

## EFFECTS OF GROWTH HORMONE (GH) ON HEPATIC ACETATE METABOLISM IN LACTATING AND NON-LACTATING SHEEP

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### ABSTRACT

The studies were undertaken to investigate effects of GH on hepatic acetate metabolism in lactating and non-lactating sheep. Five lactating and 5 non-lactating crossbred ewes were used in this experiment. Five ewes were infused continuously and simultaneously with [<sup>14</sup>C] sodium acetate (5.14 x 10<sup>4</sup> Bq/ml) at the rate of 0.3 ml/min for 4 hours (lactating ewes) and 5 hours (non-lactating ewes), via the jugular vein. Blood flows in hepatic and portal veins were measured using para-amino hippuric acid (PAH). Results showed GH did not affect hepatic metabolism of acetate in non-and lactating ewes.

Key words: Growth hormone, Acetate, Sheep

### INTRODUCTION

Growth hormone (GH) was thought to play important role in the regulation of energy metabolism in ruminants, particularly during pregnancy and lactation (Bauman *et al.*, 1982; Bines and Hart, 1982). McDowell (1985) and Johnson and Hart (1986) showed exogenous GH disturbed the concentrations of some metabolites and hormones in the circulation in both lactating and non-lactating ruminants.

In fed animals up to 75% of the acetate entering the circulation was of exogenous or gut origin, the remainder being of endogenous origin and at least 20% of that endogenous acetate was derived from the liver (Bergman and Wolff, 1971) with the major precursor being fatty acids (Palmquist, 1972). In contrast Costa *et al.* (1976) reported endogenous acetate production in lactating ewes almost 50% of acetate entry rate and net hepatic production of acetate were about 80% of the total endogenous production. This observation was supported by Pethick *et al.* (1981) who showed the liver produced about 85% of total acetate endogenous production in fasted and alloxan-diabetic sheep maintained with insulin.

Therefore, the aim of these studies was to investigate effects of GH on hepatic

acetate metabolism in lactating and non-lactating sheep.

### MATERIALS AND METHODS

#### Sheep and Rations

Experiment 1 and 2. Five lactating and 5 non-lactating multiparous crossbred ewes (Border Leicester x Merino), free of obvious abnormalities of the mammary gland, with live weight ranging from 50-60 kgs were used in these experiments. The ewes had been maintained in metabolism cages from the day of parturition when lambs were removed from their dams. All ewes were accustomed to handling and were milked by hand twice daily at 08,30 and 16,30 hours. Daily milk yields and contents of milk fat were measured.

A good quality diet {10.6 MJ/kg and 158 g crude protein per kg of dry matter} containing lucerne chaff, rolled barley grain and dairy pellets (Allied Feeds, Rhodes NSW) in the ratio of 50 : 40 : 10 was fed to meet calculated ME requirements for maintenance and milk production (MAFF, 1975). The daily ration was fed continuously by using an automatic belt feeder, to avoid post-prandial changes in metabolites and hormones. Water and salt licks were provided at all times.

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These experiments were undertaken in Dairy Research Unit at Sydney University, Camden, New South Wales (NSW) Australia.

### Experimental Procedures

**Experiment 1.** The experimental period commenced about 4 weeks after surgery. Blood and plasma constituent were measured on the last day of successive periods of 5 days when ewes were injected subcutaneously each day with 5 ml of sterile bicarbonate buffer, pH 9.5 (control period) or recombinant growth hormone (bGH) at 0.1 mg/kg liveweight. During experiment, heparinized saline was withdrawn from catheters and replaced with isotonic saline. In this way no heparin was injected in to animals 5 on the day of sampling. Five lactating ewes were infused continuously and simultaneously with [ $U-^{14}C$ ] acetate (Amersham Int.,  $5.14 \times 10^4$  Bq/ml) at the rate of 0.3 ml/min for 4 hours, via the jugular vein. Blood flows in hepatic vein (HV) and portal vein (PV) from all ewes were measured following infusion of para-amino hippuric acid (PAH, Merck West Germany) into the mesenteric vein. Pack cell volume (PCV) was measured using the microhaematocrit method.

**Experiment 2.** Five non-lactating ewes were infused continuously with ( $U-^{14}C$ ) acetate as same as in experiment 1, but for 5 hours via the jugular vein. Blood and plasma constituents were measured on the last day of successive periods of 5 days when ewes were infused continuously with sterile saline via the jugular vein (pH 9.5) at the rate 4.8 ml/h (control period) or with pituitary-derived bovine GH (extracted from bovine pituitary glands dissolved in sterile saline pH 9.5) at the same rate, circulated to be equivalent to 0.1 mg/kg liveweight/day. Blood flows were measured as described in experiment 1.

### Calculations and Statistical Analyses

The calculations used to estimate production and utilization of acetate were based on Bergman (1975), while acetate entry rate and proportion of  $CO_2$  derived from acetate was calculated by a method of Pethick *et al.* (1981).

The paired t-test (Steele and Torrie, 1980) was used to evaluate the significance of differences between mean values.

## RESULTS AND DISCUSSION

### Experiment 1

The acetate entry rate and the proportion of  $CO_2$  derived from acetate are shown in Table 1. The acetate entry rate, the proportion of  $CO_2$  derived from acetate and acetate oxidized were not significantly different ( $P > .05$ ) between saline/control and GH-treated groups. Data on acetate biokinetics were seen in Table 1 and Table 2. Although all of the parameters measured were not significantly different ( $P > .05$ ), GH-treated sheep were relatively higher compared to saline-treated group in terms of acetate entry rate (4356 vs. 4180  $\mu$  mole/min). Blood 2 concentrations in A and PV were 4.3 vs. 4.2 mM and 3.4 vs. 3.2 mM, respectively for GH and saline treated sheep. Hepatic utilization (HU) of acetate was 2345 vs. 1986  $\mu$  moles/min and blood flows (A, PV, and HV) were 1.2 vs. 0.8 L/min; 3.5 vs. 3.1 L/min; and 4.7 vs. 3.9 L/min, respectively for GH and salinetreated sheep.

Net hepatic production (NHP) of acetate accounted for 17% and 13% of acetate entry rates, 728 and 543  $\mu$  mole/min for saline and GH groups, respectively. Net portal appearance (NPA) of acetate was 2761 and 2469  $\mu$  moles/min for control/saline and GH respectively and 66% (saline) and 57% (GH) of acetate entry rates. The liver both utilized and produced acetate (Table 1).

The HU of acetate by the liver accounted for 1986  $\mu$  mole/min (saline) and 2325  $\mu$  mole/min (GH), and was 48% and 53% of acetate entry rate for control and GH, respectively. PU of acetate of 2628 and 2286  $\mu$  moles/min for saline and GH treated group respectively. GH treated ewes produced more milk and milk fat than saline treated ewes (Table 2). Blood flow appeared to be higher 13-50% in GH treated ewes, although values were not significant.

### Experiment 2.

The acetate entry rate, the proportion of  $CO_2$  derived from acetate, acetate

Table 1. Acetate production and utilization across Portal-Drained Viscera (PDV) and liver, acetate entry rate and CO<sub>2</sub> derived from acetate in lactating ewes treated with bGH (Experiment 1)

Parameter	Treatment		Change (%)	P
	Control	bGH		
Whole body				
-Acetate entry rate (u mole/min)	4180 ± 222	4356 ± 363	+ 4	NS
(u mole/min/kg LW)	76 ± 4	79 ± 7		
-CO <sub>2</sub> derived from acetate (%)	10 ± 2	8 ± 2	-23	NS
-Acetate oxidized (u mole/min)	418 ± 22	348 ± 29	-17	NS
Blood acetate concentration (u M), A	1700 ± 220	1600 ± 280	- 6	NS
PV	2600 ± 340	2200 ± 270	-15	NS
HV	2400 ± 280	2000 ± 270	-17	NS
Blood flow (ml/min) A	800 ± 180	1200 ± 200	+50	NS
PV	3100 ± 610	3500 ± 420	+13	NS
Net portal appearance (u mole/min)	2761 ± 589	2464 ± 859	-11	NS
-As prop. of ac. entry rate (%)	66	57		
Portal utilization (u mole/min)	2628 ± 640	2286 ± 402	-13	NS
-As prop. of ac. entry rate (%)	63	53		
Net hepatic production (u mole/min)	728 ± 280	543 ± 168	-25	NS
-As prop. of ac. entry rate (%)	17	13		
Hepatic utilization (u mole/min)	1968 ± 547	2325 ± 560	+17	NS
-As prop. of ac. entry rate (%)	48	53		

Experiment 1, n = 5

bGH = recombinant bovine growth hormone

P = significance level (P > .05)

prop. = proportion; ac. = acetate

oxidized and results of acetate biokinetics in the liver are given in Table 3. and Table 4. Unlike in lactating sheep, in non-lactating sheep, there was significantly different (P < .05 and P < .01) found in blood CO<sub>2</sub> concentration (Table 4). The differences of blood CO<sub>2</sub> concentration in A, PV, and HV between saline and GH treated sheep as follows: 26 vs 28.6 mM, 27.3 vs 30.4 mM, and 26.2 vs 29.7 mM, respectively. Although, there were not significantly different found in most of parameters measured, but in some parameters there were decreases due to GH administration. Acetate entry rate was lower in GH treated sheep (3511 u moles/min) compared to saline treated sheep (3737 u moles/min) as well as in blood acetate concentrations (A, PH, and HV). PU of acetate was 2497 u moles/min (saline) vs 2471 u moles/min (GH), whereas NHP of acetate was 548 u moles/min and 480 u

moles/min, for saline and GH treated groups respectively. A decrease also occurred in blood O<sub>2</sub> concentration (PV), 4.3 mM (saline) vs 4.1 mM (GH).

Likewise in lactating sheep, in non-lactating sheep there were increases for GH treated sheep compared to saline treated sheep. Net portal appearance of acetate was 1876 u moles/min (control) vs. 2068 u moles/min (GH), whereas 2068 vs. 2097 u moles/min in HU of acetate. Blood flows (A, PV, and HV) showed a same trend as well. Results of this experiment are shown in Table 3 and Table 4.

Acetate entry rate, blood concentration of acetate in A, PV, HV and oxidation of acetate in non- and lactating ewes were unaffected by bGH as seen in Table 1 and Table 3. Surprisingly, the proportion of CO<sub>2</sub> derived from acetate both in non- and lactating ewes was ranging from 8 to 11%

Table 2. Feed intake, Milk yield, Milk fat, Blood concentration of Oxygen and Carbon Dioxide in lactating ewes treated with bGH (Experiment 1)

Parameter	Treatment		Change (%)	P	
	Control	bGH			
Feed intake (g/d)	1898±160	1792± 297	- 6	NS	
Milk yield (g/d)	993± 46	1198± 54	+21	P<.01	
Milk fat (%)	5.7±0.20	7.3± 0.35	+28	P<.01	
Oxygen in blood (mM),	A	4.2±0.35	4.3 ±0.30	+ 2	NS
	PV	3.2±0.50	3.4 ±0.36	+ 6	NS
	HV	2.7±0.55	2.7 ±0.61	0	NS
Carbon dioxide in blood (mM),	A	22.9±3.78	21.6±2.79	- 6	NS
	PV	30.1±3.12	30.1± 1.79	0	NS
	HV	28.1±3.44	27.8 ± 2.22	- 1	NS

bGH = recombinant bovine growth hormone

P = significance level (P<.01, P>.05)

A = artery; PV = portal vein; HV = hepatic vein.

Experiment 1, n = 5.

which were lower compared to those obtained by King *et al.* (1985) in lactating ewes (22.5%) and Hough *et al.* (1986) in non and lactating ewes were 30 and 22%, respectively. This can be explained, may be in this study, a equilibrium state of CO<sub>2</sub> has not been reached within 4 or 5 hours of labeled acetate infusion.

Responses of milk yield, milk fat and feed intake to GH in non-and lactating ewes are plotted in Table 2 and Table 4. In this study, milk yield and milk fat were increased by 21 and 28%, respectively. These results are similar to those results obtained by Bauman *et al.* (1982), McDowell *et al.* (1983, 1987), Bitman *et al.* (1984) and Eppard *et al.* (1985), who showed increases of milk yield and milk fat were ranging from 10 to 40% in lactating cows. Another experiment (X.Y. Sun, personal communication) was using lactating ewes treated by long term GH, showed milk yield and milk fat increased by 25 and 12%, respectively. The increase of milk yield in those studies may be due to an increased Mobilization of energy stored by GH. Although statistically no effect of GH to feed intake, but decreases of feed intake were observed in these studies (Table 2 and Table 4). Surprisingly, the decrease was followed by an increase of acetate entry rate, particularly in lactating ewes, indicated GH

plays an important role in partition of nutrients in lactating ewes.

Blood acetate concentrations of A, PV, and HV in those studies were higher in saline rather than GH treated sheep both in non- and lactating sheep, suggesting that amounts of feed offered were affecting blood concentration of acetate. The decrease of blood acetate concentration in this study due to GH administration. On the other hand was increasing more concentration of free fatty acid (FFA) in A, PV, and HV in collaborative experiment by Niomsup (PhD Thesis, 1988) in lactating ewes, illustrating the lipolytic effect of GH. This extra FFA could compensate for decreases in concentration of blood acetate.

The observed tendency for increases in blood flows of A, PV, and HV were consistent with GH enhancing cardiac output as indicated by results of previous studies in which mammary blood flow showed to increase 27% in ewes (McDowell *et al.*, 1987), 18% in goats (Mephram *et al.*, 1984) and 38% in cows (McNamara *et al.*, 1983) following injections of GH. The results of blood flows in these studies in non- and lactating ewes were relatively higher compared to the previous studies. Bergman and Wolff (1971) showed arterial blood flow (0.19 L/min), hepatic blood flow (2.03

Table 3. Acetate production and utilization across Portal-Drained Viscera (PDV) and liver, acetate entry rate and CO<sub>2</sub> derived from acetate in non-lactating ewes treated with bGH (Experiment 1)

Parameter	Treatment		Change (%)	P	
	Control	bGH			
Whole body					
-Acetate entry rate (u mole/min)	3737± 930	3511± 770	- 6	NS	
(u mole/min/kg LW)	68± 17	64± 14			
-CO <sub>2</sub> derived from acetate (%)	11± 3	10 ± 4	- 9	NS	
-O <sub>2</sub> oxidized (u mole/min)	411± 102	351 ±77	-15	NS	
Blood acetate concentration (u M),A	1500±200	1200±100	-20	NS	
PV	2000± 20	1900± 10	- 5	NS	
HV	1800 ±50	1700±200	- 6	NS	
Blood flow (ml/min)	A	500 ±200	700 ±250	+40	NS
PV	2800±220	3100±200	+11	NS	
HV	3300±320	3800±400	+15	NS	
Net portal appearance (u mole/min)	1876±426	2068±512	-10	NS	
-As prop. of ac. entry rate (%)	50	59			
Portal utilization (u mole/min)	2497±337	2471±302	- 1	NS	
-As prop. of ac. entry rate (%)	67	70			
Net hepatic production (u mole/min)	548±150	480±95	-12	NS	
-As prop. of ac. entry rate (%)	15	14			
Hepatic utilization (u mole/min)	2068±420	2097±490	+ 1	NS	
-As prop. of ac. entry rate (%)	55	60			

Experiment 2, n = 5  
 bGH = recombinant bovine growth hormone  
 P = significance level (P>.05)  
 prop. = proportion; ac. = acetate; A = artery;  
 PV = portal vein; HV = hepatic vein

Table 4. Blood concentration of O<sub>2</sub>, CO<sub>2</sub> and Feed intake in non-lactating sheep treated with bGH (Experiment 2)

Parameter		Treatment		Change (%)	P
		Control	bGH		
Oxygen in blood (mM),	A	4.4 ± 0.45	4.9 ± 0.32	+11	NS
	PV	4.3 ± 0.34	4.1± 0.22	- 5	NS
	HV	3.7 ± 0.41	3.7 ± 0.36	0	NS
Carbon dioxide in blood (mM),	A	26.0 ± 1.71	28.6 ± 1.67	+10	P<.05
	PV	27.3 ± 1.7	30.4 ± 1.94	+11	P<.01
	HV	26.2 ± 2.58	29.7 ± 2.01	+13	P<.01
Feed intake (g/d)		1000 ± 80	900 ± 50	-10	NS

bGH = recombinant bovine growth hormone  
 P = significance level (P<.01, P>.05, P<.05)  
 A = artery; PV = portal vein; HV = hepatic vein.  
 Experiment 2, n = 5.hF-hF

L/min) and portal blood flow (1.84 L/min) in non-lactating sheep, whereas Costa *et al.* (1976) found hepatic blood flow (1.74 L/min) and portal blood flow (1.56 L/min). These differences may probably due to different feeding regime and diets. However, those past and present measurements of portal blood flow used PAH methods seem to be overestimate.

NPA, PU, NHP, and HV of acetate in these studies were almost equivalent as proportion of acetate entry rate between saline and GH treated sheep both in non and lactating ewes (Tables 1 and 3). These results confirmed study of Learveld *et al.* (1986) who indicated GH administration has no acute within 6 hours effects on the metabolism of acetate in sheep.

CO<sub>2</sub> in 3 blood vessels observed in non-lactating ewes was increased by GH injection (P<.05 and P<.01), but surprisingly this increase was not followed by an increase in acetate oxidized (Tables 3 and 4), suggesting GH played an important role in acetate oxidation as well.

### CONCLUSION

It was concluded in general that GH did not affect hepatic acetate metabolism in non- and lactating ewes.

### REFERENCES

- Bauman, D.E., M.J. Degeeter, C.J. Peel, G.M. Lanza, R.C. Gorewit, and R.W. Hammond. 1982. Effect of Recombinantly derived Bovine Growth Hormone (bGH) on Lactational Performance of High Yielding Dairy Cows. *J. Dairy Sci.*, 65(Suppl. 1):121.
- Bergman, E.N. and J.E. Wolff. 1971. Metabolism of Volatile Fatty Acids by Liver and Portal-derived viscera in sheep. *Am. J. Physiology*, 221:586-592.
- Bergman, E.N. 1975. *Digestion and Metabolism in the Ruminant*. (Eds. McDonald, I.W., and A.C.I. Warner). The Univ. of New England Publ. Unit, Armidale, Australia.
- Bines, J.A. and I.C. Hart. 1982. Metabolic Limits to Milk Production, Especially Roles of Growth Hormone and Insulin. *J. Dairy Sci.*, 65:1357-1389.
- Bitman, J., D.L. Wood, H.F. Tyrell, D.E. Bauman, C.J. Peel, A.C.G. Brown and P.J. Reynolds. 1984. Blood and Milk Lipid Responses Induced by Growth Hormone Administration to Lactating Cows. *J. Dairy Sci.*, 67:2873.
- Costa, N.D., G.H. McIntosh and A.M. Snosswell. 1976. *Production of Endogenous Acetate by The Liver in Lactating Ewes*.
- Eppard, P.J., D.E. Bauman, and S.N. McCutcheon. 1985. Effect of Dose of Bovine Growth Hormone on Lactation of Dairy Cows. *J. Dairy Sci.*, 68:1109-1115.
- Hough, G.M., G.H. McDowell E.F. Annison, and A.J. Williams. 1986. Acetate Metabolism in Pregnant and Lactating Ewes. *Proc. Nutr. Soc. Aust.*
- Johnson, I.D. and I.C. Hart. 1986. In: *Recent Advances in Animal Nutrition*. (Eds. W. Haresign and D.J.A. Cole). Pp.:105.
- King, K.R., J.M. Gooden, and E.F. Annison. 1985. Acetate Metabolism in the Mammary Gland of Lactating Ewe. *Aust. J. Biol. Sci.*, 38:23-31.
- Laarveld, B., D.E. Kerr and K.P. Brockman. 1986. Effects of Growth Hormone on Glucose and Acetate Metabolism in Sheep. *Comp. Biochem. Physiol.* 83A:499-502.
- MAFF. 1975. Energy Allowances and Feeding System for Ruminant. *Technical Bulletin* 33. (HMSO: London).
- McDowell, G.H., I.C. Hart, J.A. Bines, and D.B. Lindsay. 1983. Effects of Exogenous Growth Hormones in Lactating Cows. *Proc. Nuts. Soc. Aust.*, 8:165.
- McDowell, G.H. 1985. In: *Recent Advances in Animal Nutrition in Australia* (Ed. R.B. Cummings). Paper 35. Univ. of New England, Armidale.
- McDowell, G.H., I.C. Hart, J.A. Bines, D.B. Lindsay, and A.C. Kirby. 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., I.C. Hart, J.A. Bines, D.B. Lindsay and A.C. Kirby. 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. Bio. Sci.*, 40:191-202.
- McNamara, J.P., S.R. Davis, and R.J. Cotlier. 1983. Effects of Growth Hormone and Thyroxine on Plasma Lipids, Mammary lipid uptake and Milkfat in Jersey Cows. *J. Dairy Sci.*, 66:32.
- Mephram, T.B., S.E. Lawrence, A.R. Peters and I.C. Hart. 1984. Effects of Exogenous Growth Hormone on Mammary Function in Lactating Goats. *Horm. Metab. Res.*, 16:248-258.
- Palmquist, D.L. 1972. Palmitic acid as a source of endogenous acetate and B-hydroxybutyrate in fed and fasted ruminants. *J. Nutr.* 102:1401-1406.
- Pethick, D.W., D.B. Lindsay, P.J. Barker and A.J. Northrop. 1981. Acetate supply and utilization by the tissues of sheep in vivo. *Br. J. Nutr.* 46:97-110.
- Steele, R.G.D., and J.H. Torrie. 1980. *Principles and Procedures of Statistics with Special Reference to the Biological Science*. McGraw Hill Book Co. Inc. New York.