The utilisation of whole body cysteine by the mammary gland of the lactating goat

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ABSTRACT

Three lactating Saanen goats weighing 55-71 kg, producing 1.2-3.6 kg milk/day and fed *ad libitum* concentrates and hay, were infused *via* a jugular catheter with ^{35}S-cysteine (^{35}S-Cys) at 9.3μCi/min for 9 hours. Blood samples were taken hourly and mammary tissue biopsied terminally. Concentrations and specific radioactivities (SRA) of cysteine (Cys) and glutathione (GSH) were determined in whole blood, plasma and mammary tissue. Mean arterial concentrations (nmoles/g±SE) of Cys and GSH in whole blood, plasma and red blood cells were: 71±7, 79±9 and 19±8; and 823±30, 1.6±0.1 and 3426±124 respectively. Arterial SRA in whole blood at plateau (7-9h) of ^{35}S-Cys (352±13 dpm/nmole) was higher than that for GSH (5.9±0.7 dpm/nmole). However the difference between intracellular SRA for Cys and GSH in mammary gland tissue was much less (231±23 vs 146±13 dpm/nmole). Total uptake of Cys by one half of the mammary gland was 0.5±0.2 g/day. Whole body irreversible loss rate of Cys was 7.3±0.7 g/day, giving a percentage utilisation of whole body Cys by the mammary gland of 13.6±4.3.

Keywords: cysteine; glutathione; cysteine utilisation; mammary gland; lactation; goat.

INTRODUCTION

Within the dairy industry considerable emphasis is placed on milk protein production. However, realising any potential for increased protein output by the mammary gland is hindered by limited information on factors controlling milk protein synthesis and degradation. Availability of certain amino acids for synthesis of milk proteins may be one of the key rate-limiting factors in the process of milk protein production (Baumrucker, 1985; Davis and Collier, 1985). Uptake of sulphur amino acids cysteine (Cys) and methionine by the mammary gland is of interest because they appear to be provided in insufficient quantities relative to their output in milk proteins (Baumrucker, 1985). *In vivo* studies have shown that uptake as a proportion of plasma flux by the mammary gland of methionine is one of the highest of any amino acid (55-93%; Bickerstaffe *et al.*, 1974 ; Davis *et al.*, 1978; Baumrucker, 1985) but is insufficient to account for methionine output in milk. However uptake of Cys from plasma by the gland is very low, and often negative (Derrig *et al.*, 1974 ; Spires *et al.*, 1975). Such uptake studies rely on the assumption that red blood cells (rbc) do not contribute sulphur amino acids. However, Hanigan *et al.*, (1991) found significant differences between whole blood and plasma arteriovenous differences of Cys (0.4 vs - 0.4 nmoles/ml) and methionine (7.4 vs 5.9 nmoles/ml) across the mammary gland respectively. The large uptake of methionine may be partially used to supply Cys *via* the transulphuration pathway (Radcliffe and Egan, 1974) while other sources of Cys such as the tripeptide GSH may also contribute (Pocius *et al.*, 1981; Baumrucker, 1985; Vina *et al.*, 1989). The purpose of this study was to a) determine the pool size of Cys and GSH in whole blood, plasma and mammary tissue; and b) to describe the utilisation of Cys and GSH by the mammary gland of the lactating goat in relation to whole body utilisation.

METHODS

Animals and Experimental Design

Three multiparous Saanen goats in late-lactation were housed in metabolism crates and fed concentrates, chaffed lucerne hay and meadow hay *ad libitum*. Fresh water was freely available. Five weeks previously the goats were surgically modified by ligating blood vessels between udder halves, and a transit-time ultrasonic flow probe (Transonics Inc, Ithaca, NY) had been positioned around the pudic artery. Indwelling bilateral jugular vein, caudal superficial epigastric vein (milk vein) and saphenous artery catheters were also inserted under general anaesthesia 4-5 days before the experiment. Labelled ^{35}S-Cys (NEN Research Products, Du Pont, Wilmington, USA) and cold carrier (40 μM cysteine) in sterile physiological saline was infused at 0.36 g/min (9.3 μCi/min) for 9 hours into one of the bilateral jugular catheters. Blood was sampled hourly from the saphenous artery and the milk vein simultaneously by a peristaltic pump. Milk was collected by handmilking aided by intravenous administration of Oxytocin (2 x 100 mIU Oxytocin-EA, Ethical Agents Ltd., NZ). Mammary gland tissue was sampled after the 9th hour from three predetermined sites in the udder half after the goat was given a lethal overdose of barbiturate (Sodium Pentobarb, TechVet, NZ).

Sample Preparation and Analysis

Blood was sampled into vials on ice containing disodium-EDTA. Haematocrit measurements on whole blood were

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made on a subsample during collection. Extracts of whole blood, plasma and mammary tissue were prepared using a similar protocol Lee *et al.* (1993).

Concentrations and radioactivities of ^{35}S-Cys and ^{35}S-GSH in infusate, whole blood, plasma and mammary tissue extracts were determined as described by Lee *et al.* (1993). The concentration and radioactivity measurements were used to calculate SRAs (dpm/nmole) for Cys and GSH. Net uptake of Cys and GSH was calculated by multiplying arteriovenous concentration differences by blood flow. Total uptake of Cys was estimated by calculating the difference in radioactivity for the Cys fraction in the arterial and venous extracts, dividing by the Cys SRA of the precursor pool (assumed to be arterial whole blood) and multiplying by blood flow. Thus net uptake only measures the uptake of Cys and GSH from the blood as it passes through the mammary gland. The total uptake measurement, however, accounts for oxidation, protein synthesis and degradation, and export of Cys by the mammary gland. Cys irreversible loss rate (ILR) was calculated by the division of the infusion rate (dpm/min) by the SRA for Cys in whole blood (dpm/nmole).

RESULTS

Mean concentrations of Cys and GSH in whole blood, plasma and mammary gland tissue extracts are shown in Table 1. Calculated concentrations of Cys and GSH in the rbc, using the haematocrit, are also shown in Table 1. Of the Cys present in whole blood, 70-80% is found in the plasma. The concentration of GSH in plasma was very low (1-2 μM). The concentration of GSH in whole blood was 11-fold greater than that of Cys (Table 1). Glutathione concentration in mammary tissue was similar to whole blood, whereas the concentration of Cys was approximately 30% higher.

TABLE 1: Mean Concentrations (nmoles/g±se) of cysteine (Cys) and glutathione (GSH) in whole blood, plasma, red blood cells, and mammary gland tissue extracts from three lactating Saanen goats.

Extract	Cys	GSH
Whole Blood	71 ± 7	823 ± 30
Plasma	79 ± 9	1.6 ± 0.1
Red Blood Cell	19 ± 8	3,426 ± 124
Mammary Gland Tissue	106 ± 2	900 ± 45

The calculation of Cys ILR requires that the SRA of Cys in the blood extract (assumed to reflect the primary pool) is at steady state. This was verified by examining the distribution of ^{35}S label in the blood extract over the 9h collection period. The SRA for Cys and its acidic oxidation products (SO_4^{2-}, cysteinesulphinic acid and taurine) reached plateau by the 7th hour. Therefore steady state values for the SRA of Cys and GSH were calculated from samples collected at 7, 8 and 9h and are presented in Table 2. There was little difference between the SRA of Cys in whole blood and plasma. The SRA of intracellular Cys, however, was lower than that in whole blood. The SRA of GSH in whole blood was very low, while a much higher SRA of GSH was found in mammary tissue, suggesting greater interchange between intracellular Cys and GSH compared to that in whole blood.

TABLE 2: Specific radioactivities (dpm/nmole±se) of cysteine (Cys) and glutathione (GSH) in whole blood, plasma and mammary gland tissue extracts from three lactating Saanen goats after 7-9h infusion of ^{35}S-cysteine.

Extract	Cys	GSH
Whole Blood	352 ± 13	5.9 ± 0.7
Plasma	370 ± 28	n.d.
Mammary Gland Tissue	231 ± 23	146 ± 13

n.d. = not detected

Whole body ILR of Cys, net uptakes of Cys and GSH, and total uptake of Cys from whole blood by the mammary gland is shown in Table 3. The ILR of Cys calculated using the SRA data and infusion rate was 7.3 g/day, and of this, 1.0 g/day or 13.6% was taken up by the mammary gland. The net uptakes of Cys and GSH by the mammary gland were 0.3 and - 10 g/day respectively. Arteriovenous differences were however highly variable for GSH.

TABLE 3: Whole body irreversible loss rate (ILR) of ^{35}S-cysteine (^{35}S-Cys), net uptake of cysteine (Cys) and glutathione (GSH) from whole blood, total uptake of ^{35}S-Cys from whole blood, and mammary gland utilisation of whole body Cys.

ILR of ^{35}S-Cys (g/d)	7.3 ± 0.7
Net Uptake of Cys[1] (g/d)	0.3 ± 0.2
Net Uptake of GSH[1] (g/d)	-10 ± 9
Total Uptake of ^{35}S-Cys[1] (g/d)	0.5 ± 0.2
Mammary Gland Utilisation (%)	13.6 ± 4.3

[1]Uptake based on data derived from one half of the udder.

DISCUSSION AND CONCLUSIONS

Few studies investigating amino acid uptake by the lactating mammary gland have reported Cys and GSH concentrations in plasma and whole blood. Whole blood and plasma Cys and GSH concentrations found in this study were similar to other published data (Williams *et al.*, 1976; Pocius *et al.*, 1981; Cant *et al.*, 1993; Lee *et al.*, 1993). However, rbc GSH concentration was higher than those reported by Atroshi *et al.*, (1986) and Rizzi *et al.*, (1988).

The higher concentration (+30%) of Cys in mammary gland tissue compared to that in plasma and whole blood would suggest there is active transport of Cys into the mammary cell, and/or Cys derived by intracellular degradation of GSH. Intracellular GSH concentration, however, was similar to that in whole blood.

The SRA of Cys in plasma was similar to that of whole blood, perhaps indicating a rapid equilibration of Cys between the rbc and plasma. However the low SRA of GSH in whole blood suggests very little direct incorporation of Cys into GSH within the rbc, and the little ^{35}S-labelled GSH present may be accounted for by an artefact in the infusate (Lee *et al.*, 1993). In contrast, the SRA of GSH in mammary gland tissue was 30-fold higher, and although still lower than the intracellular SRA of Cys, this indicates a rapid incorporation of intracellular Cys into GSH in the mammary tissue. The lower SRA of tissue intracellular Cys in comparison with that in the whole blood reflects dilution of ^{35}S-Cys with

unlabelled Cys. This may be from a relatively slow equilibration with the blood Cys pool with a consequent failure of the SRA in the intracellular pool to reach plateau at 9h. Alternatively it could arise by an influx of unlabelled Cys from another metabolic pool.

There was large variation in the net uptake of GSH by the mammary gland as measured by arteriovenous difference, and although the mean value indicated a net release of GSH from the gland, this value was not significantly different from zero. Cant *et al.*, (1993) also found no arteriovenous difference for GSH across the mammary gland. Pocius *et al.*, (1981) however found a significant net uptake of GSH. This may have been attributed to the γ-glutamyl cycle delivering Cys to the cell (Vina *et al.*, 1989).

The net uptake of Cys by one half of the mammary gland was 0.26 g/d and is much greater than uptakes reported by Derrig *et al.*, (1974) and Spires *et al.*, (1975). Total uptake of Cys by one half of the mammary gland was 0.5 g/d. The difference between net and total uptake measurements is the degradation of structural or milk proteins, Cys oxidation, and any net production of Cys from other sources such as transulphuration or peptide utilisation. Oddy *et al.*, (1988) suggest that intracellular protein degradation in the mammary gland of goats accounts for 30% of total milk protein synthesis. If the low tissue intracellular SRA of Cys and the difference found between net and total uptake of Cys in the present study is assumed to be solely due to degradation, then 40-50% of total protein (structural and milk) synthesised in the mammary gland would be degraded.

The Cys ILR measurement is the total sum of whole body Cys utilisation for protein synthesis, oxidation and production of other metabolites, in particular bile salts (Lee *et al.*, 1993). Thus Cys ILR is an overestimate of the use of sulphur amino acids for whole body protein synthesis. Our estimate of Cys ILR (7.3 g/d) is higher than that found in sheep (Lee *et al.*, 1993), however the sheep used were of lower body weight and not lactating. Champredon *et al.*, (1990) found ILR of methionine in early lactating and dry goats to be 8.5 and 5.1 g/d respectively, *i.e.* comparable to the Cys ILR value measured in the current study.

Comparing whole body Cys ILR with total Cys uptake by the mammary gland we can calculate a percentage utilisation of whole body Cys by the mammary gland of 13.6%. This is expected to be minimum utilisation of whole body Cys by the mammary gland as the goats were in late lactation and the partitioning of amino acids to the mammary gland would be anticipated to be different in early lactation. To our knowledge no other studies have investigated the utilisation of whole body Cys by the mammary gland. Continuing studies will determine the role of methionine and GSH as sources of Cys in mammary tissue protein production.

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