The effect of dietary calcium intake of ewes in pregnancy on their Ca and phosphorus metabolism in lactation

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(*Received* 28 *March* 1977 – *Accepted* 12 *May* 1977)

1. A combination of a mineral balance and a radioactive technique has been used to study the effect of the dietary calcium intake of ewes in pregnancy on their Ca and phosphorus metabolism in lactation.

2. Ewes which had received a Ca-deficient diet in pregnancy absorbed Ca at a greater rate and with a greater efficiency in early lactation than did the control animals, which had received an adequate Ca intake in pregnancy. The apparent absorption of P was also higher in the Ca-deficient sheep.

3. Since both groups of sheep received the same high Ca intake in lactation, the increased rate of absorption of Ca must have resulted from an increased rate of active transport rather than an increased rate of diffusion.

4. Retention of both Ca and P was positive in the Ca-deficient ewes but negative in the control ewes. This difference in retention reflected a difference in bone metabolism.

5. The maximum rate of absorption possible, which is equal to the rate of irreversible loss of Ca from the rapidly exchangeable pool, was much greater in the Ca-deficient lactating ewes than was previously found in Ca-deficient wethers. This difference is due to the additional pathway of loss of Ca in the milk of the lactating animals.

6. The relationship between these findings and the prevention of milk fever in dairy cows by manipulation of the dietary Ca intake is discussed.

In spite of a plentiful supply of calcium in the diet, the sheep and dairy cow are normally unable to absorb enough to meet the peak requirements of pregnancy and lactation, and retention is usually negative at this time (Ward, Blosser & Adams, 1952; Keller, 1961; Symonds, Manston, Payne & Sansom, 1966; Braithwaite, Glascock & Riazuddin, 1969, 1970). It has been suggested that substantial losses of the skeletal reserves of Ca are necessary before the rate of intestinal absorption can increase (Braithwaite, 1976). In ewes, absorption is normally inadequate in late pregnancy and early lactation when demands for Ca are at a maximum, and it increases to a high value only in late lactation when demands have decreased (Braithwaite *et al.* 1969, 1970). This low rate of absorption of Ca by ruminants at peak demands may contribute to the development of the deficiency disorders, lambing sickness and milk fever. Since intestinal absorption of Ca is stimulated in wether sheep by a period of Ca deficiency (Braithwaite, 1974) and is higher during early lactation in ewes given a poor-quality, low-Ca diet throughout pregnancy (Sykes & Dingwall, 1975) than in ewes given an adequate diet (Braithwaite *et al.* 1969), it may be possible by dietary manipulation to promote a high rate of Ca absorption to coincide with the high requirements of pregnancy and lactation.

The present studies were undertaken to compare the effects of deficient and normal Ca intakes by ewes during pregnancy on the absorption and retention of Ca and phosphorus in early lactation.

EXPERIMENTAL

Animals, housing and diet. Eight 3-year-old pregnant ewes (Suffolk × Clun Forest) weighing 60–70 kg were used. They were randomly divided into two equal groups and when 2 months pregnant were placed in metabolism cages designed for the separate collection of urine and

Table 1. *Daily intakes of dietary ingredients by control and calcium-deficient ewes during pregnancy and the Ca and phosphorus contents of these ingredients*

Group ... Ingredient	Intake (g/kg body-wt)		Ca content (mg/g)	Total Ca (mg/kg body-wt)		P content (mg/g)	Total P (mg/kg body-wt)	
	Control	Ca-deficient		Control	Ca-deficient		Control	Ca-deficient
Hay	5	5	3·5	17·5	17·5	2·59	13·0	13·0
Barley	5	5	0·5	2·5	2·5	3·39	16·9	16·9
Maize	2·5	2·5	0·03	0·1	0·1	0·98	2·5	2·5
Bran	1·25	1·25	0·53	0·7	0·7	13·28	16·6	16·6
Linseed-oil cake	0·5	0·5	3·17	1·6	1·6	7·56	3·8	3·8
Vitamin mixture*	0·07	0·07	15·7	1·1	1·1	2·2	0·2	0·2
Disodium hydrogen phosphate	0·26	0·26	—	—	—	220·0	57·2	57·2
Calcium carbonate	0·22	—	400·0	88·0	—	—	—	—
Mineral mixture†	0·08	—	144·7	11·6	—	99·07	7·9	—
Whole diet	—	—	—	123·1	23·5	—	118·1	110·2

* Beta Vitamin No. 3a (Cooper Nutrition Products Ltd., Witham, Essex) to supply (/kg body-wt) 37·5 μg retinol equivalent, 0·775 μg cholecalciferol.

† Super Mindif (Boots Pure Drug Co., Nottingham).

faeces. All ewes had free access to distilled water and were given the same diet of hay and concentrates throughout the remainder of the pregnancy (Table 1). The P intake of both groups was adjusted by the addition of disodium hydrogen phosphate to supply the recommended requirements for pregnancy (Agricultural Research Council, 1965). The control group, in addition, received a supplement of calcium carbonate and mineral mixture.The unsupplemented diet of the Ca-deficient group contained less Ca (23·5 mg Ca/d per kg body-weight) than the 55 mg/d per kg body-weight calculated from results of Braithwaite & Riazuddin (1971) to be necessary to supply maintenance requirements. The supplemented diet of the control group on the other hand contained enough Ca (123 mg/d per kg body-weight) to supply that normally absorbed by ewes in late pregnancy (Braithwaite *et al.* 1969).

At parturition, both groups of animals were transferred to a new diet (Table 2) which contained a plentiful supply of Ca and P for both maintenance and lactational requirements. The lambs were removed 2 d after birth and the ewes were then machine milked twice daily (Treacher, 1970).

Experimental procedure. Ca kinetic studies were performed at 3 weeks lactation. A known amount (5 μCi/kg body-weight) of an aqueous solution of $^{45}CaCl_2$ (Radiochemical Centre, Amersham, Bucks.) was injected into the jugular vein immediately after the morning milking and samples of blood, urine, faeces and milk were collected for a period of 1 week as previously described (Braithwaite *et al.* 1969). During this period Ca balance measurements were made.

Methods. Kinetic analysis was done by the method of Aubert & Milhaud (1960) modified for use with sheep (Braithwaite *et al.* 1969; Braithwaite & Riazuddin, 1971). The methods used for determination of the Ca content and measurements of the radioactivity in samples of blood, urine, faeces and milk have been described previously (Braithwaite *et al.* 1969). Total P content of ashed samples of food, urine, faeces and milk was determined by the procedure of Fiske & Subbarow (1925) modified (Technicon Instruments Corporation, 1967) for use with an AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants).

Table 2. *Daily intake of dietary ingredients* by all sheep during lactation*

Ingredient	Intake (g/kg body-wt)	Total calcium (mg/kg body-wt)	Total phosphorus (mg/kg body-wt)
Hay	16	56·0	41·4
Barley	5	2·5	17·0
Maize	2·5	0·1	2·5
Bran	1·3	0·7	17·3
Linseed-oil cake	0·5	1·6	3·8
Mineral mixture	0·3	43·4	29·1
Vitamin mixture	0·07	1·1	0·2
Calcium carbonate	0·4	160·0	—
Disodium hydrogen phosphate	0·46	—	101·2
Whole diet	—	265·4	212·5

* For details of Ca and P content of these ingredients, see Table 1.

RESULTS AND DISCUSSION

All the control ewes and three of the four Ca-deficient ewes produced twin lambs. Lambs from both groups of ewes were healthy and did not differ significantly in birth weight (mean 3·8 kg/lamb).

Table 3 shows that in early lactation the Ca-deficient ewes absorbed Ca at a higher rate (75·6 mg/d per kg body-weight) and with a greater efficiency (29·2%) than did the control ewes (43·0 mg/d per kg body-weight and 16·6% respectively). Since the Ca intake was the same in lactation for both groups of sheep, this difference in absorption must have been due to the different intakes of dietary Ca in pregnancy: adequate in the control group but deficient in the Ca-deficient group.

This result is what would have been expected from previous knowledge which suggests that Ca absorption is increased to a high level only after skeletal reserves have been severely depleted (Braithwaite *et al.* 1969, 1970; Braithwaite, 1974), and it adds support to the theory of Nicolaysen (Nicolaysen, 1943; Nicolaysen, Eeg-Larsen & Malm, 1953), that Ca absorption is regulated according to the extent of skeletal saturation. Furthermore, it shows that Ca absorption can be increased to coincide with the peak Ca demands of pregnancy and lactation merely by manipulation of the previous dietary Ca intake.

Evidence now suggests that Ca absorption in ruminants, as in non-ruminants, involves two processes, a non-saturable diffusional one, related to intestinal Ca concentration, and a saturable active one, independent of concentration but related to body needs (Wasserman & Taylor, 1969; Braithwaite, 1974). Sykes & Dingwall (1975) were unable to decide whether a high rate of Ca absorption in lactating ewes, previously made Ca-deficient, was due to an increased rate of active absorption or to increased diffusion. In the present experiments, the higher rate of absorption in the Ca-deficient group must have been due to a higher rate of active absorption rather than diffusion, since both groups of animals received the same high Ca intake during lactation.

As secretion of P into the intestine was not measured, true rates of P absorption could not be calculated. It was not possible therefore to conclude whether the higher rate of apparent P absorption (P intake − total P in faeces) was effected by an increase in true absorption or by a decrease in secretion. However, since dietary P is normally absorbed in direct relation to the P intake and excess is then secreted into the intestine (Lueker & Lofgreen, 1961; Preston & Pfander, 1964; Young, Lofgreen & Luick, 1966; Young, Richards, Lofgreen & Luick 1966), it seems likely that the increased retention was due to decreased secretion

Table 3. *A comparison of the calcium and phosphorus metabolism in lactation of control and Ca-deficient ewes which received respectively an adequate Ca intake and a low Ca intake during pregnancy*†

(Mean values with their standard errors for four animals/group; tests of statistical significance determined by the *t* test)

Group ...	Control		Ca-deficient		Statistical significance of difference between means
	Mean	SE	Mean	SE	
Rate of ingestion of Ca (mg/d per kg body-wt)	259·4	4·0	258·7	3·7	NS
Rate of loss of Ca in faeces (mg/d per kg body-wt)	228·7	3·1	195·6	7·7	**
Rate of excretion of Ca in urine (mg/d per kg body-wt)	0·9	0·4	0·2	0	NS
Rate of secretion of Ca into milk (mg/d per kg body-wt)	45·2	1·3	51·2	4·8	NS
Rate of Ca retention (mg/d per kg body-wt)	−15·4	1·3	11·7	2·7	***
Rate of secretion of Ca into intestine (faecal endogenous Ca) (mg/d per kg body-wt)	12·3	0·9	12·5	0·7	NS
Rate of absorption of Ca from intestine (mg/d per kg body-wt)	43·0	2·4	75·6	5·3	**
Ca absorbed (% Ca ingested)	16·6	0·8	29·2	2·3	**
Rapidly exchangeable pool of Ca (mg/kg body-wt)	34·8	1·1	34·7	1·5	NS
Slowly exchangeable pool of Ca in bone (mg/kg body-wt)	31·8	1·5	30·7	2·1	NS
Rate of accretion of Ca into bone (mg/d per kg body-wt)	13·8	1·9	12·8	1·7	NS
Rate of resorption of Ca from bone (mg/d per kg body-wt)	29·2	2·8	1·1	2·7	***
Rate of ingestion of P (mg/d per kg body-wt)	209·5	3·1	194·1	2·9	*
Rate of loss of P in faeces (mg/d per kg body-wt)	177·2	3·7	130·4	7·0	**
Rate of excretion of P in urine (mg/d per kg body-wt)	4·7	3·4	7·8	4·3	NS
Rate of secretion of P into milk (mg/d per kg body-wt)	39·0	1·9	46·3	3·0	NS
Rate of retention of P (mg/d per kg body-wt)	−11·4	1·1	9·6	4·6	**
Apparent P absorption (P ingested − P lost in faeces) (mg/d per kg body-wt)	32·3	3·4	63·7	6·3	**

NS, not significant.
* $0{\cdot}05 > P > 0{\cdot}01$, ** $0{\cdot}01 > P > 0{\cdot}001$, *** $0{\cdot}001 > P$.
† For details, see Tables 1 and 2.

and was related to the higher rate of retention of Ca. Certainly previous results (Braithwaite, 1975), which show that Ca and P are retained in a constant ratio by Ca-deficient wethers, also show that P retention is controlled according to the rate of Ca retention.

Insufficient Ca was absorbed by the control animals to satisfy the high requirements of lactation and retention was negative (Table 3). In contrast, the Ca-deficient ewes absorbed more Ca than they needed to meet their lactational requirements, and retention was positive. Differences between the two groups in Ca and P retention reflect differences in bone metabolism. In the control group, the rate of resorption of Ca from bone was greater than the rate of accretion of Ca into bone, and bone mineral stores were mobilized to meet the deficit between the Ca absorbed and that required for lactation. In the Ca-deficient group, however, the rate of bone resorption was reduced to a negligible level, whilst the rate of accretion remained similar to that of the control ewes. In these animals, skeletal stores of Ca, presumably depleted during the previous period of Ca deficiency, were replenished.

The rate of absorption of Ca by sheep is directly related to the rate of Ca retention (Braithwaite & Riazuddin, 1971; Braithwaite, 1975) and recent studies with Ca-deficient wethers indicate that at maximum retention, which occurs when bone resorption ceases and is equal to the rate of accretion of Ca into bone, the rate of absorption of Ca reaches a maximum (Braithwaite, 1975; Braithwaite & Glascock, 1976). It was suggested that this maximum rate of absorption was due to homoeostatic mechanisms regulating Ca absorption

at a level just sufficient for maximum retention, rather than to the capacity of the intestine for absorption becoming saturated.

It can be inferred from the experiments of Braithwaite (1975) that the maximum rate of absorption of Ca (V_a) by Ca-deficient wethers is equal to the total irreversible loss of Ca from the rapidly exchangeable pool, which occurs by excretion in the urine at rate V_u, by secretion into the intestine at rate V_f, and by accretion into bone at rate V_0^+:

$$V_a = V_u + V_f + V_0^+. \quad (1)$$

In lactating ewes, milk secretion constitutes an additional pathway of loss of Ca from the rapidly exchangeable pool. For the Ca-deficient lactating ewes, therefore, it can be postulated that equation no. 1, which describes the maximum rate of absorption, must be modified to include the rate of loss of Ca in milk (V_l):

$$V_a = V_u + V_f + V_0^+ + V_l. \quad (2)$$

Since V_u and V_f remain relatively constant throughout life (Braithwaite & Riazuddin, 1971) any variations in the maximum rate of absorption must reflect changes in V_0^+ and V_l. It has already been shown that the maximum rate of absorption is lower in Ca-deficient mature wethers than in Ca-deficient young animals and that the decrease is related to a decrease with age in the rate of bone accretion (Braithwaite, 1975). Results now show that the maximum rate of absorption is increased during lactation. The rate of absorption of Ca by the Ca-deficient lactating ewes (75·7 mg/d per kg body-weight) was much greater than that (25–30 mg/d per kg body-weight) previously found in Ca-deficient mature wethers (Braithwaite, 1975), even though, in both groups of sheep, the supply of dietary Ca was plentiful and the rates of accretion into bone, resorption from bone and retention of Ca were similar. This difference is accounted for by the loss of Ca in milk (51·2 mg/d per kg body-weight).

These results provide strong evidence in support of the theory that the maximum rate of absorption of Ca is regulated according to the rate of irreversible loss of Ca from the rapidly exchangeable pool. The mean rate of absorption of Ca by the four Ca-deficient lactating ewes was 75·7 mg/d per kg body-weight and the rate of irreversible loss of Ca ($V_u + V_f + V_0^+ + V_l$) was 76·7 mg/d per kg body-weight. Further support for this theory comes from the results of individual sheep. In one ewe, with a lower than average milk yield and in consequence a lower rate of secretion of milk Ca (37·5 mg/d per kg body-weight compared with a mean of 55·7 mg/d per kg body-weight for the other three ewes), the rate of absorption of Ca was correspondingly lower (63·1 mg/d per kg body-weight compared with a mean for the other ewes of 79·9 mg/d per kg body-weight).

Differences in the prepartum dietary intake of Ca had no effect during lactation on the size of the rapidly exchangeable pool of Ca nor on the size of the slowly exchangeable pool of Ca of bone. Neither was there any effect on the rate of secretion of Ca into the milk and intestine or on the rate of excretion of Ca in the urine.

Prevention of milk fever. It has been claimed recently that milk fever can be prevented in dairy cows by feeding them with a low-Ca diet throughout most of pregnancy and then increasing the Ca intake at parturition (Goings, Jacobson, Beitz, Littledike & Wiggers, 1974; Westerhuis, 1974; Pickard, 1975; Pickard, Care, Tomlinson & O'Riordan, 1975). The present results show that in the sheep such treatment does result in an increased rate of absorption of Ca from the intestine. Furthermore, since it has also previously been shown that bone resorption is stimulated by Ca deficiency (Braithwaite, 1974), animals given a Ca-deficient diet in pregnancy should be well prepared to meet the increased demands arising at the onset of lactation. Such treatment results in a transfer of the inevitable period of bone resorption and negative retention from the period of peak demands

to a period in early pregnancy when demands for Ca are normally low. The increased absorptive capacity which follows can then be exploited if the increase in dietary intake is made to coincide with the increase in demands.

The author thanks Dr R. F. Glascock for his encouragement, Mrs A. F. A. Jones, Miss S. G. Neville and Mr R. J. Ranson for skilled technical assistance and Mr A. R. Jones for the care and maintenance of the experimental ewes.

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Printed in Great Britain