Original article

Changes in endogenous urea recycling and the handling of renal urea in pregnant and lactating Sardi sheep kept on a constant feeding level

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Summary — The kinetics of endogenous urea were compared during the last month of pregnancy, lactation, and a nonpregnant, nonlactating control period in Sardi sheep kept on a constant feed level.

Urea entry rate estimated by injections of [^{14}C]urea rose by 36% during pregnancy. Renal urea excretion was reduced by 40% during pregnancy and by 28% during lactation. Consequently, fractional urea recycling was greater during pregnancy and, to some extent, during lactation than during the control period.

In a second series of experiments, glomerular filtration rate increased by 48% and urea filtration rate rose by 17% during pregnancy. During lactation, both glomerular filtration rate and urea filtration rate were close to control levels. It appears that the decreased renal urea excretion during pregnancy and lactation was due mainly to increased tubular reabsorption of urea.

Rumination time increased by 15% during pregnancy. Rumen ammonia concentration was elevated in both pregnant and lactating ewes above the control period level.

The results suggest that Sardi sheep possess a high potential for the conservation of nitrogen during pregnancy and lactation periods.

urea, endogenous — urea, renal — pregnancy — Sardi sheep

Résumé — Changements du recyclage endogène de l'urée et du contrôle rénal de l'urée chez des brebis Sardi gestantes et allaitantes soumises à un régime alimentaire constant. *La cinétique de l'urée endogène a été comparée au cours du dernier mois de gestation, au cours de la lactation et durant une période de contrôle chez des brebis Sardi soumises à un régime alimentaire constant. Le renouvellement de l'urée endogène estimé par des injections d'urée marquée au carbone 14 a augmenté de 36% au cours de la gestation. L'excrétion rénale d'urée a diminué de 40% durant la gestation et de 28% durant la lactation. Comme conséquence, la fraction d'urée endogène recyclée a été plus élevée durant la gestation et, à un moindre degré, durant la lactation que durant la période de contrôle. Dans une seconde série d'expériences, le débit de filtration glomérulaire a augmenté de 48% et la quantité d'urée filtrée de 17% durant la gestation. Durant la lactation, le débit de filtration glomérulaire et la quantité d'urée filtrée ont été voisines des valeurs de contrôle. Il apparaît que la baisse de l'excrétion rénale d'urée est principalement due à un accroissement de la réabsorption tubulaire de l'urée. La durée journalière de rumination a augmenté de 15% durant la gestation. La concentration ruminale en ammoniaque chez les brebis*

gestantes et allaitantes a dépassé les valeurs de la période de contrôle. Les résultats suggèrent que les brebis Sardi possèdent un potentiel élevé de conservation d'azote durant les périodes de gestation et de lactation.

urée endogène — urée rénale — gestation — brebis Sardi

Introduction

In ruminants, endogenously produced urea is partly secreted into the digestive tract and partly excreted in the urine. The ammonia produced in the rumen from secreted urea can be used for microbial protein synthesis (Egan *et al.,* 1986). The proportion of urea secreted into the digestive tract (urea recycling) and that excreted in the urine is influenced by the diet and the physiological state of the animals. In sheep and goats low-protein diets decrease plasma urea concentration and its glomerular filtration rate (GFR), which reduces the proportion of urea that is excreted in the urine. Renal tubular reabsorption also increases, but its contribution to nitrogen economy seems less important than the above factors (Rabinowitz *et al.*, 1973; Ergene and Pickering, 1978; Eriksson and Valtonen, 1982).

Pregnancy and lactation increase the protein requirement. Renal urea clearance decreases in pregnant and lactating sheep (Nolan and Leng, 1970; Benlamlih *et al.,* 1985). The urea recycling rate is greater in pregnant than in nonpregnant Corriedale sheep kept on a similar feeding level (Nolan and Leng, 1970). The proportion of recycled urea increases during lactation in Merino sheep, but there is no difference between pregnant and dry ewes (Oddy *et al.,* 1983). However, the level of nitrogen intake was different among the three physiological states in the latter study. Thus, we compared urea recycling in the same ewes kept on a constant feeding regime

throughout pregnancy, lactation, and a nonpregnant, nonlactating period. We used the Sardi sheep, a Moroccan breed adapted for grazing in semi-arid zones where nitrogen conservation may be important.

GFR generally increases during pregnancy in humans (Davison and Dunlop, 1980), dogs (Robb *et al.,* 1970), rabbits (Woods *et al.,* 1987), and rats (Atherton, 1983). If a similar increase in GFR occurred in pregnant sheep, it would increase renal urea clearance unless urea reabsorption became more efficient. Therefore we compared factors controlling the renal excretion of urea during pregnancy, lactation, and the control period.

Urea enters the forestomach through the epithelia or with the saliva (Egan *et al.,* 1986). Salivary secretion increases with feeding and rumination (Church, 1979). Thus, we monitored feeding and ruminating behavior as well as rumen ammonia concentration.

Preliminary results have been reported previously (Benlamlih and Oukessou, 1986).

Materials and Methods

Animals

Thirteen Sardi ewes (body wt 42—52 kg) were used, 6 for measurements of urea recycling and 7 for measuring GFR and feeding activity. The animals were transported from the university farm to the laboratory 7—9 wk after

mating. After arrival they were kept in metabolic cages. Room temperature was 16—23 °C and relative humidity was 60 ± 5%. The animals had free access to water unless otherwise noted. They were fed 0.8 kg of lucerne and 0.5 kg of grain daily. The diet provided 11.5 MJ metabolizable energy with 145 g of digestible protein per day. The energy and protein requirements of Sardi ewes are not yet known, but according to the INRA (Tissier and Thériez, 1980), the estimated requirements during the last month of pregnancy, during early lactation, and during the control period are, respectively, 11, 14, and 7 MJ of metabolizable energy and 100, 150, and 50 g of digestible protein. Half of the food was given at 8:30 h and the other half at 17:00 h, when 6 g NaCl plus 10 g of a commercial mixture of vitamins and minerals (CMV, Cerna, Morocco) were also given.

Three ewes lambed twins and the other 10 had singletons. The lambs were kept with their mothers in the metabolic cages for the first 8 wk, during which they could not reach the manger. The lambs were then removed. The ewes became completely dry after 3—6 wk, when they were subjected to the control experiments.

Urea recycling experiments

Each sheep was subjected to urea recycling experiments during pregnancy (12—40 d before parturition), during lactation (20—35 d after parturition) and during the control period (20—50 d after removal of young).

The experiments started at 10:00 h, after the morning feed and post prandial watering. Polyethylene catheters were introduced bilaterally into the jugular vein, one for injection of labeled urea and the other for blood sampling. Urine was collected *via* a bladder catheter. The water bucket was removed at the start of the experiment and was replaced with the afternoon feed after the last blood and urine samples were taken.

Parameters of urea kinetics were estimated using single injections of [^{14}C]urea (Cocimano and Leng, 1967). After a blood sample (5 ml) was taken for a blank, 2 ml of 0.15 M NaCl solution containing 40 μCi of [^{14}C]urea (0.001 mg urea/μCi; Amersham, U.K.) was injected, and the syringe was flushed by withdrawal and reinjection of blood to ensure that all the isotope was infused. Blood samples (5 ml) were taken in heparinized (100 IU) tubes hourly for 9 h. Urine was collected at 3-h intervals

during this 9-h period. The urine volume was measured and urine samples were taken for urea analysis.

GFR and renal urea clearance

Each of the other seven ewes was subjected to GFR and renal urea clearance measurements once during pregnancy (12—30 d before parturition), during lactation (20—35 .d after parturition), and during the control period (20—45 d after removal of young). Venous and bladder catheters were prepared as described above.

The single injection method without urine collection was used for the evaluation of GFR (Mercer *et al.*, 1978; Fettman *et al.*, 1985). After a blood sample (5 ml) for a blank had been taken, 50 μCi of [^{3}H]inulin (343 μCi/mg Amersham, U.K.) diluted in 10 ml of 0.15 M NaCl solution was injected into the jugular vein. Blood samples (5 ml) were taken in heparinized (100 IU) tubes 1, 2, 4, 8, 15, 30, 45, 60, 75, 90, 105, and 120 min after isotope administration.

Urine was collected during the 120-min experimental period. Urea concentrations in the plasma and urine were also determined. The blood sample taken 60 min after the injection was used for the calculation of renal urea filtration.

Recordings of jaw movements and sampling of rumen fluid

Feeding activity was recorded in the seven ewes in the second experiment. A recording was taken for 48 consecutive hours in each animal twice during the last month of pregnancy, during lactation, and during the control period. The jaw movements were detected with a balloon attached in the submandibular space and connected to a pressure transducer (Statham P23, Gould Inc, Oxnard, CA) to record on a chart recorder (Beckman R511, Schiller Park, IL).

Rumen liquor was taken during d2 of the above recording period. Three hours after the morning feeding 50 ml of rumen liquor were aspirated *via* a stomach tube introduced into the rumen.

Analysis

For determinations of [^{3}H]inulin and [^{14}C]urea, 0.5-ml aliquots of plasma were transferred to

scintillation vials to which a liquid scintillator (10 ml/vial, Scintiverse E, Fisher Company) was added. Radioactivities of the sample were counted with a liquid scintillation counter (Beckman LS 3133T). Correction for quenching was made by means of the external channel-ratio (I.A.E.A., 1985). Standards were prepared for [^{14}C]urea (2 μCi/l) and for [^{3}H]inulin (3 μCi/l) and their radioactivities were analyzed as for the plasma.

Urea concentration was determined by the method of Foster and Hochholzer (1971).

The rumen samples were immediately filtered through gauze and the pH was measured. Ammonia concentration was determined by steam distillation over magnesium oxide using a Kjeldahl apparatus.

Mathematical analysis of isotope data

The parameters of urea recycling experiments were calculated according to Cocimano and Leng (1967). Urea pool size and urea entry rate were derived from the 0-time intercept concentration A_0 (in μCi/mmol urea) and the slope k (min^{-1}) of the plasma disappearance curve of radioactivity :

Urea pool size = injected dose (μCi) / A_0.

Urea entry rate   = urea pool size x k.
                  = urea recycling rate
                  + renal urea excretion rate.

Fractional urea recycling= urea recycling rate /
                  urea entry rate.

The urea secreted in the milk was not measured since the estimated urea loss in the milk would have been < 1% of urea entry rate, given a daily milk production of 1 l (Sardi ewes having twins produce a maximum of 1.25 l/day).

GFR was calculated according to Fettman et al. (1985). The area under the plasma disappearance curve of [^{3}H]inulin was divided into $A1$ and $A2$ (before and after 45 min). $A1$ was determined graphically. $A2$ was calculated by the following equation :

$A2 = C \times k,$

where C is the plasma specific activity of [^{3}H]inulin at 45 min and k is the fitted slope of the second part of the curve. The GFR was then determined as :

GFR = injected dose of radioactivity / ($A1$ + $A2$).

Urea filtration rate = GFR x plasma urea concentration;

Urea reabsorption rate = urea filtration rate —
                  urea excretion rate;

Fractional urea reabsorption = urea reabsorption / urea filtration.

Statistical analysis

Data are presented as means ± SEM.

The means of the pregnancy, lactation, and control periods were statistically compared by analysis of variance followed by multiple mean comparison with Fisher's least-significant difference test. P values < 0.05 were considered significant.

Results

Urea recycling experiments

Body weight, urea pool size, and urea entry rate were higher during pregnancy than during lactation or control periods (Fig. 1). The renal urea excretion rate was lower in both pregnant and lactating ewes than in the control period. Both urea recycling rate and fractional urea recycling were higher in the following order of decreasing magnitude: during pregnancy, during lactation, during the control period.

Renal handling of urea

GFR and GFR/kg body wt were higher during the last month of pregnancy than during the control period (Fig. 2), but no significant difference was noticed between the lactation and the control periods.

Plasma urea concentration was 6.9 ± 0.5, 5.5 ± 0.3 (NS), and 6.8 ± 0.3 mmol/l (NS), respectively, during the control period, during pregnancy, and during lactation. Urea filtration rate did not change significantly during the three periods. Urea excretion rate was significantly lower during pregnancy and lactation. Thus, absolute urea reab-sorption and fractional urea reabsorption
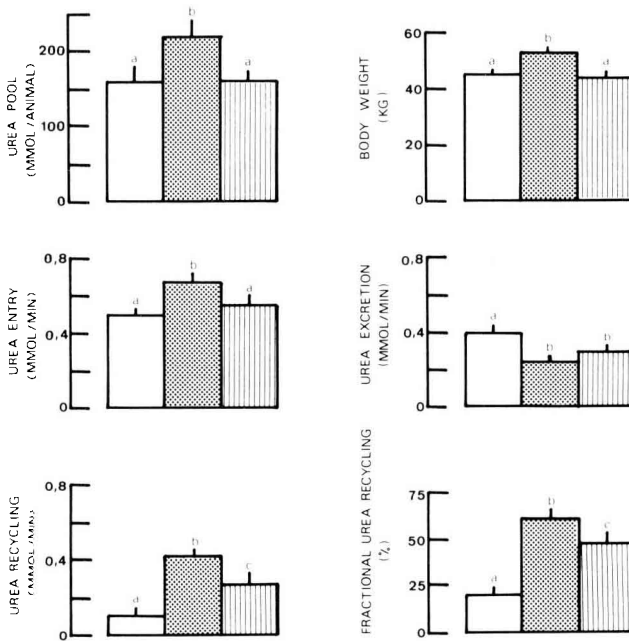
Fig. 1. Urea kinetics during the control period (□), during pregnancy (▥), and during lactation (▨) in sheep: mean with SEM. $N = 6$ ewes. **a,b,c** values with different letters over the bars differed significantly ($P < 0.05$).

by the kidneys were higher during pregnancy and lactation (only fractional urea reabsorption) than during the control period.

Urine flow rates were 1.6 ± 0.2, 2.4 ± 0.1 ($P < 0.05$), and 1.9 ± 0.3 ml/min (NS), respectively, during the control period, pregnancy, and lactation.

Feeding activity and rumen liquor composition

The ewes spent about 3 h a day eating two daily meals in all periods (Table I). Pregnant animals spent 40—50 min more ruminating than lactating and non-pregnant, nonlactating ewes. The rumination cycles started 5—30 min after the end of the meal during pregnancy, whereas rumination started 45—90 min

after the end of the meal during lactation and control periods.

Rumen ammonia concentration 3 h after the morning feed was increased during pregnancy and lactation as compared to the control period. The pH was similar in the three physiological states.

Discussion

The present results show that Sardi sheep kept on constant energy and nitrogen intakes increase their capacity to recycle urea when they become pregnant or are lactating. Fractional urea recycling increases when the levels of nitrogen intake decreases (Cocimano and Leng,
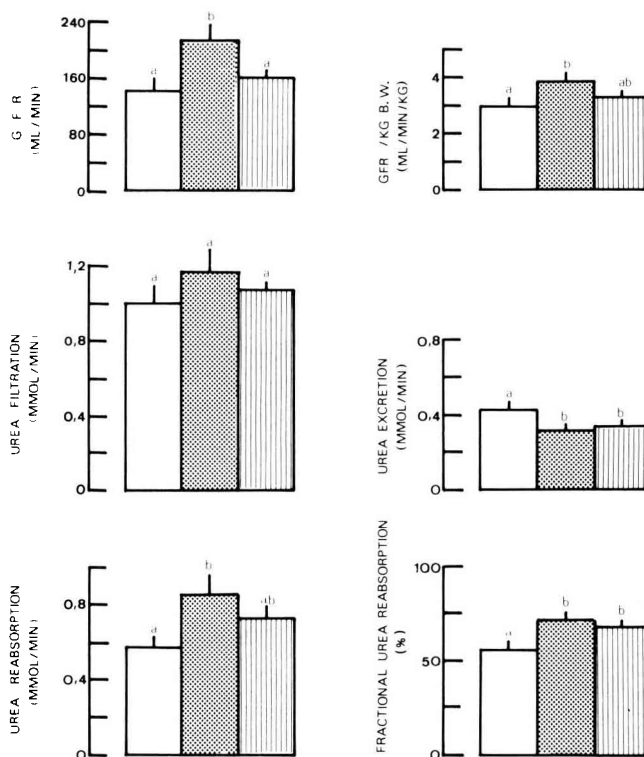
Fig. 2. Glomular filtration rate (GFR) and renal urea handling during the control period (□), during pregnancy (▦), and during lactation (▨) in sheep: mean with SEM. $N = 6$ ewes. **B.W.** = body weight. **a,b,c** values with different letters over the bars differed significantly ($P < 0.05$).

1967). The nitrogen intake fulfilled 145%, 97% and 290% of the estimated requirement, respectively, during pregnancy, lactation, and control periods. These differences might have led to the increased fractional urea recycling seen in the pregnant and lactating ewes. However, with increased nitrogen intake during lactation, a rise in fractional urea recycling was still observed (Oddy *et al.,* 1983). On the other hand, the nitrogen requirements of lactation are much higher than those of pregnancy, and yet fractional urea recycling was more elevated in the pregnant than in the lactating ewes of the present study.

The amount of urea recycling, estimated by use of [14C]urea, represents the degradation of endogenous urea in the whole digestive tract (Egan *et al.,* 1986). Blood urea enters the rumen *via* the saliva and the rumen wall; both routes seem to be enhanced by pregnancy. The secretion of saliva increases during mastication, especially when the animal is ruminating (Church, 1979). The daily rumination time was about 15% longer in pregnant than in lactating and control ewes. Therefore, additional amounts of urea could enter the rumen *via* saliva during pregnancy. Furthermore, the increase in forestomach blood flow in sheep with advancing

Table I. Daily feeding and rumination times and rumen liquor composition 3 h after the distribution of morning feed during pregnancy, lactation, and the control period in sheep.

	Control	Pregnancy	Lactation
Feeding (min/d)	184 ± 5 [a]	183 ± 7 [a]	182 ± 15 [a]
Rumination (min/d)	320 ± 19 [a]	368 ± 18 [b]	329 ± 25 [a]
Rumen pH	6.4 ± 0.1 [a]	6.5 ± 0.1 [a]	6.6 ± 0.1 [a]
Rumen ammonia (mmol/l)	8.8 ± 0.6 [a]	14.8 ± 1.2 [b]	15.1 ± 1.6 [b]

[a, b] Values with different superscripts within rows differed significantly ($P < 0.05$).
Means with SEM $N = 7$ ewes.

pregnancy (Alexander *et al.,* 1987) would favor blood urea clearance there. Although rumen ammonia concentration is mainly influenced by the kinetics of proteolysis and protein synthesis in the rumen, the increase observed during pregnancy might also indicate that part of the additional urea, which was recycled during this period, had been transferred to the rumen.

During lactation, mastication and rumination times were similar to those in the control period. Yet the urea recycling rate and the ammonia concentration of the rumen fluid were elevated. The enhanced urea recycling rate during lactation may be achieved by an increased capacity of urea transfer across the digestive mucosa.

The reduction of urea excretion by the kidneys is a prerequisite for enhanced urea recycling during pregnancy and lactation. The decreased renal excretion of urea by low-protein diets accompanies a lower plasma urea concentration and reduced GFR (Rabinowitz *et al.,* 1973; Ergene and Pickering, 1978; Eriksson and Valtonen, 1982). The increased fractional urea reabsorption in such a situation is attributed to increases in the permeability of the collecting duct of urea (Rabinowitz *et al.,* 1973) and in the active transport of urea from the medullary collecting ducts (Schmidt-Nielsen *et al.,* 1958; Ergene & Pickering, 1978). However, the actual quantity of urea reabsorbed is one-fifth to one-tenth of the quantity reabsorbed by animals on a high-protein diet (Ergene and Pickering, 1978; Eriksson and Valtonen, 1982). Thus, the contribution of urea reabsorption to nitrogen economy of ruminants on a low-protein diet is far less significant than the reduction in the urea filtration rate. During pregnancy and lactation, the relative contribution of the two mechanisms to a reduction in renal urea excretion appeared to be different. GFR in our sheep during the control period were relatively high, but still within the means of 1.9 to 5.3 ml/min/kg reported in sheep and goats on high-protein diets (Rabinowitz *et al.,* 1973; Ergene and Pickering, 1978; Eriksson and Valtonen, 1982; Godwin and Williams, 1984; Wittenberg *et al.,* 1986). During pregnancy, the GFR increased, a result similar to that found in other species (see

Introduction). The urea filtration rate, however, did not increase significantly. During lactation, the urea filtration rate was similar to the value of the control period. Therefore, the reduction in renal urea excretion during pregnancy and lactation was achieved mainly by increased renal tubular reabsorption of urea.

Renal urea excretion is reported to be positively correlated with urine flow rate in sheep (Cocimano and Leng, 1967; Godwin and Williams, 1984). In spite of the increased urine flow in the present pregnant and lactating ewes, renal urea excretion was reduced. It is not excluded that morphological and/or functional changes in the kidney occurring during pregnancy and lactation may have induced increased renal reabsorption of urea.

The magnitude of the increase in fractional urea recycling during pregnancy and lactation in Sardi sheep was higher than that reported in pregnant Corriedale ewes (Nolan and Leng, 1970) and in pregnant and lactating Merino sheep (Oddy et al., 1983). The high potential for nitrogen conservation during the two productive states could be of special importance for breeds such as the Sardi, which usually graze vegetation poor in nitrogen.

Acknowledgements

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