Br. J. Nutr. (1983), **49**, 475

The effect of 1α-hydroxycholecalciferol on the placental transfer of calcium and phosphate in sheep

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(*Received 1 November 1982 – Accepted 14 December 1982*)

1. The calcium and phosphorus concentrations in foetal tissue or the placental transfer of ^{45}Ca and ^{32}P, or both, were studied in fifty-five control or 1α-hydroxycholecalciferol (1α-(OH)D_3)-treated (0·1 μg/kg body-weight per d for 12 d) ewes between 77 and 140 d of gestation.

2. Treatment resulted in a significant increase in the concentration of Ca and P in foetal tissues at all stages of gestation except at 140 d when, it is suggested, foetal mineralization may approach a maximum value.

3. This increase in Ca and P concentration in foetal tissues was associated with an increased placental transfer of Ca, though at 111 and 120 d gestation this increase was not significant. P transfer, which was only measured at 140 d gestation, was also significantly higher in treated animals.

4. The concentrations of Ca and P in both maternal and foetal plasma were increased significantly by the 1α-(OH)D_3 treatment.

5. Whereas the concentration of Ca in the plasma of foetuses was always greater than in their dams, the concentration of plasma P in treated animals, unlike controls, was lower in foetuses than dams. This suggests that the increased placental transfer of P, unlike that of Ca, may be a passive rather than an active process.

It is well-known that hypercalcaemia induced by calcium infusion into pregnant ewes (Bawden & Wolkoff, 1967; Garel *et al.* 1974) and cows (Barlet *et al.* 1979) does not affect Ca concentration in foetal plasma. However, the intravenous injection of 1α-hydroxycholecalciferol (1α-(OH)D_3); a synthetic analogue of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$), the biologically active metabolite of cholecalciferol, into ewes during the last month of gestation increases both maternal and foetal plasma Ca and plasma phosphate (P) concentrations (Barlet *et al.* 1978). These increases in maternal Ca and P concentrations result mainly from increased intestinal absorption of these elements following 1α-(OH)D_3 treatment (Braithwaite, 1978, 1980). The concomitant increases in foetal calcaemia and phosphataemia, however, could be due either to stimulation of placental transfer of Ca and P by the 1α-(OH)D_3 in the same way that 1,25-$(OH)_2D_3$ stimulates placental Ca transfer in guinea-pigs (Durand *et al.* 1983) or to a direct action of the 1α-(OH)D_3 on foetal mineral metabolism, since vitamin D_3 metabolites can readily cross the sheep placenta (Ross *et al.* 1979).

The experiments reported here show that placental transfer of Ca and P is stimulated by 1α-(OH)D_3 administration to pregnant ewes.

EXPERIMENTAL

Animals, housing and diet

Forty-seven primiparous 16-month-old Limousine ewes and eight 4-year-old Suffolk ewes bearing one or two foetuses were divided equally into control and treatment groups. A single experiment was performed on each sheep and experiments were carried out during days 70–77, 90–97, 98–105, 104–111, 113–120, 121–128 and 133–140 of gestation. Suffolk ewes were used only at 140 d. The stage of pregnancy was calculated from the time of mating and the number of foetuses present was determined by X-radiography. Each ewe was housed in an individual metabolism cage and allowed at least 15 d to adapt to the experimental diet (hay and concentrate) which provided a daily intake of 100, 70 and 0·002 mg/kg body-weight respectively of Ca, P and cholecalciferol.

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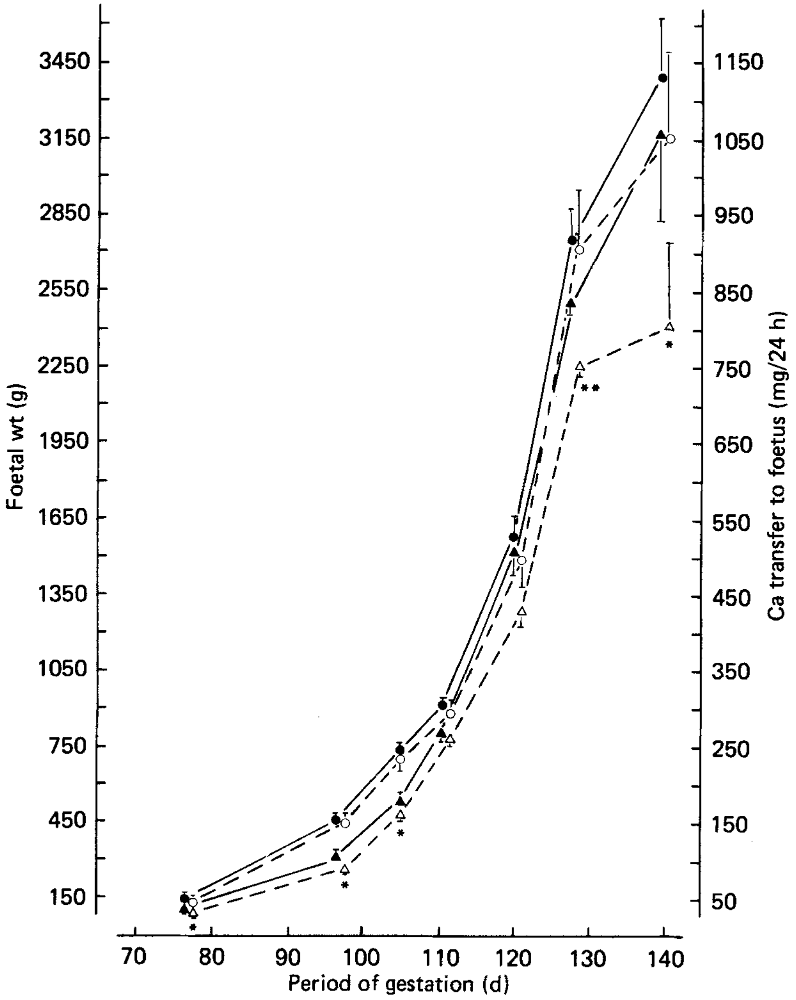

Fig. 1. Evolution of foetal weight (1α-hydroxycholecalciferol-treated (●—●) and control (○---○) animals) and of calcium transfer from dam to foetus (treated (▲—▲) and control (△---△) animals) between days 77 and 140 of gestation. Points are mean values with their standard errors; mean values for treated animals were significantly different from those for control animals: * $P < 0.05$, ** $P < 0.01$. The numbers of animals in each group are given in Table 3.

Treatment

Intramuscular injection of 1α-(OH)D_3 (Teva Pharmaceutical Industries, Jerusalem) in propylene glycol (0·1 μg/kg body-weight per d) was begun in each of the treatment group animals, 5 d before the start of the studies to measure the placental transfer of Ca and P. Control ewes received in the same way the same volume (0·1 ml/kg body-weight per d) of propylene glycol.

Measurement of placental transfer of Ca and P

The procedure used to measure Ca and P transfer from the dam to the foetus was that employed by Wasserman *et al.* (1957) and modified by Twardock (1967). At 7 d before slaughtering (5 d after the beginning of 1α-(OH)D_3 or propylene glycol treatment) each ewe received, in the right jugular vein, an injection of ^{45}Ca, as calcium chloride in saline (9 g

Table 1. *Concentrations of calcium and phosphorus (mmol/l) in plasma of control and 1α-hydroxycholecalciferol-treated (0·1 μg/kg body-weight per d for 12 d) ewes at the start of the experimental period and of the same ewes and their foetuses at the end of the 12 d experimental period*

(Mean results from sheep at all stages of gestation; no. of determinations in parentheses)

	Plasma Ca				Plasma P			
	Control		Treated		Control		Treated	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ewes at start of experimental period	2·59 (28)	0·04	2·55 (27)	0·02	3·39 (28)	0·13	3·29 (27)	0·27
Ewes at end of experimental period	2·52 (28)	0·03	2·84** (27)	0·07	3·62 (28)	0·31	6·13** (27)	0·38
Foetuses at end of experimental period	3·08 (43)	0·03	3·24** (42)	0·06	3·85 (43)	0·02	4·71** (42)	0·20

Mean values for treated animals were significantly different from those for control animals: ** $P < 0·01$.

sodium chloride/l) solution (0·5 mCi/animal). Placental transfer of P was also measured in ewes slaughtered on day 140 of gestation. They were injected in the same way with ^{32}P, as orthophosphate (0·5 mCi/animal). The transfer rate of ^{45}Ca (or ^{32}P) during the experimental period (7 d) was calculated by dividing the total radioactivity transferred to the foetus during this period by the mean specific radioactivity of the plasma Ca (or P) during this same period. Thus,

$$Ca_f = \frac{(^{45}Ca_f)\,(Ca_{mp})}{^{45}Ca_{mp}},$$

where Ca_f is the total Ca transferred from dam to foetus during the experimental period, $^{45}Ca_f$ is the total ^{45}Ca found in the foetus at the end of this period, $^{45}Ca_{mp}$ is the average ^{45}Ca concentration in maternal plasma during the experimental period and Ca_{mp} is the Ca concentration of the maternal plasma.

The mean plasma Ca specific radioactivity during the experimental period was calculated from the radioactivity of forty-two venous blood samples: thirty during the first 24 h following tracer injection and twelve during the next 6 d (Braithwaite *et al.* 1969).

Methods

At 12 d after the first 1α-$(OH)D_3$ injection (7 d after the injection of ^{45}Ca and ^{32}P) the animals were killed by exsanguination. The foetuses were removed, weighed, ashed at 600° for 12 h and the ash dissolved in a known volume of 2 M-hydrochloric acid.

Blood was collected on heparin by puncture of the left maternal jugular vein (and the foetal umbilical artery at slaughtering). After centrifugation a part of the plasma was frozen for mineral analysis and the other part was used for determination of ^{45}Ca and ^{32}P.

^{45}Ca or ^{32}P was radioassayed in duplicate in each sample of maternal blood plasma and in dissolved foetuses (Braithwaite *et al.* 1970; Braithwaite, 1980). Ca concentration in blood plasma and in dissolved foetuses was measured by atomic absorption spectrophotometry (Perkin-Elmer 400). Inorganic P was determined by the procedure of Fiske & Subbarow (1925) modified (Technicon Instruments Corporation, 1967) for use with an autoanalyser.

Results are expressed as means with their standard errors. The statistical significance of differences observed between treated and control animals was evaluated using a Mann and Whitney U test.

Table 2. *The effect of 1α-hydroxycholecalciferol injections into pregnant ewes (0·1 μg/kg body-weight per d for 12 d) on foetal calcium and phosphorus concentrations (mg/g fresh weight)*

Period of gestation† (d)	Group	No. of Ewes	No. of Foetuses	Foetal wt (g) Mean	Foetal wt (g) SE	Foetal concentrations Ca Mean	Ca SE	P Mean	P SE
77	Treated	2	3	151	17	4·34*	0·09	3·74*	0·13
	Control	2	3	143	17	3·69	0·08	3·33	0·16
97	Treated	3	4	470	25	5·30*	0·15	4·23**	0·14
	Control	3	4	450	18	4·33	0·10	3·71	0·13
105	Treated	3	4	745	45	6·11*	0·20	4·68**	0·18
	Control	3	4	710	22	4·92	0·18	3·91	0·12
111	Treated	5	8	1006	104	6·96**	0·50	5·05**	0·30
	Control	6	9	934	79	5·55	0·17	4·15	0·22
120	Treated	2	3	1595	78	8·36*	0·17	5·68**	0·18
	Control	2	3	1495	106	7·44	0·18	4·61	0·16
128	Treated	5	8	2437	122	10·04*	0·45	5·94*	0·23
	Control	5	8	2077	241	9·21	0·32	5·10	0·24
140	Treated	7	12	3048	229	10·01	0·56	6·28	0·27
	Control	7	12	3163	347	10·13	0·31	6·35	0·16

† At the time of slaughtering mean values for treated foetuses were significantly different from those for control foetuses at the same stage of gestation: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

Table 3. *The effect of 1α-hydroxycholecalciferol injections into pregnant ewes (0·1 μg/kg body-weight per d for 12 d) on placental transfer of calcium*

Period of gestation† (d)	Group	No. of Ewes	No. of Foetuses	Foetal wt (g) Mean	Foetal wt (g) SE	Ca Transfer from dams to foetuses (mg/24 h) Mean	(mg/24 h) SE	(mg/24 h per kg foetal weight) Mean	(mg/24 h per kg foetal weight) SE
77	Treated	2	3	151	17	42*	2	276*	2
	Control	2	3	143	17	32	3	223	2
97	Treated	3	4	470	25	107*	2	227**	2
	Control	3	4	450	18	90	3	200	2
105	Treated	3	4	745	45	179*	6	237*	2
	Control	3	4	710	22	160	7	226	4
111	Treated	2	3	921	33	274	11	298	14
	Control	3	4	902	58	259	14	287	21
120	Treated	2	3	1595	98	490	31	307	17
	Control	2	3	1496	106	431	23	288	14
128	Treated	2	3	2760	122	834**	12	302*	12
	Control	3	4	2721	241	752	15	276	12
140	Treated	4	6	3438	208	1056*	116	310*	22
	Control	4	6	3087	333	806	112	255	14

† At the time of slaughtering mean values for treated animals were significantly different from those for control animals at the same stage of gestation: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

RESULTS

Foetal weight increased steadily between 77 and 140 d of gestation (Fig. 1), the greatest rate of increase (130 g/d) occurring between 105 and 128 d. No significant differences were observed between the rate of gain of treated and control animals.

The concentrations of Ca and P in maternal plasma at the start of each treatment period were not significantly different in control or 1α-$(OH)D_3$-treated ewes and were unaffected by the stage of gestation (Table 1). However, after 12 d of 1α-$(OH)D_3$ treatment at a rate of 0·1 μg/kg body-weight per d, maternal plasma levels of both Ca and P were higher ($P < 0{\cdot}01$) at all stages of gestation than in control sheep. Simultaneously, plasma Ca and P concentrations in foetuses from treated ewes were higher ($P < 0{\cdot}01$) than in foetuses from control animals. Whereas the concentration of Ca in the plasma of foetuses was always greater than in their dams, irrespective of treatment, the concentration of plasma P in treated animals, unlike controls, was lower ($P < 0{\cdot}01$) in foetuses than in dams.

Between days 77 and 140 of gestation, Ca and P concentrations in foetal tissues (mg/g fresh weight) increased gradually from 3·69 ± 0·08 to 10·13 ± 0·31 ($P < 0{\cdot}01$) and from 3·33 ± 0·16 to 6·35 ± 0·16 ($P < 0{\cdot}01$) respectively. Ca and P concentrations in foetal tissues from ewes injected with $1\ \alpha$-$(OH)D_3$ were always significantly higher than those measured in foetal tissues from control dams, except on day 140 of gestation (Table 2).

Ca transfer from dams to foetuses was also higher in treated than in control ewes, but at 111 and 120 d of gestation the increase was not significant (Fig. 1 and Table 3).

P transfer from dams to foetuses was only measured at 140 d of gestation but was found to be greater ($P < 0{\cdot}05$) in treated animals (12·3 ± 0·8 mg/kg body-weight per d) than in controls (9·6 ± 0·7).

DISCUSSION

The concentrations of Ca and P in the tissues of control foetuses at different stages of gestation (Ca: 3·69 mg/g fresh weight at 77 d rising to 10·13 mg/g at 140 d; P: 3·33 mg/g fresh weight at 77 d rising to 6·35 mg/g at 140 d) are very similar to those reported by other workers (Field & Suttle, 1967; McDonald *et al.* 1979). These concentrations were significantly increased by 1α-$(OH)D_3$ treatment at all stages of gestation except at 140 d. The reason for this lack of effect at 140 d may be that the concentration of minerals in foetal tissues (and hence foetal mineralization) approaches a maximum value at this time. If this is so, then $1\ \alpha$-$(OH)D_3$ treatment at 128 d resulted in an increase in foetal Ca and P tissue concentrations up to their maximal level.

The finding that the foetal plasma Ca concentration was always greater than the corresponding maternal value agrees well with previous reports (Bawden *et al.* 1965; Braithwaite *et al.* 1972) and is consistent with the view that placental transfer of Ca from mother to foetus is by a mechanism involving active transport (Comar, 1956). It seems less likely, however, that P transfer occurs by an active process, since the plasma inorganic P concentration was virtually the same in control foetuses and dams and was considerably lower in treated foetuses than their dams. It is indeed possible that the rise in placental transport of P during treatment could be due entirely to increased passive diffusion resulting from the increased maternal plasma concentration.

Since placental transfer of Ca and P in ruminants, unlike other species, is virtually a one-way process (Symonds *et al.* 1966; Braithwaite *et al.* 1972), any problems due to back transfer from foetus to dam can be ignored. Treatment of ewes with $1\ \alpha$-$(OH)D_3$ resulted in an increased placental transfer of Ca at all stages of gestation, though at 111 and 120 d the increases were not significant. These findings suggest that the increased concentration of Ca in foetal tissue of treated ewes must occur as a direct result of a stimulation of the placental transfer of Ca. Furthermore, the increase in foetal P concentration in treated ewes is probably also a result of increased placental transfer of P, though, as discussed previously, this increased transfer may be due merely to an increased rate of passive diffusion.

Although it is not possible to decide from these experiments whether 1 α-(OH)D_3 or 1,25-$(OH)_2D_3$ is responsible for the stimulation of placental transfer of Ca, it seems likely that the active metabolite is 1,25-$(OH)_2D_3$. Certainly 1α-(OH)D_3 is very quickly converted in the liver to 1,25-$(OH)_2D_3$ and this hydroxylation, unlike that involved in the conversion of 25-hydroxycholecalciferol to 1,25-$(OH)_2D_3$, is unlikely to be self-limiting (Holick *et al.* 1976). A Ca-binding protein (CaBP) has been isolated from rat (Bruns *et al.* 1978; Marche *et al.* 1978) and human (Tuan, 1982) placentas, and there is some evidence that this CaBP plays a major role in placental Ca transport and that its production is vitamin D-dependent (Delorme *et al.* 1979; Garel *et al.* 1981). Furthermore, 1,25-$(OH)_2D_3$ receptors have been isolated in rat and human placentas (Christakos & Norman, 1980; Pike *et al.* 1980); at birth, maternal 1,25-$(OH)_2D_3$ plasma levels and neonatal plasma Ca and P concentrations are positively correlated (Goff *et al.* 1982). Thus, as already suggested (Barlet *et al.* 1978; Ross *et al.* 1979), one physiological role for 1,25-$(OH)_2D_3$ during gestation might be to stimulate Ca and P transport across the placenta.

In conclusion, 1α-(OH)D_3 injected into pregnant ewes increased the Ca and P concentrations in foetal tissues and this increase in foetal mineral concentration was associated with a stimulation of Ca and P transfer from dam to foetus.

The authors wish to thank Dr Z. Mazor (Teva Pharmaceutical Industries, Jerusalem) for the gift of 1α-(OH)D_3 used in this experiment.

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Printed in Great Britain