Aust. J. Biol. Sci., 1985, **38**, 23–31

Acetate Metabolism in the Mammary Gland of the Lactating Ewe

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Abstract

Acetate metabolism in the mammary gland of lactating ewes was studied by continuous infusion of radioisotopic [U-^{14}C]sodium acetate and measurement of mammary gland arteriovenous difference and blood flow. Entry rate of acetate into the whole body averaged 75 ± 7 μmol min^{-1} kg^{-1} liveweight and $22 \cdot 1 \pm 2 \cdot 7$% of total CO_2 production was derived from acetate. Acetate was both utilized and produced by the mammary gland. Acetate uptake was related linearly ($r^2 = 0 \cdot 94$) to arterial concentration and gross utilization of acetate accounted for $16 \cdot 2 \pm 2 \cdot 6$% of whole-body entry rate. Endogenous acetate production by the mammary gland increased linearly ($r^2 = 0 \cdot 90$) as milk yield rose, and accounted for $25 \cdot 6 \pm 2 \cdot 7$% of the gross mammary utilization of acetate. The proportion of mammary CO_2 derived from acetate ($22 \cdot 5 \pm 3 \cdot 9$%) was similar to that of the whole body. The uptake of acetate, 3-hydroxybutyrate, esterified fatty acids and plasma free fatty acids accounted for about 25, 13, 60 and 4% of milk fatty acid carbon respectively, after correction for the oxidation of acetate, but not of the other substrates. Metabolism of acetate in the mammary glands of lactating ewes appears quantitatively more important than that in cows, but similar to that in goats.

Introduction

Acetate and 3-hydroxybutyrate taken up by the mammary gland of the goat are utilized for the *de novo* synthesis of the short- and medium-chain fatty acids, and for about half of the palmitate which appears in milk fat triacylglycerols (Annison 1984). Ewes' milk has a higher fat content (Jenness 1974) and somewhat greater proportions of short- and medium-chain fatty acids in milk fat than in cow and goat milk fat (Christie 1978), suggesting that at equivalent milk yields, the ewe mammary gland may use relatively more acetate and 3-hydroxybutyrate.

Pethick and Lindsay (1982) showed that arterial concentrations and mammary extraction rates of acetate and 3-hydroxybutyrate in the ewe were similar to those reported for the goat and cow. Net use of acetate by the udder of the lactating ewe, in relation to whole-body utilization, was similar to that of goats, but higher than that of cows. In the absence of data on endogenous acetate production (see Annison 1984) and acetate oxidation, the actual contribution of acetate to milk fat synthesis could not be assessed, and further, the output and fatty acid composition of milk fat was not measured (Pethick and Lindsay 1982).

In this study entry rate and oxidation of acetate in the whole animal and the production, oxidation and utilization of acetate in the mammary glands of lactating ewes were measured. In addition, the contributions of acetate, 3-hydroxybutyrate, plasma free fatty acids (FFA) and esterified fatty acids to milk fat synthesis are reported. Some of these results have been presented in a preliminary form (King *et al.* 1983).

0004-9417/85/010023$02.00

Materials and Methods

Experimental Animals

Six multiparous crossbred ewes (Border Leicester × Merino), free from obvious abnormalities of the mammary glands, were housed indoors for at least 50 days before the start of the experiment. They were accustomed to handling so that blood sampling and hand milking (0830 and 1600 h daily) could be performed without restraint, and with minimum stress. Ewes were fed continuously a pelleted ration (45% lucerne hay, 40% crushed oats, 15% wheaten chaff (w/w), plus 1% (w/w) sodium chloride; metabolizable energy (ME) 8·8 MJ/kg dry matter; crude protein 179 g/kg dry matter; fatty acid content 25 g/kg dry matter) to calculated requirements for maintenance of liveweight and milk production (Anon. 1975).

At the time of the experiment, milk yield, feed intake, liveweight and stage of lactation were 1·6 (mean) ± 0·17 (s.e.m.) kg/day, 16·9 ± 0·68 MJ day/ME, 58 ± 1·9 kg and 32 ± 6·9 days, respectively.

Experimental Procedure

One week before the start of the experiment all ewes were fitted, under local anaesthesia, with an indwelling catheter (0·86 mm i.d., 1·27 mm o.d., Dural Plastics, Sydney) in a femoral artery via the saphenous artery. Two days before the experiment, catheters were inserted into a jugular vein and the medial subcutaneous mammary vein by the percutaneous Seldinger technique. Labelled [U-^{14}C]sodium acetate (Amersham International; 37 kBq/ml, 0·1 mg/ml) in 0·9% (w/v) sterile sodium chloride was infused continuously (0·3 ml/min) for 7–9 h into four ewes in early lactation and two ewes in mid-lactation. Constant specific radioactivity (SRA) of circulating acetate was achieved after about 2–3 h. Four pairs of arterial and mammary venous blood samples were withdrawn simultaneously into heparinized syringes at 30-min intervals during the final 1·5 h of infusion and immediately cooled in crushed ice. A portion of blood was deproteinized and used to determine acetate and D(−)-3-hydroxybutyrate concentrations. Care was taken to ensure that blood acetate isolated by steam distillation was stored in the sodium form. The remaining blood was centrifuged at 4°C and the plasma stored at −16°C.

The transfer of radioactivity into milk fat during the infusion of labelled acetate was monitored in the two ewes studied in mid-lactation. Ewes were weighed weekly and hand-milked following the intravenous injection of 0·4 i.u. of Syntocinon (Sandoz, N.S.W., Australia) immediately after blood samples were taken. Milk samples (3 ml) were stored at −16°C.

Blood Analysis

Blood acetate concentration and specific radioactivity

Acetate was isolated after steam distillation as the sodium salt from 3 ml of blood by the method of Annison (1954). An aliquot was removed for scintillation counting on a Philips PW 4540 liquid scintillation analyser with external standard channels ratio for quench correction and a counting efficiency of 74%. The remainder of the sample was analysed for acetate concentration on a Varian 3700 gas–liquid chromatograph using isobutyric and isovaleric acids (British Drug Houses, Poole, England) as internal standards for calculation of concentration and amount of acetate counted, respectively.

The sample was dissolved in 100 μl of formic acid (6·5 M) and 3 μl injected onto a 4 mm by 2 m glass column packed with Chromosorb 101 (Varian). Temperatures for the injector, detector and column were 200, 250 and 145°C, respectively. Nitrogen gas flow was 25 ml/min. A removable glass pre-column insert (Varian) half-filled with Ballotini beads was used to reduce column contamination. Peak areas were measured using a computing integrator (SP 4100, Spectraphysics, California, U.S.A.). With this method the coefficient of variation associated with estimates (n = 8) of acetate concentration and radioactivity for a sample of pooled plasma were 6·1 and 7·1%, respectively.

D(−)-3-Hydroxybutyrate

D(−)-3-Hydroxybutyrate concentration in blood was measured by the method of Zivin and Snarr (1973).

Plasma free fatty acids

Plasma free fatty acids (FFA) determinations in plasma were by the method of Dole (1956).

Glucose

Glucose concentration was measured in duplicate aliquots (100 μl) of the supernatant obtained by adding perchloric acid (1·8 ml, 0·33 M) to plasma (0·2 ml). Glucose oxidase, peroxidase, 2,2′-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS, Boehringer, Mannheim, W. Germany) were mixed as described in the method of Bernt and Lachenicht (1974). The absorbance was read at 675 nm.

Esterified fatty acids

Lipid was extracted from plasma by the method of Folch *et al.* (1957) after the addition of an internal standard (heptadecanoic acid; Sigma Chemical Co.). The isolated lipid was methylated with methanolic-HCl (Christie 1976) and total concentration of fatty acids determined by gas–liquid chromatography using a stainless-steel column (2 m by 4 mm) containing 15% by weight EGSSX on Chromosorb P, 100–120 mesh (Applied Science Laboratories, Pennsylvania, U.S.A.) at 170°C. Peak areas were measured by a computing integrator (SP 4100, Spectraphysics, California, U.S.A.). Because this method measured total plasma fatty acids, the concentration of esterified fatty acids (EFA) was calculated after correction for plasma FFA measured as described above.

Blood CO_2 specific radioactivity

Blood CO_2 was isolated as $BaCO_3$ (Leng and Leonard 1965) and assayed for SRA using the modification of Hinks *et al.* (1966). Distilled water (1·5 ml) and 12 ml of scintillation cocktail were added to the solubilized $BaCO_3$. External standard channels ratio was used for quench correction using a counting efficiency of 74%.

Blood gases

Blood was analysed within 30 min of collection on a 165/2 pH blood gas analyser (Corning, Massachusetts, U.S.A.). The pH and partial pressures of O_2 and CO_2 were measured and the concentration of O_2 and CO_2 calculated as described by Oddy *et al.* (1984). Haemoglobin was determined using a colorimetric test kit (No. 124729, Boehringer Mannheim, W. Germany).

Milk and Feed Analyses

Milk

Lipid was extracted from milk by the method of Folch *et al.* (1957) after the addition of an internal standard (triheptadecanoin, Nu-Chek-Prep, Inc., Minnesota, U.S.A.) and the fatty acids determined by gas–liquid chromatography by the method of Parodi (1970).

Feed

Metabolizable energy was calculated from *in vitro* digestibility (Tilley and Terry 1963) and crude protein by multiplying the Kjeldahl nitrogen (%) by 6·25. Fatty acids were measured by gas–liquid chromatography after addition of an internal standard (heptadecanoic acid, Sigma Chemical Co.), using the direct methylation technique of Outen *et al.* (1976).

Calculations

Acetate biokinetic data were calculated by the procedures (equilibrium method) outlined by Pethick *et al.* (1981). All calculations of metabolite utilization by mammary tissue involved a blood flow estimate (ml/min) derived from the ratio of blood flow to milk yield of 487 (±64) : 1. This value was the average obtained in this laboratory from 15 lactating ewes in which mammary blood flow (millilitres per minute per 100 g) was measured using tritiated water (see Oddy *et al.* 1984) and mammary gland mass was measured after slaughter and dissection. Where uptake of a plasma metabolite was determined (e.g. FFA, EFA and glucose) blood flow was corrected to plasma flow by measuring packed cell volume and assuming that the metabolite was confined to plasma.

Results

Acetate Metabolism

Whole animal

The mean entry rate of acetate, and the proportion of CO_2 derived from acetate are shown in Table 1. Entry rate and the proportion of CO_2 derived from acetate were not significantly ($P > 0·05$) related to milk yield, feed intake or arterial concentration.

Mammary gland

Data on acetate biokinetics in mammary tissue are given in Table 1. Arterial concentration of blood acetate averaged 1·56 ± 0·10 mmol/l and 60·0 ± 3·0% of the

acetate was extracted by the mammary gland. The arteriovenous (AV) difference (mmol/l) was related to arterial concentration of acetate (A_A, mmol/l) by the following equation:

$$\text{Arteriovenous difference} = -0{\cdot}59 + 0{\cdot}98 \text{ (s.e. } \pm 0{\cdot}13)A_A ;$$
$$r^2 = 0{\cdot}94, P < 0{\cdot}01 .$$

The AV difference was not related to milk yield, butterfat yield or feed intake ($P > 0{\cdot}05$).

The mammary glands both utilized and produced acetate (Table 1). Gross and net utilization of acetate by the mammary glands accounted for, on average, $16{\cdot}2 \pm 2{\cdot}6\%$ and $12{\cdot}3 \pm 2{\cdot}1\%$, respectively, of whole-animal entry rate. Although there was no significant ($P > 0{\cdot}05$) correlation between milk yield and gross or net utilization, the proportion of acetate entry rate used by the mammary gland appeared to increase as milk yield increased; the range in daily milk yield was $1{\cdot}10 \pm 0{\cdot}05$–$2{\cdot}26 \pm 0{\cdot}07$ kg, with corresponding values of $8{\cdot}3 \pm 0{\cdot}2$ and $21{\cdot}0 \pm 0{\cdot}5\%$, and $6{\cdot}4 \pm 0{\cdot}4$ and $14{\cdot}6 \pm 1{\cdot}5\%$ for gross and net utilization, respectively.

Table 1. Acetate biokinetics in lactating ewes

Parameter	Mean ± s.e.m. ($n = 6$)
Whole body	
Acetate entry rate (μmol min^{-1} kg^{-1} liveweight)	75 ± 7
CO_2 from acetate (%)	22·1 ± 2·7
Mammary gland	
Arterial concentration of acetate (mM)	1·56 ± 0·10
Acetate arteriovenous concentration difference (mM)	0·94 ± 0·10
Extraction (%)	60·0 ± 3·0
Net utilization of acetate (μg C min^{-1} kg^{-1} liveweight)	214 ± 29
As proportion of entry rate (%)	12·3 ± 2·1
Gross utilization of acetate (μg C min^{-1} kg^{-1} liveweight)	279 ± 36
As proportion of entry rate (%)	16·2 ± 2·6
Endogenous production of acetate (μg C min^{-1} kg^{-1} liveweight)	66 ± 11
As proportion of gross utilization	25·6 ± 2·7
As proportion of entry rate	3·9 ± 0·6
CO_2 from acetate (%)	22·5 ± 3·9
Acetate oxidized (%)	23·8 ± 5·8
Arterial concentration of CO_2 (mM)	22·6 ± 0·7
As production (μmol min^{-1} kg^{-1} liveweight)	23 ± 2·2
Arterial concentration of O_2 (mM)	6·0 ± 0·1
As uptake (μmol min^{-1} kg^{-1} liveweight)	15·0 ± 2·1
Respiratory quotient	1·5 ± 0·1

Endogenous acetate production by the mammary glands comprised a constant proportion ($25{\cdot}6 \pm 2{\cdot}7\%$) of the gross mammary utilization of acetate and accounted for, on average, 3·9% of whole-animal entry rate. The proportion of entry rate accounted for by endogenous production (%) in the mammary gland increased as milk yield, (Y, kg/day) increased, and the relationship was described by the following equation:

$$\text{Endogenous production} = -3{\cdot}17 + 4{\cdot}37 \text{ (s.e. } \pm 0{\cdot}73)\ Y ;$$
$$r^2 = 0{\cdot}90, P < 0{\cdot}01 .$$

Oxidation accounted for 23·8±5·8% of the acetate used by the udder and the percentage of CO_2 derived from the oxidation of acetate within the mammary gland was 22·5±3·9%, a value similar to that for the whole animal.

Mammary Metabolism of other Blood Metabolites

Arterial concentration and mammary AV differences for blood 3-hydroxybutyrate, plasma FFA, esterified fatty acids and glucose are given in Table 2. Mean extraction rates for 3-hydroxybutyrate, plasma FFA, esterified fatty acids and glucose were 42·1±2·9, 11·7±3·4, 12·4±1·4 and 30·1±3·0%, respectively.

Table 2. Arterial concentration (A; mM), mammary arteriovenous difference (AV; mM), extraction rate[A] (E; %) and uptake (U, μg C min^{-1} kg^{-1} liveweight) of circulating 3-hydroxybutyrate, plasma esterified fatty acids [expressed as palmitic and palmitoleic acids (P), stearic acids (S) and oleic acid (O)], plasma FFA and glucose in lactating ewes

Values are means ± s.e.m. for six ewes

Metabolite		A	AV	E	U
3-Hydroxybutyrate		0·62±0·06	0·26±0·03	42·1±2·9	117±16
Plasma esterified fatty acids	P	0·76±0·06	0·081±0·005	10·6±0·7	95±13
	S	0·64±0·04	0·054±0·007	8·3±2·8	81·9±15
	O	0·63±0·04	0·053±0·008	8·4±1·9	80·3±21
Plasma FFA		0·23±0·01	0·026±0·007	11·7±3·4	35±9
Plasma glucose		4·01±0·08	1·204±0·111	30·1±3·0	576±55

[A] Extraction rate = (AV/A) × 100.

Esterified fatty acids of chain length C_{16}–C_{18} which comprised 73% of the total fatty acids in arterial plasma were the major fraction contributing to the mammary uptake of long-chain fatty acids. The fatty acid composition of milk fat and production of milk fatty acids are shown in Table 3. Those fatty acids synthesized *de novo* (i.e. short- and medium-chain fatty acids, plus half of palmitic—assuming that lipogenesis from acetate is similar in goat and ewe mammary tissue) comprised about half of the total.

Table 3. Fatty acid composition (wt %) and milk fatty acid production (μg C min^{-1} kg^{-1}) in six ewes

Values are means ± s.e.m.

Fatty acid chain length	Composition	Production
4–15	34·6±1·6	290±20
16	25·5±0·6	216±26
18	38·4±1·3	342±17
>18	1·5±0·1	14±2
Total		862±93

Production of Milk Fat

After correction for the amount of acetate oxidized, but without correcting for the oxidation of the other substrates, all of the milk fat produced was accounted for by

the uptake of acetate, 3-hydroxybutyrate, esterified fatty acids and plasma FFA: the contributions of each precursor to milk fat were 25·4, 13·2, 59·9 and 4·0%, respectively (Table 4).

Table 4. Carbon balance (%) for the production of milk fat in fed lactating ruminants

Collated data for goat and cow from Linzell (1974). Data for sheep from present study

Substrate	Goats	Cows	Sheep
Acetate	16·1	25·0	25
3-Hydroxybutyrate	14·4[A]	11·8[B]	13
Plasma FFA	—	—	4
Triacylglycerol	61·8	49·6	60
Total	92·3	86·4	102

[A] Corrected for oxidation (Annison and Linzell 1964; Annison 1970).
[B] Data from Palmquist *et al.* (1969).

The uptake of acetate and 3-hydroxybutyrate, however, accounted for only 81% of the milk fatty acids synthesized *de novo* (Table 5), whereas the remaining milk fatty acids could be accounted for by uptake of their respective long-chain fatty acids from esterified fatty acids and plasma FFA.

Table 5. Contribution of acetate and 3-hydroxybutyrate to milk fatty acids synthesized *de novo*

Animal	Milk fatty acids synthesized *de novo* (%)	Proportion of fatty acids accounted for by acetate and 3-hydroxybutyrate
Goat	43·7[A]	0·70[B]
Cow	36·5[A]	1·01[B]
Sheep (this study)	47·4[C]	0·81

[A] Data from Christie (1978).
[B] Data from Table 4.
[C] Assumes 50% of palmitic acid synthesized *de novo*.

Discussion

Entry rates of acetate in lactating ewes were similar to those reported for sheep (Oddy 1978; Pethick and Lindsay 1982), and for lactating goats (Annison *et al.* 1967) and cows (Annison *et al.* 1974). Previous workers have established relationships between entry rate and both intake (fed or fasted) and arterial concentration of blood acetate (Annison *et al.* 1967; Pethick *et al.* 1981; Pethick and Lindsay 1982). These relationships could not be examined in the present study because the sheep were fed to calculated requirements and intakes and arterial levels varied only over a limited range.

The relationship found between arterial concentration and AV difference across the mammary gland is in agreement with earlier results (McClymont 1951; Linzell 1960), and consistent with the concept that circulating concentration is a major determinant of acetate utilization by peripheral tissues (see Bell 1980).

Although the mammary gland extracted a large proportion of arterial acetate, total utilization of acetate by the udder, expressed as a percentage of entry rate and for a mean milk yield of 1·62 litres per day, was only 16%. This value is similar to the value for net utilization of 17% reported by Pethick and Lindsay (1982) for ewes producing 1·57 litres of milk per day. Values for net utilization in goats and cows are 20 ± 6 and 10 ± 4%, respectively (Bickerstaffe *et al.* 1974), suggesting that utilization of acetate by the udder is similar for goats and sheep, and greater than that for the cow. Increased mammary utilization of acetate by the goat and sheep, relative to most cows, would be consistent with higher milk fat production (Jenness 1974) and increased proportions of milk fatty acids synthesized *de novo* in these species (Christie 1978).

Data collated by Linzell (1974) for arterial concentration and mammary extraction and oxidation of the blood precursors of milk fat have been used to calculate a carbon balance for goats and cows (Table 4). The percentage of milk fatty acids synthesized *de novo* varies between species, and it would appear that only in the cow is the contribution of acetate and 3-hydroxybutyrate sufficient to account for the synthesis of the short- and medium-chain fatty acids (Table 5). Annison and Linzell (1964) showed that in goats, the uptake of acetate, after subtraction of the amount oxidized, accounted for 17–41% of the fatty acids in milk, but three of the four animals had values between 17–29%. These data, and those obtained in the present study suggest that in the sheep and goat, there may be a shortfall of carbon for *de novo* synthesis of milk fatty acids. A possible additional carbon source is lactate, which has been shown to be incorporated into lipids of ruminant adipose tissue (Prior 1978) and into milk fat by bovine (Rodriguez *et al.* 1983) and caprine mammary tissue (Baldner *et al.* 1982).

When expressed as a percentage of entry rate, total utilization and endogenous production of acetate by the mammary gland increased as milk yield increased. There was no change in the extraction rate of arterial acetate (mean ± s.e.m., 60 ± 3%), or of the percentage of total utilization accounted for by endogenous production ($25 \cdot 6 \pm 2 \cdot 7$%).

The simultaneous uptake and release of acetate by many tissues is a puzzling phenomenon. The relationship between milk yield and endogenous acetate production shown here is the first reported correlation between level of metabolic activity and acetate production. This possibility was alluded to by Costa *et al.* (1976). We have no explanation for the release of acetate by tissues beyond suggesting that the process allows acetate in excess of immediate requirements to be made available to other tissues.

Acknowledgments

The skilled technical assistance of Miss S. Baker is gratefully acknowledged. Financial support was provided by the Australian Dairying Research Committee (A.D.R.C.). One of us (K.R.K.) was an A.D.R.C. Post-graduate Scholar.

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Manuscript received 13 August 1984, accepted 19 December 1984