Regulation of fatty acid metabolism and gluconeogenesis by growth hormone and insulin in sheep hepatocyte cultures

Effects of lactation and pregnancy

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Primary monolayer hepatocyte cultures derived from non-mated, pregnant and lactating sheep were used to investigate the interactions between the effects of growth hormone and insulin on (i) the partitioning of fatty acid metabolism between oxidation and esterification, and (ii) the rate of gluconeogenesis. In hepatocytes from lactating sheep the rates of gluconeogenesis, ketogenesis and very-low-density lipoprotein secretion were approx. 2-fold higher than in cells from non-mated or pregnant animals. There was no apparent difference in the rates of fatty acid uptake between the three groups of sheep cells. Growth hormone stimulated gluconeogenesis only in hepatocytes from non-mated sheep. It had no effect on the flux of fatty acid towards ketone body formation. Growth hormone inhibited intracellular accumulation of acylglycerol from exogenous fatty acid. Insulin alone had no such effect, but it blunted the effect of growth hormone when the two hormones were present together. The data suggest that major differences may exist between ruminants and non-ruminants in the responses of liver metabolism both to lactation *per se* and to the effects of growth hormone and insulin.

INTRODUCTION

There is increased interest in the metabolic effects of growth hormone, particularly in ruminant species, because of the effect of the hormone to increase milk yield in lactating animals. Although a clear lactopoeitic effect of growth hormone has been repeatedly demonstrated for ruminants *in vivo* [1–7], and the importance of continued growth hormone action in the maintenance of full lactation in the rat has been established [8], we are not aware of studies in which the direct effects of growth hormone on liver preparations from ruminants have been demonstrated. Direct effects of growth hormone on the liver are expected to be important with respect to both the overall adaptation of the female animal to lactation and the pharmacological effects of the hormone when administered to lactating animals. The increased milk production that accompanies either of these conditions (i.e. via the actions of endogenous and/or exogenous growth hormone) can be presumed to be accompanied by an increased production by the liver of substrates for utilization by the mammary gland for the synthesis of the major milk constituents. It would appear desirable, therefore, to investigate whether growth hormone directly stimulates the production of glucose (which in the ruminant is largely used as a substrate for lactose synthesis in the mammary gland), very-low-density lipoprotein (VLDL)-triacylglycerols and ketone bodies. Acetoacetate and D-3-hydroxybutyrate are important substrates for milk fat synthesis *de novo* in ruminants [9–11], whereas triacylglycerols secreted by the liver as VLDL are a source of preformed fatty acids for the mammary gland.

The liver is expected to be involved in the adaptation of the lactating animal to increased triacylglycerol mobilization from adipose tissue under conditions characterized by elevated circulating growth hormone concentrations [7,12]. Under these conditions the rate of delivery of fatty acids to the liver is increased, raising the possibility of the induction of adverse effects on

hepatic metabolism, such as the occurrence of fatty liver. This condition occurs frequently in early-lactating high-yielding cows [13–18]. However it appears to be very effectively counteracted *in vivo* [16,19,20], such that it is totally reversed beyond the earliest periods of lactation. Moreover, maximally effective growth hormone treatment of lactating cows does not appear to result in either the induction of a fatty liver or ketosis. These observations raise the question as to the mechanisms whereby, in ruminants, the growth-hormone-induced increase in fatty acid delivery to the liver is managed so as to minimize the effects of what could otherwise result in conditions that would impair liver function and lactational performance in general, if extended over a prolonged period.

In this work we have used primary monolayer cultures of sheep parenchymal hepatocytes to investigate the effects of growth hormone on (i) the partitioning of fatty acid metabolism between oxidation and esterification, and (ii) the rate of gluconeogenesis from propionate the major gluconeogenic precursor in fed ruminants [21–25]. We have investigated the interactions between the effects of growth hormone and insulin and have examined these parameters for cells obtained from non-lactating, pregnant and lactating sheep. The usefulness of cultured hepatocytes in the observation of the long-term effects of growth hormone has already been successfully demonstrated with rat hepatocytes [26]. The data suggest that major differences may exist between the rat and the sheep with respect to the responses of liver metabolism both to lactation *per se* and to the effects of growth hormone and insulin.

MATERIALS AND METHODS

Materials

Sheep insulin, collagenase (type 1) and trypsin inhibitor (crude) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); sheep growth hormone (NIDDK-oGH-15) was a gift

Abbreviations used: VLDL, very-low-density lipoprotein; MEM, minimal essential medium; ASM, acid-soluble material.

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(NIDDK, Bethesda, MD, U.S.A.). [^{14}C]Palmitic acid and [^{14}C]propionic acid (sodium salt) were from Amersham International (Amersham, Bucks., U.K.) and [^3H]glycerol was from Du Pont (Stevenage, Herts., U.K.). Fatty-acid-poor BSA (fraction V) was obtained from Boehringer Mannheim (Lewes, E. Sussex, U.K.) and was dialysed [27] before use. Waymouth's medium MB721/1 was from Flow Laboratories (Rickmansworth, Herts., U.K.). Propionic acid was purchased from BDH (Poole, Dorset, U.K.). Sources of other materials were as described previously [28].

Animals

Sheep were 3–4-year-old Finn $\times$ Dorset Horn crossbred ewes, all of which were multiparous, and were fed as described previously [29]. Sheep were anaesthetized with an intravenous injection of sodium pentobarbitone before excision of the caudate lobe of the liver.

Preparation and culture of sheep parenchymal hepatocytes

Parenchymal hepatocytes were prepared essentially as previously described [30]. Briefly, the caudate lobe was immediately cleared of blood upon removal by perfusion with Ca^{2+} -free buffer containing 140 mM-NaCl, 6.7 mM-KCl, 10 mM-Hepes, 2.5 mM-glucose and 0.5 mM-EDTA, pH 7.4, during which the liver was transferred to the perfusion equipment. This was followed by a recirculating collagenase perfusion with buffer containing 30 mg of collagenase/100 ml, 2.5 mg of trypsin inhibitor/ml, 140 mM-NaCl, 6.7 mM-KCl, 30 mM-Hepes, 2.5 mM-glucose and 5 mM- CaCl_2 . Digestion was continued for various periods (30–70 min), depending on the size of the lobe, until a suitable consistency was obtained. Hepatocytes were filtered and washed as described previously [31], except that, due to the smaller size of sheep parenchymal hepatocytes compared with those of the rat (V. A. Zammit & N. Emmison, unpublished work), centrifugation was performed at 50 *g* for 2–3 min. Routinely, cell viabilities exceeded 85% as assessed by Trypan Blue exclusion, although cell yields were variable.

The hepatocytes were cultured in monolayer in collagen-coated 24-well plates (Corning Glass Works, Corning, NY, U.S.A.) at a density of $(0.8\text{--}1.0) \times 10^5$ cells/ cm^2 in minimum essential medium (MEM) containing 5% (v/v) foetal bovine serum [28]. After cell attachment (6–10 h) the hepatocyte monolayers were precultured (24 h) in serum-free Waymouth's MB721/1 medium containing 10 nM-dexamethasone and either without or with sheep insulin and/or sheep growth hormone, with one change of medium at 12 h. The use of Waymouth's MB721/1 medium at this stage was found to prolong cell viability compared with the use of other commonly used hepatocyte culture media.

Assessment of metabolic parameters

For measurement of metabolic parameters, the hepatocytes were cultured for further periods as indicated in the legends to the Tables with 0.5 ml of MEM containing 0.5 mM-L-carnitine, 1 mM-glycerol, 1 mM-palmitic acid (complexed to BSA, 12.5 mg/ml) and hormones as indicated. Rates of palmitate uptake were obtained by measurement of medium palmitate concentrations using a commercial kit (NEFA C; Wako, Tokyo, Japan). For the study of rates of palmitate oxidation to acid-soluble material (ASM), [^{14}C]palmitic acid was added (0.05 Ci/mol). Incubations (4 h) were terminated by addition of 80 μl of HClO_4 (0.33 M) to 400 μl of medium, and the radioactivity in a portion of protein-free supernatant was measured as an index of the incorporation of radioactivity into ASM [32]. Preliminary studies indicated that the rates of fatty acid utilization and of ASM production by the cells were linear for at least 6 h

under these conditions. ASM is represented primarily (approx. 95%) by ketone bodies [32]. L-Carnitine availability was not limiting for these optimal rates. For the measurement of the incorporation of glycerol into acylglycerols, [^3H]glycerol (3 Ci/mol) was added to the medium. The cells were washed once with cold NaCl (0.9%, w/v) and extracted for measurement of lipid-associated radioactivity as described in [33], with modifications [34]. Cell protein was measured by the method of Bradford [35]. Part of the medium was used for measurement of secreted [^3H]triacylglycerols as described above.

For the determination of [^{14}C]glucose production, the culture medium contained only 2 mM-[^{14}C]propionate (0.1 Ci/mol). Incubations (3 h) were terminated by aspiration of the medium, and the cells were washed (see above) and extracted with 0.5 ml of 0.1 M-NaOH for cell protein determinations. The medium was acidified (see above) and, after neutralization of the protein-free supernatant, was used for the measurement of the ^{14}C radioactivity specifically associated with glucose, as described previously [36].

RESULTS

Effect of lactation on fatty acid metabolism and gluconeogenesis

Hepatocytes isolated from lactating sheep showed several important differences from those isolated from either non-mated or pregnant animals when incubations were performed in the absence of hormones. The rate of production of ASM from 1 mM-[^{14}C]palmitate was 2-fold higher in cells from lactating animals compared with those from non-mated controls (Table 1). Pregnancy did not affect this parameter. This enhanced rate of oxidation was not accompanied by an increased rate of uptake of palmitate from the medium, although in lactating animals there was a tendency for growth hormone to increase the rate of fatty acid uptake, particularly in the presence of insulin (Table 1). Despite these observations, however, lactation resulted in a significant increase in the proportion of fatty acid metabolized to oxidation products (Table 1). Interestingly, lactation also resulted in a significant increase (2.3-fold) in the proportion of synthesized triacylglycerols that were secreted into the medium as VLDL (Table 2). There was no statistically significant change in the amount of cellular acylglycerol accumulation. Consequently it appears that lactation in sheep specifically stimulates the process of VLDL secretion.

Hepatocytes from lactating sheep also showed a 2-fold increase in the rate of incorporation of [^{14}C]propionate into [^{14}C]glucose compared with that observed in cells isolated from control non-mated animals (see Table 4). Consequently, lactation increased the production of all three important substrates for milk fat synthesis, i.e. glucose, VLDL triacylglycerol and ketone bodies.

Effects of growth hormone

Growth hormone (1–100 nM) had no effect on the rate of palmitate oxidation to ASM in any of the cell preparations tested, irrespective of the physiological state of the donor animals (Table 1). By contrast, growth hormone inhibited significantly the cellular accumulation of acylglycerol (Tables 2 and 3). This effect was most pronounced in the absence of insulin and in cells isolated from lactating animals (Tables 2 and 3). In general, growth hormone did not affect the amount of VLDL-triacylglycerol secreted by any of the cell preparations tested, such that its net effect was to increase the proportion of synthesized triacylglycerols that were secreted. In this respect the effects of growth hormone resembled those of lactation.

Growth hormone addition resulted in a significant increase in the rate of gluconeogenesis from propionate in cells isolated from non-mated control animals (Table 4). This effect was only

Table 1. Effects of insulin and growth hormone (GH) on the rates of ASM production and palmitate utilization by hepatocyte cultures derived from non-mated, pregnant and lactating sheep

Hepatocytes were precultured (24 h) with 10 nM-dexamethasone and the hormones indicated. They were then incubated (4 h) with 1 mM-[U-¹⁴C]palmitate, 1 mM-glycerol, 0.5 mM-L-carnitine and hormones as shown. Rates of ASM formation and palmitate uptake were determined as described in the Materials and methods section and are expressed as nmol of ASM/h per mg of cell protein and nmol of palmitate utilized/h per mg of cell protein respectively. Values are means $\pm$ S.E.M. for five, two and four cultures derived from non-mated (control), pregnant and lactating sheep respectively. Statistics (*t* test): **P* < 0.05, ***P* < 0.005 relative to respective controls (i.e. absence of hormone); †*P* < 0.005 for unpaired observations with non-mated control animals.

Hormone status	Rate of ASM production (nmol/h per mg)			Rate of palmitate uptake (nmol/h per mg)		
	Control	Pregnant	Lactating	Control	Pregnant	Lactating
Control	12.8 $\pm$ 0.9	12.7 $\pm$ 1.8	22.8 $\pm$ 1.3†	120 $\pm$ 5	159 $\pm$ 8	111 $\pm$ 9
1 nM-GH	12.4 $\pm$ 0.8	10.2 $\pm$ 2.5	22.1 $\pm$ 1.4†	131 $\pm$ 7	169 $\pm$ 6	106 $\pm$ 14
10 nM-GH	13.2 $\pm$ 0.9	15.1 $\pm$ 2.9	22.4 $\pm$ 1.6†	158 $\pm$ 20	170 $\pm$ 13	156 $\pm$ 15**
100 nM-GH	12.8 $\pm$ 0.9	13.5 $\pm$ 2.1	22.8 $\pm$ 1.4†	137 $\pm$ 8	147 $\pm$ 6	116 $\pm$ 9
1 nM-Insulin	13.2 $\pm$ 0.9	12.8 $\pm$ 1.4	22.7 $\pm$ 1.6†	126 $\pm$ 6	153 $\pm$ 7	117 $\pm$ 7
+ 1 nM-GH	12.0 $\pm$ 0.8	13.4 $\pm$ 1.4	21.9 $\pm$ 1.6†	137 $\pm$ 15	174 $\pm$ 12	144 $\pm$ 16*
+ 10 nM-GH	12.4 $\pm$ 0.9	13.4 $\pm$ 1.9	22.3 $\pm$ 1.7†	117 $\pm$ 9	152 $\pm$ 6	123 $\pm$ 9*
+ 100 nM-GH	11.7 $\pm$ 0.7*	13.5 $\pm$ 2.6	22.0 $\pm$ 1.5†	143 $\pm$ 15	159 $\pm$ 2	156 $\pm$ 10*
10 nM-Insulin	14.0 $\pm$ 1.1*	13.3 $\pm$ 1.5	25.1 $\pm$ 1.6*†	128 $\pm$ 9	154 $\pm$ 9	124 $\pm$ 5
+ 1 nM-GH	14.3 $\pm$ 1.1*	12.6 $\pm$ 1.6	23.7 $\pm$ 1.6†	135 $\pm$ 11	157 $\pm$ 8	115 $\pm$ 6
+ 10 nM-GH	14.4 $\pm$ 1.2	12.6 $\pm$ 1.7	23.6 $\pm$ 1.5†	153 $\pm$ 16	143 $\pm$ 5*	134 $\pm$ 12*
+ 100 nM-GH	13.0 $\pm$ 1.2	14.0 $\pm$ 2.3	23.8 $\pm$ 1.2†	123 $\pm$ 9	149 $\pm$ 6	117 $\pm$ 5
100 nM-Insulin	14.1 $\pm$ 1.1	13.2 $\pm$ 2.0	25.1 $\pm$ 2.1†	136 $\pm$ 10	149 $\pm$ 9	121 $\pm$ 5
+ 1 nM-GH	14.0 $\pm$ 1.3	12.3 $\pm$ 1.8	25.1 $\pm$ 2.4†	146 $\pm$ 18	145 $\pm$ 11	151 $\pm$ 10**
+ 10 nM-GH	13.0 $\pm$ 1.1	13.2 $\pm$ 2.1	24.5 $\pm$ 2.3†	137 $\pm$ 15	143 $\pm$ 13	150 $\pm$ 7**
+ 100 nM-GH	13.3 $\pm$ 1.2	11.3 $\pm$ 1.6	25.5 $\pm$ 2.2†	144 $\pm$ 17	135 $\pm$ 11**	151 $\pm$ 10**

Table 2. Effects of insulin and growth hormone (GH) on the metabolism of palmitate to cellular VLDL-triacylglycerol by cultured hepatocytes from non-mated, pregnant and lactating sheep

Hepatocytes were precultured (24 h) with 10 nM-dexamethasone and the hormones indicated. They were then incubated (8 h) with 1 mM-palmitate, 1 mM-[2-³H]glycerol, 0.5 mM-L-carnitine and the hormones as shown. [³H]Triacylglycerol remaining in the cells and culture medium was determined at the end of the incubation and is expressed as nmol of [2-³H]glycerol incorporated into lipid/8 h per mg of cell protein. Statistics (*t* test): **P* < 0.05, ***P* < 0.005 relative to respective controls (i.e. absence of hormone); †*P* < 0.005 for unpaired observations with non-mated control animals.

Hormone status	Cellular incorporation of [2- ³ H]glycerol into cellular acylglycerols (nmol/8 h per mg)			Incorporation of [2- ³ H]glycerol into VLDL-triacylglycerol (nmol/8 h per mg)		
	Control	Pregnant	Lactating	Control	Pregnant	Lactating
Control	117 $\pm$ 10	97 $\pm$ 4	144 $\pm$ 12	3.2 $\pm$ 0.2	4.4 $\pm$ 0.3	7.2 $\pm$ 0.9†
1 nM-GH	108 $\pm$ 8*	87 $\pm$ 4	123 $\pm$ 12**	2.7 $\pm$ 0.3	4.2 $\pm$ 0.2	5.8 $\pm$ 1.1†
10 nM-GH	102 $\pm$ 8**	82 $\pm$ 4	110 $\pm$ 10**	2.8 $\pm$ 0.2	4.9 $\pm$ 0.4	5.0 $\pm$ 0.4†
100 nM-GH	110 $\pm$ 8*	77 $\pm$ 4	109 $\pm$ 4**	3.8 $\pm$ 0.4	5.0 $\pm$ 0.4	5.6 $\pm$ 0.5†
1 nM-Insulin	111 $\pm$ 9	91 $\pm$ 5	138 $\pm$ 12	3.3 $\pm$ 0.3	3.8 $\pm$ 0.1	5.4 $\pm$ 0.5*†
+ 1 nM-GH	100 $\pm$ 7**	86 $\pm$ 6	119 $\pm$ 10**	2.4 $\pm$ 0.2*	4.1 $\pm$ 0.3	5.1 $\pm$ 0.7†
+ 10 nM-GH	109 $\pm$ 8*	87 $\pm$ 6	126 $\pm$ 11*	2.6 $\pm$ 0.3*	4.9 $\pm$ 0.6	5.8 $\pm$ 0.9*†
+ 100 nM-GH	99 $\pm$ 6**	86 $\pm$ 6	122 $\pm$ 8*	3.2 $\pm$ 0.5	5.8 $\pm$ 0.7	4.8 $\pm$ 0.5*†
10 nM-Insulin	116 $\pm$ 9	90 $\pm$ 4	139 $\pm$ 9	2.7 $\pm$ 0.2*	3.2 $\pm$ 0.3	4.6 $\pm$ 0.9*†
+ 1 nM-GH	114 $\pm$ 11	78 $\pm$ 5	116 $\pm$ 10**	2.5 $\pm$ 0.2*	3.1 $\pm$ 0.2	4.7 $\pm$ 0.6*†
+ 10 nM-GH	107 $\pm$ 9	79 $\pm$ 5	118 $\pm$ 12*	3.2 $\pm$ 0.2	3.4 $\pm$ 0.2	4.8 $\pm$ 0.4**†
+ 100 nM-GH	100 $\pm$ 9**	77 $\pm$ 5	124 $\pm$ 11*	2.4 $\pm$ 0.2**	3.9 $\pm$ 0.4	4.9 $\pm$ 0.5†
100 nM-Insulin	117 $\pm$ 11	93 $\pm$ 4	133 $\pm$ 10	2.4 $\pm$ 0.1**	3.2 $\pm$ 0.3	3.8 $\pm$ 0.3**†
+ 1 nM-GH	102 $\pm$ 9**	83 $\pm$ 5	111 $\pm$ 10**	2.3 $\pm$ 0.2**	2.9 $\pm$ 0.2	4.0 $\pm$ 0.5**†
+ 10 nM-GH	91 $\pm$ 10**	77 $\pm$ 5	109 $\pm$ 11*	2.5 $\pm$ 0.3*	2.9 $\pm$ 0.2	4.3 $\pm$ 0.4**†
+ 100 nM-GH	92 $\pm$ 7**	77 $\pm$ 5	113 $\pm$ 11*	2.6 $\pm$ 0.3*	3.5 $\pm$ 0.4	4.2 $\pm$ 0.5**†

seen in the absence of insulin. In cells from lactating sheep, however, in which the basal rate of gluconeogenesis was already relatively high (see above), there was no further stimulatory effect of growth hormone on this parameter.

Effects of insulin

Insulin, in contrast with growth hormone, inhibited VLDL-triacylglycerol secretion but did not affect intracellular acyl-

Table 3. Effects of insulin and growth hormone (GH) on the partition of fatty acid metabolism in cultured sheep hepatocytes

Hepatocytes were precultured and incubated as in Tables 1 and 2. Cell-associated [³H]triacylglycerol was determined as for Table 2, except that incubations were terminated at 4 h, when the rate of fatty acid uptake was linear with time. Statistics (*t* test): **P* < 0.05, ***P* < 0.005 relative to respective controls (i.e. absence of hormone); †*P* < 0.05, ††*P* < 0.005 for unpaired observations with non-mated control animals.

Hormone status	ASM (% of palmitate uptake)			Cellular triacylglycerol (% of palmitate uptake)		VLDL-triacylglycerol (% of total triacylglycerol)		
	Control	Pregnant	Lactating	Control	Lactating	Control	Pregnant	Lactating
Control	10.5±0.6	7.3±0.8	22.6±2.1††	48±3	57±4	2.7±0.3	4.3±0.4	4.8±0.9†
1 nM-GH	9.9±0.9	7.0±0.9	25.6±3.7††	46±5	43±2*	2.4±0.4	4.6±0.3	4.5±1.0†
10 nM-GH	10.3±1.2	7.7±0.7	23.1±0.9††	44±5	40±3**	2.7±0.3	5.6±0.5	4.3±0.5†
100 nM-GH	9.7±0.7	6.7±0.2	21.6±1.5††	45±5	50±5*	3.3±0.5*	6.0±0.5	4.9±0.6†
1 nM-Insulin	10.6±1.0	8.5±0.9	21.6±1.5††	49±5	58±5	2.9±0.4	4.0±0.3	3.8±0.6
+ 1 nM-GH	9.0±0.9	7.5±0.8	19.3±1.4††	47±4	43±4*	2.3±0.3	4.6±0.3	4.1±0.8†
+ 10 nM-GH	10.8±0.7	6.3±0.2	20.3±1.6††	50±5	45±4**	2.3±0.3	5.3±0.7	4.4±1.0†
+ 100 nM-GH	9.3±0.8	6.2±0.5	15.6±1.5††	49±4	41±2*	3.1±0.6	6.3±0.8	3.8±0.6
10 nM-Insulin	11.1±1.2	8.7±0.5	20.2±1.9††	51±4	48±3	2.3±0.3	3.4±0.4	3.2±1.0
+ 1 nM-GH	12.1±1.5	8.2±0.7	21.8±1.4††	51±4	55±5	2.1±0.3*	3.8±0.3	3.9±0.7†
+ 10 nM-GH	10.5±1.3	8.8±0.8	19.2±1.7††	49±3	47±4	2.9±0.3	3.8±0.3	3.9±0.7
+ 100 nM-GH	11.5±1.3	9.7±1.5	20.8±1.4††	49±3	47±2	2.3±0.3	4.8±0.5	3.8±0.6†
100 nM-Insulin	11.7±1.5	8.7±0.8	26.8±2.2††	53±3	52±2	2.0±0.1*	3.3±0.3	2.8±0.4**†
+ 1 nM-GH	11.3±1.4	8.2±0.6	20.4±1.4††	51±3	44±2	2.2±0.2	3.4±0.3	3.5±0.6†
+ 100 nM-GH	11.8±1.7	9.0±0.6	19.5±1.3††	50±5	36±2**	2.6±0.4	3.6±0.3	3.8±0.5
+ 100 nM-GH	11.8±1.7	8.3±0.8	20.7±1.2††	49±3	35±2**	2.7±0.4	4.3±0.5	3.6±0.6

Table 4. Effects of insulin and growth hormone (GH) on the rate of gluconeogenesis in hepatocyte cultures derived from non-mated and lactating sheep

Hepatocytes were precultured (24 h) with 10 nM-dexamethasone and hormones as indicated. They were then incubated (3 h) with 2 mM-[2-¹⁴C]propionate and the hormones shown. The rate of [¹⁴C]glucose formation was determined as described in the text and is expressed as nmol of [2-¹⁴C]propionate incorporated into [¹⁴C]glucose/h per mg of cell protein. Statistics (*t* test): **P* < 0.05 relative to respective controls (i.e. absence of hormone); †*P* < 0.005 for unpaired observations with non-mated control animals.

Hormone status	Rate of [2- ¹⁴ C]propionate incorporation into [¹⁴ C]glucose (nmol/h per mg)	
	Control	Lactating
Control	76±6	159±5†
1 nM-GH	90±6*	154±10†
10 nM-GH	94±7*	156±11†
100 nM-GH	93±7*	221±34†
1 nM-Insulin	81±7	153±10†
+ 1 nM-GH	80±7	159±19†
+ 10 nM-GH	89±6*	168±13†
+ 100 nM-GH	72±8	138±12†
10 nM-Insulin	70±4	141±14†
+ 1 nM-GH	79±4	148±20†
+ 10 nM-GH	78±5	139±17†
+ 100 nM-GH	64±2	191±9**†
100 nM-Insulin	85±4	181±10**†
+ 1 nM-GH	77±5	177±5†
+ 10 nM-GH	80±5	177±9†
+ 100 nM-GH	73±4	166±5†

glycerol accumulation (Table 3). The inhibitory effect of insulin on VLDL secretion was most marked (50%) for cells from lactating animals. Insulin was without effect on the rate of production of

ASM from palmitate in any of the preparations tested (Table 1). There was a tendency for high concentrations of insulin (100 nM) to increase the rate of gluconeogenesis from propionate. This reached statistical significance only for cells isolated from lactating animals (Table 4).

DISCUSSION

The importance of growth hormone *in vivo* in promoting the repartitioning of substrates during lactation so as to favour the synthesis of milk constituents is well established, particularly for ruminants [1–7]. These changes at the level of peripheral tissues would be expected to be accompanied by complementary changes in the liver so as to facilitate the provision of substrates to the mammary gland. In ruminants, a major role of the liver in the normal fed animal is the synthesis of glucose from gluconeogenic substrates, particularly propionate, which originates in the gut as a product of fermentation. The increased gluconeogenic rate is as expected from previous results obtained *in vivo* [37,38], and demonstrates that cultured sheep hepatocytes have the capacity to retain characteristics of the donor liver. Increased glucose production rates in lactation would enable the animal to cope with the increased demand for glucose by the mammary gland.

Other important substrates for ruminant mammary tissue are ketone bodies, which are used as substrates for fatty acid synthesis *de novo* [9–11]. It was interesting, therefore, to observe that in cultured hepatocytes isolated from lactating sheep both the rate of gluconeogenesis and the production of ASM by palmitate metabolism were markedly higher than in cells from non-mated animals. If the observation that cells from lactating sheep gave an increased utilization of exogenous fatty acid substrate for the production of oxidation products can be extrapolated to the situation *in vivo*, it would suggest that this is a specialization of ruminants. In the rat, ketone bodies are not important substrates for milk fat synthesis [39,40], and lactation is associated with decreased rates of ketogenesis in the liver [41,42]. Consequently, the increased rates of ketogenesis in the livers of lactating sheep suggest that the extensive use of ketone bodies as substrates for

milk fat synthesis is complemented by a hepatic adaptation enabling an increase in the supply of ketones from a source (i.e. hepatic) additional to the rumen. The mechanism for this increased oxidation rate requires further elucidation. It appears to result from a greater fractional utilization of fatty acids for oxidation, as it is not accompanied by increased fatty acid uptake (Table 1). It is noteworthy, however, that oxidation product formation represents a small proportion of total fatty acid utilization, such that relatively minor changes in the rates of fatty acid esterification could account for proportionately greater changes in the rates of fatty acid oxidation. Thus, although we did not observe an inverse relationship between esterification and oxidation of fatty acids, this may have been masked by experimental variation coupled with the difference in magnitude between the two parameters. The increase in absolute rates of palmitate uptake by hepatocytes from lactating sheep elicited by growth hormone (Table 1, last column) did not result in any increase in ASM production. At high insulin concentrations (100 nM) the growth hormone effect on palmitate uptake was most pronounced (Table 1) and tended to result in a higher rate of VLDL-triacylglycerol secretion.

VLDL-triacylglycerol secretion by ruminant liver is generally thought to be extremely low [43]. In the present studies we found that pre-culture of sheep hepatocytes in Waymouth's medium (which contains a richer mixture of substrates than other conventionally used culture media, e.g. 28 mM-glucose and 5 × the amino acid concentration) resulted in substantial rates of VLDL-triacylglycerol secretion. These were much higher than those reported previously for cultured goat hepatocytes [43]. In cells from lactating sheep, VLDL-triacylglycerol secretion was increased 2-fold compared with controls, suggesting that increased VLDL-triacylglycerol production may be another adaptation of the liver in lactating sheep. Such an increase would favour the delivery of preformed fatty acids to the mammary gland through the action of mammary lipoprotein lipase on VLDL.

The use of cultured cells has enabled us to monitor the long-term effects of growth hormone on hepatocyte metabolism. Growth hormone is thought to exert its effects primarily through long-term processes such as changes in DNA transcription rates [44]. The present data suggest that, when added as the sole hormone to cells from non-mated animals, growth hormone mimics some of the effects of lactation but not others. Thus it stimulated the rate of gluconeogenesis only in cells from non-mated animals. In cells from lactating sheep, in which the rate of gluconeogenesis was already high, it had no further effect. Consequently it would appear that cells from lactating animals were operating close to their maximal gluconeogenic capacity with propionate as substrate. This does not exclude an effect of growth hormone on gluconeogenesis from other substrates. However, it is possible that the higher levels of circulating growth hormone *in vivo* that are associated with lactation in ruminants [7,12] may account for the lack of a response of gluconeogenesis to growth hormone *in vitro*, since a recent study [26] demonstrated stimulation of ketogenesis by growth hormone in cells cultured from hypophysectomized rats (which *in vivo* are growth-hormone-deficient), but not in those from normal rats.

In contrast with lactation, growth hormone had no effect on the proportion of fatty acids metabolized to oxidation (acid-soluble) products (excluding CO₂ which normally in sheep hepatocyte suspensions accounts for approx. 9% of total oxidation products [32]). However, it decreased the extent of accumulation of cellular acylglycerols. These two observations may explain two aspects of growth hormone action *in vivo* that have hitherto appeared paradoxical, namely that, in spite of the increased release of fatty acids into the circulation due to the

hormone's lipolytic action, growth-hormone-treated animals do not show a tendency to develop fatty liver syndrome or ketosis. The present data suggest that growth hormone may counteract the development of intracellular lipid deposition in the liver and that, in spite of this, it does not increase the flux of fatty acid towards oxidation (ketogenesis). Similarly, growth hormone achieves a reduction in cellular acylglycerol accumulation without decreasing the rate of VLDL-triacylglycerol secretion, which, as discussed above, is required as a source of exogenous preformed fatty acids for the mammary gland.

Insulin tended to antagonize the effects of growth hormone on acylglycerol intracellular accumulation and secretion, and on gluconeogenesis. Because of the reciprocal changes in the circulating concentrations of these two hormones that occur during lactation (an increase in growth hormone and a decrease in insulin), it can be envisaged that lactation will be characterized predominantly by the effects of growth hormone on the liver. It is evident, however, that other factors must determine the metabolic profile of the liver during lactation, as illustrated by the fact that, whereas neither insulin nor growth hormone affected the rate of fatty acid oxidation, cells from lactating animals showed a marked increase in this parameter.

Comparison of the present data with previous observations obtained *in vivo*

As far as we are aware, this is the first study in which cultured sheep hepatocytes have been used to study the effects of physiological state and hormone action on carbohydrate and lipid metabolism. It is of interest, therefore, to compare our present observations with previous studies conducted *in vivo*. Our data on the effects of lactation on gluconeogenesis are as expected from the increased gluconeogenic capacity observed by direct measurement *in vivo* in sheep liver (for review see [45]). Similarly, the increased output of D-3-hydroxybutyrate by the livers of lactating cows [46] can be explained by a combination of an increased rate of delivery of fatty acids to the liver and our present observations that lactation increases the proportion of fatty acid which is metabolized to ASM (mostly ketone bodies, see above). In contrast with the conclusions drawn from the present study, however, the rate of hepatic triacylglycerol secretion *in vivo* does not appear to be altered by lactation in cattle [47]. The lack of effect of growth hormone on the rate of formation of ketone bodies in cultured hepatocytes (Table 1) explains the observation obtained *in vivo* that, in lactating cows, there is no increase in ketone body production rate after growth hormone treatment [7], or any evidence of the induction of ketosis [48]. By contrast, previous observations on the effects of growth hormone treatment on hepatic glucose production *in vivo* have been equivocal (see [49] for review). This may be related to our observation that when gluconeogenesis from propionate is already stimulated (e.g. by lactation) growth hormone does not elicit an additional effect.

In cultured hepatocytes, growth hormone inhibited the accumulation of intracellular acylglycerol. This may explain why lactating cows treated with growth hormone do not tend to develop a fatty liver in spite of increased availability of fatty acids to the liver [7]. However, the isolated finding that the hepatic triacylglycerol secretion *in vivo* is decreased in growth hormone-treated sheep [50] is difficult to rationalize on the basis of our data.

In general, we conclude that the cultured hepatocyte system is a useful model for the study of long-term effects of growth hormone and their interaction with the effects induced by different physiological states of the donor sheep.

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