 0·81	14·3 ± 5·52	10·0 ± 7·55	7·6 ± 2·72	8·5 ± 0·47	7·5 ± 0·70
C18:3	1·9 ± 0·86	0·7 ± 0·60	0·7 ± 0·29	0·1 ± 0·07	2·3 ± 0·38	1·9 ± 0·12
Other	0·2 ± 0·01	0·3 ± 0·01	0 0	0 0	0·4 ± 0·09	0·5 ± 0·15

Table 5. Whole-body irreversible losses (WB-IL) of glucose and palmitate and proportions of CO_2 derived from oxidation of glucose and palmitate in the whole-body (WB), leg-muscle (L) and mammary (M) tissue during injections of saline or growth hormone (GH)

Values are means ± s.e.m. for measurements made on day 5 of each treatment period for three ewes given infusions of appropriate ^{14}C-labelled metabolite. Values which differ significantly are indicated: $^{*}P < 0{\cdot}1$; $^{**}P < 0{\cdot}05$; n.s., no significant difference. $LW^{0{\cdot}75}$, liveweight$^{0{\cdot}75}$

Parameter		Injected with saline	Injected with GH	Significance
WB-IL glucose (μmol min^{-1})		846 ± 146·0	872 ± 56·0	ns
(μmol min^{-1} per kg $LW^{0{\cdot}75}$)		36 ± 8·5	37 ± 0·8	ns
CO_2 from glucose (%):	WB	6·2 ± 1·07	5·9 ± 0·08	ns
	L	25·9 ± 3·51	37·3 ± 9·94	ns
	M	20·4 ± 1·60	16·8 ± 1·72	ns
WB-IL palmitate (μmol min^{-1})		136·2 ± 3·78	180·6 ± 13·70	*
(μmol min^{-1} per kg $LW^{0{\cdot}75}$)		6·3 ± 0·39	8·4 ± 1·05	*
CO_2 from palmitate (%):	WB	1·6 ± 0·13	3·4 ± 0·30	**
	L	0·7 ± 0·29	0·6 ± 0·49	ns
	M	1·3 ± 0·15	4·6 ± 1·40	*

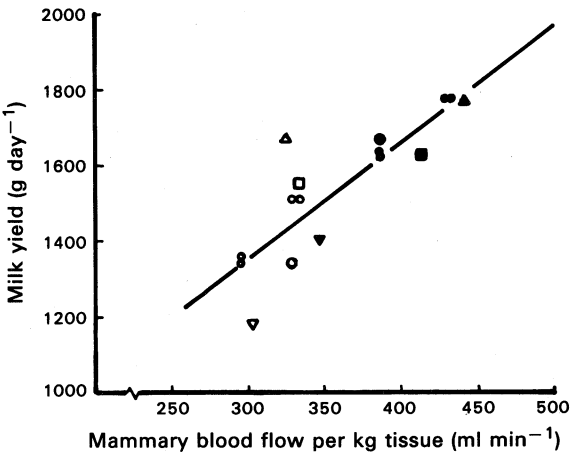

Fig. 1. The relationship between milk yield and mammary blood flow. Values for individual ewes are indicated by symbols of different shapes. Values measured during injection of saline and growth hormone are represented by open and closed symbols respectively.

Table 6. Blood flow to leg-muscle (LBF) and mammary (MBF) tissues, milk yield (MY) and MBF:MY ratio during injection of saline or growth hormone (GH)

Values are means ± s.e.m. for six ewes. Values for individual parameters which differ significantly are indicated: $^{*}P < 0{\cdot}1$; $^{**}P < 0{\cdot}05$; $^{***}P < 0{\cdot}01$; ns, not significant. Mammary tissue mass was estimated as 1·43 ± 0·072 and was derived by assuming a 1:1 relationship between milk yield and the mass of mammary tissue, no change in response to growth hormone and using the average milk yield for 5 days for each ewe

Parameter	Injected with saline	Injected with GH	Significance
LBF (ml kg^{-1} min^{-1})	130·0 ± 5·03	152·4 ± 3·82	**
MBF (ml kg^{-1} min^{-1})	300·0 ± 6·46	402·0 ± 14·14	***
(ml min^{-1})	460·4 ± 29·48	581·4 ± 48·27	***
MY (ml min^{-1})[A]	1·00 ± 0·050	1·14 ± 0·039	**
MBF:MY ratio	460·8 ± 9·28	505·0 ± 28·88	ns

[A]Derived from average milk yield for 5 days for each ewe.

Tissue Blood Flows

Blood flow to leg muscle and mammary tissues increased significantly during treatment with GH (see Table 6). Although the procedure used to measure blood flow did not provide absolute values (viz. ml min^{-1}), it is possible to derive estimates. In the case of the mammary gland, it is reasonable to assume that the ratio of milk yield : mass of mammary tissue approximates 1:1 (G.M. Hough and K.R. King, personal communication). Using this relationship, and making the further assumption that the mass of mammary tissue was not affected by GH treatment, it is possible to compute the relationship between milk yield and mammary blood flow (see Table 6). It is apparent that the ratio of blood flow : milk yield increased during GH injections. Moreover, milk yield and mammary blood flow were significantly and linearly related as shown in Fig. 1. The relationship between milk yield (Y, g day^{-1}) and mammary blood flow (F, ml kg^{-1} min^{-1}) was as follows:

$$Y = 441{\cdot}781 + 3{\cdot}044\ F\ (r = 0{\cdot}811,\ P < 0{\cdot}01)$$

Discussion

The present results are consistent with results of a limited number of previous studies which have shown that exogenous GH is galactopoietic in the lactating ewe (Dracy and Jordan 1954; Jordan and Shaffhausen 1954; Hart *et al.* 1985; McDowell *et al.* 1987*c*). Thus, milk yield and the yields of the major milk constituents were increased and milk fat content was raised during injections of GH. In these respects, the data show that the lactating ewe responds to exogenous GH in much the same manner as the lactating cow (see McDowell 1985; Johnsson and Hart 1986).

It is clear that plasma concentrations of GH were increased significantly during injections of GH, and that there was an accompanying increase in plasma insulin. The latter is of interest in relation to previous observations that GH is diabetogenic in the ruminant (Hart 1983). Although plasma insulin was increased, there was an increase in plasma glucose during injections of GH suggesting that GH exerted 'anti-insulin' or diabetogenic effects as shown recently by Leenanuruksa and McDowell (1986).

The marked changes in milk production and production of milk components were accompanied by changes in supply to, and utilization of, nutrients in muscle and, more particularly, mammary tissue. These changes in supply and utilization of nutrients by muscle and mammary tissue were broadly similar to those observed in mid-lactating cows by McDowell *et al.* (1987*a*). Although GH did not affect the A-V difference for glucose across leg muscle, as previously observed in lactating cows (McDowell *et al.* 1987*a*) and growing calves (Leenanuruksa *et al.* 1985), there was a suggestion that glucose utilization by leg muscle was altered. Thus, there was a tendency for lactate output from leg muscle to decrease in response to injection of GH. In conformity with results of others (Williams *et al.* 1963; Kronfeld 1965; Hart 1983; McDowell *et al.* 1987*a*) there were obvious lipolytic effects of GH, with arterial concentrations of NEFA increasing dramatically during GH injections. The tendency for blood 3-hydroxybutyrate to increase in response to GH injections is consistent with the previous report by Kronfeld (1965) that GH increases blood concentrations of ketone bodies in dairy cows. In spite of the marked lipolytic effect of GH, there were no changes in proportions of individual NEFA constituting the pool of NEFA in plasma.

The increased mammary A-V difference for NEFA during treatment with GH is similar to previous observations in dairy cows (McDowell *et al.* 1987*a*). It can be calculated that the additional NEFA taken up across the mammary gland exceeded the amount of NEFA required to account for extra synthesis of milk fat occurring during treatment with GH, even if all the additional fatty acids in milk fat were derived from plasma NEFA extracted by the mammary gland. In fact, the data for plasma concentrations of total- and VLDL-triglycerides (see Table 3) suggest that plasma NEFA were relatively more important precursors of milk fatty acids during injections of GH than of saline. The latter observation is of interest because VLDL normally contribute most of the pre-formed fatty acids (approximately half the total fatty acids) of milk triglycerides in the ruminant (see Annison 1983).

It seems reasonable to suggest that exogenous GH reduced the synthesis of VLDL by the liver and thereby decreased circulating concentrations of total- and VLDL-triglycerides. In this connection, there are data which indicate that, in humans, exogenous GH influences hepatic triglyceride synthesis indirectly by promoting glucagon release which, in turn, decreases synthesis of triglycerides (Blackett *et al.* 1982). This effect of GH, mediated by glucagon, apparently is the result of an effect on synthesis of apolipoprotein, and in particular the apolipoprotein of VLDL. Indeed, Blackett *et al.* (1982) found that exogenous GH exerted different effects on synthesis of the different apolipoproteins in humans. Clearly, concentrations of the individual fatty acids comprising VLDL- and total-triglycerides did not change during injections of GH (see Table 4).

Exogenous GH did not significantly affect whole-body irreversible loss or oxidation of glucose in the body or individual organs, but there was a suggestion that oxidation of glucose by mammary tissue was reduced. There were marked effects of GH on whole-body irreversible loss and oxidation of palmitate and in oxidation of palmitate in the mammary gland but not leg muscle tissue (see Table 5). These observations suggest that exogenous GH spares glucose oxidation, by increasing oxidation of palmitate and other NEFA, thereby increasing the availability of glucose for lactose synthesis and possibly for the glycerol moieties of milk triglycerides.

It is evident from Table 6 that exogenous GH increased blood flow to the mammary and leg muscle tissues. The increased blood flow to mammary tissues during administration of GH has been described previously for lactating goats (Hart *et al.* 1980; Mepham *et al.* 1984) and cows (McNamara *et al.* 1983; Davis and Collier 1985; McDowell *et al.* 1987*a*). There is only one previous report of effects of exogenous GH on blood flow to leg muscle tissue in the ruminant (McDowell *et al.* 1987*a*). The effect of GH on blood flow probably results from an increased cardiac output (Mepham *et al.* 1984) and would have resulted in supply to the tissues of additional nutrients, even in the absence of effects of GH on metabolite concentrations in blood.

The calculated ratios for mammary blood flow : milk yield presented in Table 6 are similar to values reported previously for lactating ruminants (Linzell 1974; King 1983; McDowell *et al.* 1987*a*). It is of interest that the value for this ratio was higher during injection of GH than of saline, as found recently for cows by McDowell *et al.* (1987*a*).

In conclusion, the results of the present study provide further direct evidence that exogenous GH is galactopoietic by virtue of several physiological changes induced by the hormone. First, GH affects the partition of nutrients and changes plasma concentrations of key metabolites for milk biosynthesis. Secondly, effects of GH on tissue blood flow (possibly via altered cardiac output) lead to supply of additional metabolites to mammary tissue. Finally, utilization of nutrients in the mammary gland is altered, such that glucose oxidation is spared by increased oxidation of plasma NEFA.

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