

## Mammary Glucose Uptake in the Lactating Ewe and the Use of Methionine Arterio-Venous Difference for the Calculation of Mammary Blood Flow

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### Abstract

(1) The validity of using the arterio-venous concentration difference of methionine to calculate mammary blood flow in the ewe, on the basis of the Fick principle, is discussed.

(2) Calculation of mammary blood flow in the lactating Merino ewe indicated that blood flow per unit weight of tissue and the ratio of blood flow : milk yield were approximately twice that found in the lactating cow and goat.

(3) Calculated mammary blood flow in Merino ewes was used in conjunction with glucose arterio-venous difference to determine mammary glucose uptake. Glucose uptake per unit weight of tissue in the ewe was almost double that found in the cow and goat. The ratio of mammary glucose uptake to lactose output was also higher in the ewe than that found in the cow and goat. The utilization of glucose by the mammary gland of the ewe is discussed in relation to the possible greater requirement for NADPH and glycerol for milk fat synthesis in this species.

### Introduction

A number of methods have been developed for the determination of mammary blood flow in ruminants, namely continuous thermodilution, electromagnetic flowmeter, antipyrine absorption and nitrous oxide diffusion. Of these the continuous thermodilution method has been shown to be the most accurate (Reynolds *et al.* 1968). However, the complexity and cost of the equipment used and the surgical preparation and training of animals required for the thermodilution method are not attractive to the investigator wishing to make only occasional determinations of mammary blood flow.

Linzell (1974) suggested that the arterio-venous (AV) concentration difference of certain essential amino acids could be used to calculate mammary blood flow on the basis of the Fick principle. Previously the AV difference of glucose (Linzell 1934) and calcium (Linzell 1960) has been used to estimate mammary blood flow on this basis. However, the mammary extraction (AV difference as a percentage of arterial concentration) of calcium is extremely low (*c.* 2.8%) making the error of AV difference determination correspondingly high. On the other hand, glucose, while having a higher extraction (25-30%), is not transferred entirely to lactose from blood plasma, but also enters into oxidative pathways in the mammary cells and contributes carbon for glycerol and amino acid synthesis (see Mephram 1971; Linzell 1974).

The present paper discusses the validity of using methionine AV difference to estimate mammary blood flow in the lactating ewe and the application of such

data to the measurement of mammary glucose uptake. Mammary blood flow has not previously been measured during full lactation in sheep although flow rates determined with the use of an electromagnetic flowmeter immediately *post partum* have been reported (Burd *et al.* 1976).

## Methods

### *Analytical Methods and Experimental Procedures*

Plasma glucose was assayed by a glucose oxidase method (Beckman Glucose Analyser, Beckman Instruments, California, U.S.A.). All other methods and procedures were as described by Davis *et al.* (1978).

### *Calculation of Mammary Blood Flow*

On the basis of the Fick principle mammary blood flow may be calculated from the following equation:

$$F = \frac{CPM}{(A-V)} \times \frac{10}{(100-H_{CT})},$$

where  $F$  is mammary blood flow (litres per hour),  $C$  is the methionine content of milk protein (g/100 g protein),  $P$  is the milk content (g/100 g milk) of mammary synthesized proteins [86% of total milk protein (Kataoka and Nakae 1972)],  $M$  is milk yield (ml/h),  $H_{CT}$  is haematocrit (%), and  $A-V$  is the arterio-venous concentration difference of methionine ( $\mu\text{g/ml}$  plasma).

### *Validity of Calculation*

The use of amino acid AV difference in conjunction with the Fick principle for the accurate estimation of mammary blood flow necessitates quantitative definition of the relationship between AV difference and the rate of secretion of the amino acid in question. The extent of utilization of many amino acids for milk protein synthesis is difficult to assess because of their synthesis (non-essential amino acids) or catabolism (certain essential and non-essential amino acids) in the mammary gland (see Mephram 1971, 1976).

However, in ruminants it would appear that five of the essential amino acids (methionine, phenylalanine, tyrosine, histidine and threonine) are transferred from plasma to milk without undergoing appreciable catabolism (Kellaway *et al.* 1974; Clark 1975; Mephram 1976; Davis *et al.* 1978) and are thus suitable, collectively or individually, for use in the calculation of mammary blood flow.

The accuracy of determination of AV difference increases with increasing mammary extraction. Of the group cited above, methionine usually has the highest extraction in ruminants, consistently so in the ewe [ $> 70\%$  (Davis *et al.* 1978)]. Perfusion of the isolated udder of sheep with [ $^{35}\text{S}$ ]methionine in the perfusate has substantiated the view that the methionine is transferred from blood plasma to milk without undergoing appreciable catabolism (Verbeke *et al.* 1967). Thus methionine was chosen for the routine calculation of mammary blood flow.

Blood samples must be taken under 'steady-state' conditions for the accurate determination of AV differences (see Zierler 1961; Mephram and Linzell 1974). By placing the lamb in close proximity to its mother and by using teat catheters for milk removal, disturbance of the ewe can be kept to a minimum (Davis *et al.* 1978). Inaccuracies may also arise from failure to ensure that a sample of pure mammary venous blood is obtained. Procedures for sampling mammary venous blood in the ewe and the nature of mammary venous drainage in ruminants have been discussed elsewhere (Linzell 1974; Davis *et al.* 1978).

Determination of methionine output in milk includes the secretion of methionine in proteins which are derived directly from blood plasma. Ovine milk protein contains up to 14% serum albumin and immunoglobulins (Kataoka and Nakae 1972) neither of which are synthesized in the mammary gland (see Mephram 1971). As the amino acid composition of these proteins is similar to that of mammary synthesized milk protein (Kataoka and Nakae 1972) correction for errors arising from this source may be made on the basis of total protein content.

Table 1. Mammary blood flow and glucose uptake in lactating Merino ewes

Expt. No. <sup>a</sup>	Methionine AV diff. (µg/ml)	Milk protein content (g/100 g)	Mean haemato-crit (%)	Calc. mammary blood flow (l/min) <sup>b</sup>	Blood flow Milk yield <sup>c</sup>	Plasma glucose concn (mg/100 ml)	Glucose extraction (%)	Glucose uptake (g/h)	Glucose uptake Lactose output <sup>d</sup>
1 (6)	1.28	3.93	25	0.91	898 (10)	57.6	21	5.2	1.65
2 (8)	1.62	4.70	31	1.0	750 (8)	74.1	35	11.0	2.75
3 (7)	1.45	3.96	31	0.91	755 (15)	54.9	28	5.8	1.71
4 (7)	1.21	4.10	28	1.43	998 (32)	56.6	23	8.2	1.90
5 (3)	1.08	4.08	23	0.88	980 (42)	71.3	22	7.2	2.74
6 (3)	1.22	3.80	26	1.23	948 (45)	50.4	26	7.2	1.63
7 (3)	1.88	4.80	28	1.05	722 (30)	80.4	26	9.7	2.15
8 (4)	0.91	5.24	25	0.77	1527 (65)	58.9	19	4.0	2.78
9 (3)	1.27	4.24	23	0.53	908 (70)	68.0	16	2.8	1.67
10 (4)	1.24	4.06	25	0.66	694 (66)	60.3	19	3.4	1.53
11 (3)	1.05	5.87	23	0.86	1053 (71)	67.1	8	2.8	1.70

<sup>a</sup> Values in parentheses are the number of AV sample pairs.

<sup>b</sup> Gland weights in experiments 1, 3, 4 and 5 were 868, 925, 1094 and 940 g respectively.

<sup>c</sup> Values in parenthesis are the number of days *post partum* when each experiment was performed.

<sup>d</sup> Experiments 7-11 calculated assuming a lactose content in Merino ewe milk of 5.1 g/100 g milk (Davis *et al.* 1978).

Losses of methionine from the mammary gland via the lymph are negligible (Linzell 1974) as are losses of methionine as free residues in milk. The free methionine concentration in ovine milk is less than 10% of the plasma concentration (Davis, unpublished data).

## Results

### *Mammary Blood Flow*

Mammary blood flow calculated on the basis of at least three measurements of methionine AV difference in five animals on 11 occasions was found to vary between 0.53 and 1.43 litres/min (Table 1). On four occasions when mammary weight was estimated from measurements of mammary volume by a water displacement method (Linzell 1966) it was calculated that mammary blood flow was 105, 98, 94 and 131 ml per 100 g per minute. In the goat and cow at peak lactation mammary blood flow is 50–60 ml per 100 g per minute (Linzell 1974).

The AV difference of methionine did not vary significantly with milk protein yield indicating that, as in the cow and goat, mammary blood flow and milk yield are highly correlated.

Table 2. Comparison of mammary glucose uptake and blood flow in sheep, cows and goats

Parameter	Sheep	Cow <sup>A</sup>	Cow <sup>B</sup>	Goat <sup>C</sup>
Mean arterial plasma glucose concentration (mg/100 ml)	63.6	52.0	64.4	60.0
Mean glucose extraction (%)	22	28	21	33
Mammary uptake of glucose (mg g <sup>-1</sup> h <sup>-1</sup> )	6.9	3.6	3.4	4.3
Ratio of glucose uptake to lactose output	2.0	1.4	0.9	1.8
Mammary blood flow [ml (100 g) <sup>-1</sup> min <sup>-1</sup> ]	107	42	55	44
Milk yield (ml g <sup>-1</sup> day <sup>-1</sup> )	1.84 <sup>D</sup>	1.90	1.70	1.75
Ratio of blood flow to milk yield	870	457	390	460

<sup>A</sup> Calculated from Table 2, Bickerstaffe *et al.* (1974).

<sup>B</sup> Calculated from the data for cows 8–13, Table 4, Paterson and Linzell (1974).

<sup>C</sup> From Linzell (1974).

<sup>D</sup> From Davis *et al.* (1978).

The ratio of blood flow to milk yield was in the range 694–1053 : 1 (mean 870), with one exception. In this animal where the blood flow : milk yield ratio (1527 : 1) was considerably greater than the mean, milk production from the right udder half was negligible so that a higher blood flow : milk yield ratio might be expected.

In the lactating cow and goat the blood flow : milk yield ratio is approximately 500 : 1 (Bickerstaffe *et al.* 1974; Linzell 1974), although this ratio increases as yield falls with advancing lactation. In the ewe there was no correlation between stage of lactation and the blood flow : milk yield ratio, or with mammary blood flow alone.

### *Mammary Glucose Uptake*

Data pertaining to the mammary glucose requirements of the lactating ewe are shown in Table 1, and are compared with data for the cow and goat in Table 2. Arterial plasma glucose concentrations were similar to those found previously in the lactating cow and goat and declined throughout each experiment. Glucose AV difference was relatively constant during the experimental period (Fig. 1). Mammary glucose extraction was similar in the sheep and cow but is higher in the

goat (Table 2). However, glucose uptake per unit weight of tissue by the sheep udder was approximately double that found in the cow and goat whilst the ratio of glucose uptake to glucose output in lactose was greater in the sheep than in the other species.

No correlation was apparent between glucose arterial concentration and AV difference although mammary glucose uptake tended to be lower in those experiments carried out on ewes in late lactation (experiments 8–11, Table 1). The ratio of mammary glucose uptake to lactose output was not significantly affected by yield or stage of lactation.

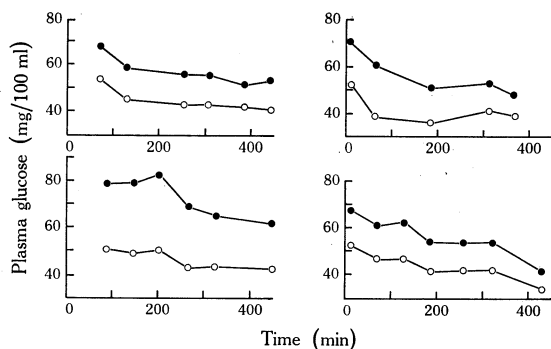


Fig. 1. Hour to hour variation in arterial (●) and mammary venous (○) plasma glucose concentrations in four lactating Merino ewes.

## Discussion

The use of methionine AV difference measurement to calculate mammary blood flow according to the Fick principle indicates that mammary blood flow per unit weight of tissue in the ewe is approximately double that of the lactating cow and goat in which flow has been measured by the thermodilution method (see Bickerstaffe *et al.* 1974; Linzell 1974).

Comparison of calculated flow with direct measurement was not possible in these experiments with the ewe. Mammary blood flow in ewes has been measured, immediately *post partum*, by the use of an electromagnetic flowmeter (Burd *et al.* 1976) and values in the range of 300–400 ml/min for the half-udder were reported. These are of a similar magnitude to the values calculated here (Table 1).

A direct comparison may be made between the thermodilution method and methionine AV difference calculation in studies made on the cow (Bickerstaffe *et al.* 1974) and goat (Mephram and Linzell 1966). In the goat, mammary methionine uptake (AV difference  $\times$  mammary blood flow by thermodilution) balanced well with methionine output in milk. However, in the study on dairy cows there was a deficit in the mammary uptake of most essential amino acids as compared with their output in milk protein, although the pattern of uptake was similar to that found in the sheep (Davis *et al.* 1978) and goat (see Mephram 1976). This deficit was particularly marked in the case of methionine and in four experiments mammary uptake ranged from 55 to 93% of output. Deficits of this magnitude would seem too great to explain solely on the basis of analytical error, particularly as essential amino acids other than methionine, e.g. phenylalanine, showed similar discrepancies.

These data suggest that the thermodilution method occasionally underestimates mammary blood flow in dairy cows. In the study of Bickerstaffe *et al.* (1974) comparison of the thermodilution method with a Fick principle method based on

mammary urea clearance showed that, on average (in both cows and goats), the urea method gave values almost 10% greater than the thermodilution method. This finding indicates that this proportion of blood leaves the udder via minor veins, undetected by the thermodilution technique. That this error may be greater in certain instances is also suggested from the data of Paterson and Linzell (1974) where mammary glucose uptake was determined in dairy cows in conjunction with the thermodilution method. These data indicate that, on average, mammary glucose uptake is only 90% of the output of lactose in milk (see Table 2). Ruminant mammary tissue has a substantial requirement for glucose for purposes other than lactose synthesis (see Davis and Bauman 1974) and its rate of gluconeogenesis is low (Scott, *et al.* 1976). The data of Bickerstaffe *et al.* (1974) suggest a more realistic ratio of glucose uptake to lactose output of 1.35 (average of six cows) although some individual results gave a ratio only marginally greater than unity.

While calculation of mammary blood flow in the ewe from methionine AV difference requires verification by direct measurement, the results calculated here suggest that the efficiency of milk synthesis (in terms of blood volume required per unit volume of milk synthesized) is less in the ewe than that found in the goat and cow. The cow and goat might be expected to be more efficient than the ewe in the terms defined above because of both genetic improvement and a higher plane of nutrition. The total solids content of ewe's milk is substantially higher than that of the cow and goat, mainly due to a higher fat content [7.5 g/100 g milk (Corbett 1968)]. Thus the substrate requirement for each unit volume of milk synthesized is increased in the ewe and must be met, at least in the case of protein, from relatively low substrate levels in plasma. For example, it has been proposed that methionine is the amino acid first-limiting for milk protein synthesis in the ewe (Davis *et al.* 1978) and the mean plasma concentration in the lactating ewe (1.70 µg/ml) is considerably less than that found in studies on the cow [mean 3.50 µg/ml (Bickerstaffe *et al.* 1974)] and goat [mean 2.70 µg/ml (Mephram and Linzell 1966)].

Mammary glucose uptake per unit weight of tissue is greater in the ewe than in either the cow or goat as is the ratio of glucose uptake to lactose output (Table 2). The increased mammary production of fat in the ewe might be expected to increase mammary glucose utilization for production of NADPH and glycerol (see Bauman and Davis 1974). The deficit of non-essential amino acid uptake relative to output in milk protein (Davis *et al.* 1978) might also be expected to increase the mammary glucose requirement of ovine mammary tissue.

While the methionine AV difference method would appear to be suitable for estimating mammary blood flow in ruminants, this would not appear to be the case for the pig. Mammary blood flow in the lactating sow is similar, per unit weight of tissue, to that found in the cow and goat (Linzell *et al.* 1969). However, calculation of the balance between uptake and output of essential amino acids across the udder of the sow (Davis 1974) indicates that methionine is taken up in excess of the requirement for milk synthesis in both reported studies (Linzell *et al.* 1969; Spincer *et al.* 1969).

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