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Influence of moderate food restriction on calcium metabolism in pregnant rats

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Summary — Two groups of female rats, non-pregnant (NP) and pregnant (P1), were fed *ad libitum*. A second group of pregnant rats (restricted diet, P2) were restricted for 14 d to the same intake as NP, and their ration was then increased by 5% from d 14 to d 21. For the first 3 d P1 and P2 absorbed a higher percentage of calcium than NP. During the final wk of pregnancy serum calcium decreased and 1,25-dihydroxycholecalciferol increased regardless of the dietary regimen. In both P1 and P2, femur calcium was higher than in NP on d 7 and apparent bone density increased during the 2nd wk of pregnancy and decreased from then on, this decrease being more pronounced in P2. On d 21, P2 fetuses were smaller and contained a smaller amount of calcium than those in P1, although the calcium concentration in the body remained similar in both groups of fetuses.

calcium / 1,25-dihydroxycholecalciferol / calcium balance / prenatal undernutrition / rat

Résumé — Influence d'une restriction alimentaire modérée sur le métabolisme calcique chez la ratte gravide. *Deux groupes de rattes, gravides (P1) est non gravides (NP), ont été alimentées* ad libitum. *Un autre groupe de rattes gravides, avec alimentation restreinte (P2), a reçu le même régime en quantités égales à celles consommées par le groupe NP pendant les 14 premiers jours de gestation, puis un apport supplémentaire de 5% du 14e au 21e jour. Pendant les 3 premiers jours, l'absorption du calcium était meilleure pour P1 et P2 que pour NP. Le calcium sérique a diminué et le 1,25-dihydroxycholécalciférol a augmenté pendant la dernière semaine de gestation, quel que soit le régime. Par rapport au groupe NP, la calcémie était plus faible dans le groupe P2. Le contenu en calcium du fémur au 7e jour était plus élevé dans P1 et P2 que dans NP et la densité osseuse apparente augmentait pour P1 et P2 pendant la 2e semaine, puis diminuait, cette diminution étant plus prononcée pour P2. Au 21e jour, les fœtus de P2 étaient plus petits et contenaient moins de calcium que ceux de P1, mais la teneur en calcium rapportée au poids vif était similaire dans les 2 lots.*

calcium / 1,25-dihydroxycholecalciférol / bilan calcique / dénutrition prénatale / rat

INTRODUCTION

Pregnancy requires high amounts of dietary calcium in both humans and animals. The calcium-regulating hormones, including vitamin D metabolites, play an important role in providing adequate amounts of minerals to the mother and developing fetus by acting on the absorption phase, kidney transport and bone turnover of calcium, phosphorus and magnesium.

Parathyroid secretion increases in the mother during gestation, which enhances 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) synthesis and therefore stimulates calcium absorption (Garel, 1987). There is some evidence that estrogen, prolactin and growth hormone stimulate, either directly or indirectly, the renal production of $1,25(OH)_2D_3$ (Holick and Adams, 1990). During pregnancy calcitonin also increases, which protects the maternal skeleton from PTH-induced resorption (Pitkin, 1985; Garel, 1987). The fetus also has some autonomy for regulating its own calcium metabolism. Although the fetus depends totally on its mother for calcium, its own serum calcium levels appear relatively independent of the mother's (Garel, 1987). The fetus's ability to produce calcitonin and PTH accounts for higher serum calcium levels at the end of the gestational period, yet these hormones do not pass across the placenta. Conversely, vitamin D metabolites do cross the placenta, although fetal rat kidneys can convert 25-hydroxyvitamin D to $1,25(OH)_2D_3$. Nevertheless, these metabolites are not involved in the control of fetal serum calcium, possibly because of the relatively large influx of $1,25(OH)_2D_3$. Nevertheless, these metabolites are not involved in the control of fetal serum calcium, possibly because of the relatively large influx of $1,25(OH)_2D_3$ across the placenta from both maternal synthesis and placental production (Danan *et al,* 1982; Stevenson, 1983). The placenta would then act both as a target and a source of vitamin D metabolites.

It is well known that voluntary food intake increases throughout pregnancy, thus providing a higher supply of calcium. The calcium absorption rate can also increase. In rats this may occur by d 14 (Graves and Wolinski, 1980; Toraason, 1983), d 20 (Halloran and DeLuca, 1980b) or at other times (Chef, 1969; Lai *et al,* 1984).

There is also some controversy with respect to bone changes. On the one hand, in several species including rats (Miller *et al,* 1986) and women (Heany and Skillman, 1971) a storage of calcium in the skeleton may precede intensive calcium accretion in the fetus. This is considered to be an adjustment anticipating further calcium mobilization, associated with an increase in calcitonin levels (Miller *et al,* 1986). On the other hand, Halloran and DeLuca (1980a) have pointed out that while they observed minimal variations in the femurs of vitamin D-replete rats on d 18 and 19, in vitamin D-deficient rats the volume of the femur, total bone calcium and bone calcium per unit volume tended to increase in the course of pregnancy. Pregnant rats fed on a calcium-deficient diet exhibit less bone mineral content than those fed on a standard diet (Bawden and McIver, 1964; Rasmussen, 1977; Bruns *et al,* 1987).

Several experiments were carried out in order to determine the influence of individual dietary factors on calcium homeostasis. On the practical side, calcium deficiencies are often linked to malnutrition. Severe malnutrition can affect pregnancy (Rosso and Cramoy, 1979; Munro *et al,* 1983), causing various inborn diseases, as observed in developing countries. In western countries pregnant women may also suffer from nutritional deficiencies as a result of inadequate dietary restrictions

aimed at controlling weight and preventing obstetric problems during delivery. Intrauterine growth retardation can result from this limited food intake (Rasmussen and Fellows, 1985; Leury *et al*, 1990) and is related to calcium utilization (Alvarez-Ordás *et al*, 1988).

The aim of the present work was to study the effects of moderate food restriction on the absorption and retention of calcium in pregnant rats as well as on mineral accretion in the maternal skeleton and the fetus.

MATERIALS AND METHODS

Animals and diet

Ninety female virgin Wistar rats (initial body weight 160 ± 1 g) were kept in an environmentally controlled chamber maintained at 20–22°C, with a 12-h light–12-h dark cycle and 55–70% humidity.

All the animals had free access to demineralized water. A semi-synthetic diet (table I) was prepared according to the recommendations of the National Research Council (1978).

Experimental procedure

The rats were kept in stainless-steel cages with grid bottoms for a 5-d adaptation period. They were then mated with adult males from 17.00 to 09.00 h (2 males per female rat). The 24-h period immediately following the appearance of copulation plugs was regarded as d 1 of pregnancy. Rats which did not become pregnant were used as controls.

Two groups of rats, non-pregnant (NP, n = 33) and pregnant (P1, n = 25), were fed *ad libitum*. Subsequently another pregnant group (P2, n = 31) was restricted for the first 2 wk to the intake level of NP. During the 3rd wk they were provided with 5% more food than was consumed by NP. This was done to avoid any important effects occurring as a result of severe restriction regimens throughout pregnancy, since the 3rd wk is the period of maximum intake under normal circumstances. Food restriction regimens with a tighter restraint during the first 2 wk than during the final wk have been assayed previously in rats (Jones *et al*, 1986; Pond and Mersmann, 1988). In our experiments the level of food supply during the 3rd wk for group P2 was based on the observation of the evolution of the intake of group P1 (4–5% higher intake during the 3rd wk than during the other 2).

Body weight was measured on d 1, 4, 7, 14, 18 and 21 and food intake on d 3, 5, 7, 10, 14, 16, 18 and 21. In NP (n = 10) and P1 (n = 10) faeces and urine were collected on d 3, 5, 7, 10, 14, 16, 18 and 21 and calcium balance was calculated for each wk as well as for the periods 1–3, 14–18 and 18–21 d. In P2 (n = 10) faeces and urine were only collected for the intervals

Table I. Composition of the diet.

Ingredients (g/kg)	
Casein	158
DL-methionine	2
Cellulose	53
Wheat starch	374
Sucrose	346
Olive oil	50
Sunflower oil	5
Mineral mix*	38
Vitamin mix**	15
Chemical analysis (g/kg dry matter)	
Crude protein (nitrogen x 6.25)	135
Ether extract	61
Crude fiber	50
Calcium	6
Phosphorus	5
Magnesium	0.6

* Contents (g/kg diet): $CaCO_3$: 10; $CaHPO_4$: 6.8; KH_3PO_4: 8.2; $KHCO_3$: 6.1; NaH_2PO_4: 2.3; $MgSO_4 \cdot 7H_2O$: 2.3; $(MgCO_3)_4 \cdot (OH)_2Mg \cdot 5H_2O$: 0.89; NaCl: 0.90; $FeSO_4 \cdot 7H_2O$: 0.20; $MnSO_4 \cdot H_2O$: 0.17; $ZnCO_3$: 0.03; $CuSO_4 \cdot 5H_2O$: 0.03; NaF: 0.002; Na_2CrO_4: 0.001; Na_2SeO_3: 0.0002; ** Contents (mg/kg diet): choline chloride: 1 341; folic acid: 1, nicotinic acid: 20; calcium pantothenate: 8; riboflavin: 3; thiamin hydrochloride: 5.1; pyridoxine: 6; cyanocobalamin: 0.05; retinol (4 000 IU): 1.2; cholecalciferol (1 000 IU): 0.03; DL-α-tocopheryl acetate: 30; menadione: 0.07; wheat starch: 13 584 (as excipient).

1–3 14–18 and 18–21 d and calcium balance was calculated accordingly.

Urine was collected in 0.5 M HCl. Laparotomies were performed under sodium pentobarbital anesthesia on d 7, 14, 18 and 21 of the assay. Maternal blood was taken by cannulation of the carotid artery. Serum was obtained by centrifugation for 15 min at 3 000 *g* and stored frozen at –20°C until analysis. Maternal femurs and the conceptus were removed. On d 18 and 21 individual fetuses were separated from their corresponding uterus–placentae complexes. Fetuses, uterus–placentae complexes and all other tissues were weighed separately. On the 21st d 5 litters from P1 and 8 litters from P2 were used to assess fetus mineral content. Uterus, serum and femurs of NP rats were also studied on the same days.

Apparent calcium absorption was calculated as the difference (intake – fecal excretion); calcium absorption rate as the ratio (intake – fecal excretion) x 100 / intake ; and calcium balance, or retention, as the difference (apparent calcium absorption – urinary calcium).

Analytical methods

Femurs, placentas, fetuses, duplicate feces and triplicate diet samples were dry-ashed and dissolved with $HCl/HNO_3/H_2O$ (1/1/2). Calcium was determined by atomic absorption spectrometry (Perkin–Elmer 420, Norwalk, CT) using 0.5% lanthanum ($LaCl_3$, E Merk AG) to avoid interference with phosphate and sulphate. A certified serum (Monitrol I-Normatest, American-Dade, Miami, FL) and a pool of rat feces were used as controls. The interassay variation was 1.2%.

Ionic calcium was determined in fresh blood collected directly from a cannula inserted in the carotid artery into heparinized glass capillary tubes (C951 7.5–130 Clinitubes™, Radiometer, Copenhagen). The determination was performed immediately in a ICa 1 analyzer (Radiometer, Copenhagen).

Serum 1,25-dihydroxycholecalciferol (1,25 $(OH)_2D_3$) was analyzed by radioreceptor assay (Immuno Nuclear Corp, Stillwater).

Volume and apparent density of femurs were determined *via* Archimede's principle by weighing each left femur in the air and under distilled water (any changes due to temperature were corrected).

Statistical analysis

The results of calcium balance in NP and P1 have been reported for each week. The differences between both pregnant groups were studied for those periods of gestation where maximal changes were observed in the normal pregnant group, *ie* 1–3, 14–18 and 18–21 d and compared with non-pregnant rats.

The values were analyzed for effects of either time or group by 1-way analysis of variance (ANOVA). Two sample comparisons were made by the unpaired Student's *t*-test or the non-parametric Mann–Whitney test. Correlation and regression analysis were used to assess the dependency between 2 variables. The significance of the results was established at the $p < 0.05$ level. Data were processed with the statistical programmes SAS (SAS Institute Inc, 1985) and BMPD (Biomedical Computer Programme) series 'p' (Dixon *et al*, 1983).

RESULTS

Pregnant rats in group P1 increased their food intake, with maximum values observed in the 3rd wk of gestation and a slight decrease near term (table II). The P2 rats consumed all their food, except for a few animals on the first 2 d. The mean intake values were therefore lower than in group NP but the differences were not significant. Body weight followed an exponential growth pattern in both pregnant groups ($r = 0.990$, $p < 0.01$ and $r = 0.997$, $p < 0.01$ in P1 and P2 respectively) (table III). Maternal body weight was, however, lower in P2 than in P1 throughout pregnancy. Nevertheless, there was no significant difference between these 2 groups in conceptus weight on d 7, 14, 18 and 21 (fig 1). Food efficiency for the whole gestation period (food intake/body weight gain ratio) was

Table II. Food intake in g/d (mean values and SEM for 10 animals per group).

Gestation period (d)	*Non-pregnant (NP)*		*Pregnant*			
			P1		*P2*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
1–3	15.5	1.2	19.1*	1.6	13.4[b]	0.7
3–5	16.9	0.6	20.0*	1.0	16.0[b]	0.7
5–7	16.0	0.8	20.1**	0.5	15.8[c]	0.5
7–10	16.4	0.4	20.0***	0.5	16.2[c]	0.6
10–14	15.7	0.5	21.0***	1.0	16.0[c]	0.5
14–16	16.1	0.4	21.4*	2.2	16.2[a]	0.4
16–18	16.2	0.7	21.5***	0.8	17.0[c]	0.3
18–21	16.7	0.9	19.8*	0.7	17.9[a]	0.8

Significantly different from NP: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; significantly different from P1: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.

0.28 ± 0.01 and 0.26 ± 0.01 in P1 and P2 respectively (NS). This efficiency was significantly higher ($p < 0.05$) than in NP (0.11 ± 0.01).

Serum calcium levels declined slightly in pregnant rats (P1 and P2), but the differences with NP were only significant on d 21 (table IV). Serum $1,25(OH)_2D_3$ concentrations in P1 and P2 were higher than in NP controls in the 3rd wk, whilst a significant decrease in Ca^{2+} levels was observed on d 21 in the restricted-diet group.

Table III. Changes in body weight of non-pregnant and pregnant rats fed a diet *ad libitum* and in pregnant rats submitted to moderate food restriction (mean values and SEM for 10 animals per group).

Day of gestation	*Non-pregnant (NP)*		*Pregnant*			
			P1		*P2*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
1	168.8	1.8	167.6	1.3	165.5	1.4
4	174.9	0.8	181.9*	1.3	170.5**	1.5
7	180.6	1.3	196.2*	1.9	182.0**	1.2
14	192.1	1.8	224.7*	2.3	209.0*,**	1.4
18	196.4	2.4	246.5*	5.1	228.8*,**	3.5
21	203.3	2.2	283.9*	5.4	254.2*,**	2.0

* Significantly different from NP ($p < 0.001$); ** significantly different from P1 ($p < 0.001$).

Table IV. Influence of food restriction during pregnancy on serum calcium and 1,25-dihydroxycholecalciferol concentrations in rats (mean values and SEM).

	Non-pregnant (NP)			*Pregnant*					
				P1			*P2*		
	n	*Mean*	*SEM*	n	*Mean*	*SEM*	n	*Mean*	*SEM*
Ca (mmol/l)									
7 d	6	2.61[a]	0.09	3	2.54[a]	0.04	3	2.44[a]	0.01
14 d	6	2.44[a]	0.05	3	2.49[a]	0.06	3	2.11[b]	0.01
18 d	4	2.54[a]	0.04	3	2.29[a]	0.16	3	2.33[a]	0.08
21 d	12	2.54[a]	0.02	8	2.12[a*]	0.18	12	2.11[b*]	0.06
Ca^{2+} (mmol/l)									
7 d	7	1.35[a]	0.01		–		6	1.35[a]	0.01
14 d	4	1.26[ab]	0.03		–		6	1.22[b]	0.07
18 d	8	1.36[a]	0.03		–		5	1.35[a]	0.01
21 d	4	1.33[a]	0.05		–		6	1.13[c*]	0.03
1-25-DHD (pmol/l)									
7 d	3	86.4[a]	13.0	5	78.0[a]	11.3	3	92.1[a]	1.8
14 d	4	68.5[a]	3.2	4	133.3[b*]	16.0	4	147.9[ab*]	32.1
18 d	3	88.8[a]	7.1	3	131.2[ab]	32.7	3	123.8[ab]	16.9
21 d	3	110.0[a]	21.7	6	216.9[c*]	17.0	4	210.3[c*]	18.9

Time effect: within each group, means without a common superscript letter are significantly different ($P < 0.05$); group effect: * significantly different ($P < 0.05$) from the corresponding NP value.

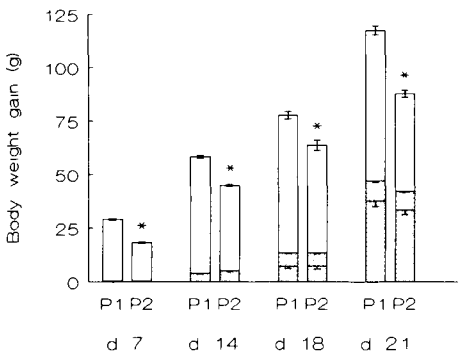

Fig 1. Weight partition between rat dam and conceptus. The total bars are divided into litter weight (▒), uterus–placentae weight (▨), and net dam body weight (▯). Values represented are means with their SEM for 4 animals per group, except for 5 and 8 animals in P1 and P2 respectively on d 21. * $p < 0.05$ from P1.

Table V shows the weekly calcium balance during normal pregnancy. Because of the higher overall food intake, the amount of calcium entering the body of pregnant rats in group P1 was greater than in NP controls. However, the differences in the calcium absorption rate for each week did not reach statistical significance (table V). Urinary calcium increased in P1 and was positively correlated with its absorption ($r = 0.715$, $p < 0.05$). Consequently the overall effect of pregnancy was an increase in the amount of calcium absorbed and retained.

A marked elevation in the amount of calcium absorbed was observed for the period 1–3 d in pregnant rats (P1 and P2) independently of dietary treatment, since the

Table V. Calcium balance of non-pregnant and pregnant rats fed on a diet *ad libitum* (mg/d) (mean values with their SEM for 10 animals per group).

Gestation period (d)	*Non pregnant (NP)*						*Pregnant (P1)*					
	1–7		*7–14*		*14–21*		*1–7*		*7–14*		*14–21*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Intake	87.1	5.2	89.1	2.4	88.7	2.2	109.0**	3.0	115.1***	3.0	116.4***	4.3
Faecal	67.7	5.2	64.9	3.0	68.3	4.9	75.0	5.2	85.7***	2.6	87.4**	4.0
Absorption	19.5	6.2	24.2	1.9	20.4	4.7	34.0*	4.3	29.4*	2.7	28.9*	2.5
% Absorption	22.9	5.7	27.3	2.1	23.0	5.1	31.8	4.2	25.2	2.1	25.0	2.0
Urinary	1.2	0.7	1.1	0.3	1.9	0.5	2.2*	1.2	1.8*	0.2	2.1	0.2
Balance	18.2	6.0	23.1	1.8	18.5	4.3	31.8*	4.2	27.1	2.6	26.9*	2.4

Significantly different from non-pregnant rats at corresponding periods: * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table VI. Influence of food restriction on calcium balance at the beginning of pregnancy and in the 3rd wk (mg/d) (mean values for 10 animals per group).

Gestation period (d)		*1–3*			*14–18*			*18–21*		
		NP	*P1*	*P2*	*NP*	*P1*	*P2*	*NP*	*P1*	*P2*
Intake	Mean	85.6	105.4*	73.9[a]	88.1	120.2***	91.6[b]	91.9	112.6***	97.8**
	SEM	4.4	8.2	7.8	2.1	6.5	2.9	2.2	2.9	3.6
Faecal	Mean	67.4	53.8***	31.7[a]*	67.7	89.8*	72.1[a]	69.1	85.0*	78.1
	SEM	3.0	9.7	4.1	4.7	6.2	3.5	5.0	3.3	4.4
Absorption	Mean	18.2	51.6***	42.2*	20.4	30.4	19.5[a]	22.8	27.5	19.9
	SEM	2.6	7.1	9.8	4.6	3.8	3.1	4.8	1.5	4.4
% Absorption	Mean	21.2	49.0***	57.1***	23.2	25.3	21.3	24.8	24.4	20.1
	SEM	2.3	4.1	8.1	5.0	3.1	3.1	5.1	1.5	3.4
Urinary	Mean	1.2	2.9*	1.8	1.8	2.2	1.4	1.9	2.0	2.2
	SEM	0.4	0.5	0.5	0.5	0.3	0.3	0.5	0.2	0.3
Balance	Mean	17.0	48.8***	40.4*	18.6	28.2	18.1[a]	20.9	25.4	17.7
	SEM	2.6	7.0	9.9	4.3	3.7	3.2	4.5	1.4	4.3

Significantly different from NP at corresponding periods: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; significantly different from P1 at corresponding periods: [a] $p < 0.05$; [b] $p < 0.001$.

calcium absorption rate was higher in P1 and P2 than in NP (table VI). Urinary calcium excretion during this period was slightly lower (NS) in P2 than in P1, so that calcium balance did not differ significantly between both pregnant groups and was higher than that of NP.

In the 3rd wk of pregnancy the percentage of calcium absorption was similar in all 3 groups of rats, and the balance results therefore paralleled the amount of calcium ingested. Accordingly, restricting the feeding of the pregnant rats P2 caused the values for absorbed and retained calcium to be slightly lower than in P1; this decrease was significant during the period 14–18 d. Urinary excretion of Ca was only 5–10% of the absorbed amount and did not show any significant differences among groups during this same period.

Weight, volume and ashes of femurs tended to increase with experimental time in all 3 groups of rats. However, on d 7, femur calcium content was higher in P1 and P2 than in NP. The relative ash content of femurs (ashes/weight) never varied in NP and P2, but in P1 it was highest on d 14 and 18. Apparent bone density in P1 increased during the second wk of pregnancy and then decreased during the 3rd wk. P2 followed the same pattern throughout pregnancy, but on the 14th d the density was higher than that of P1, and on d 18 and 21 it reached its lowest values (table VII).

Conceptus calcium accretion was minimal until d 7 and remained very small until d 14 (fig 2), but during the last wk of gestation it became 100-fold larger. No significant differences between P1 and P2 were noticed, nor was there any difference in the placentas between groups on d 18 and 21 of pregnancy (table VIII). However, on the 21st d individual fetuses from rats of group P2 were smaller, had less ashes and a lower calcium content, although the relative calcium content (mg of calcium/g fetus) was unchanged (table IX).

DISCUSSION

Both groups of pregnant rats exhibited an exponential body weight gain, as widely described (Fairweather-Tait and Payne, 1984; Marcos *et al*, 1987). However, the weight increase was lower in the restricted-diet than in the *ad libitum* group of pregnant rats presumably because their food intake was ≈ 20% less, both groups having an equivalent food efficiency during the whole pregnancy. Rasmussen and Fellows (1985) submitted pregnant rats to food level restrictions ranging from 20 to 50% of normal intake and observed a decrease in food efficiency during the first 10 d of pregnancy but not thereafter. The conceptus weight accounted for a higher proportion of body weight gained in the underfed rats than in the control pregnant rats, as shown in figure 1. This tendency is consistent with previous studies which show that under nutritional deficit conditions the maternal

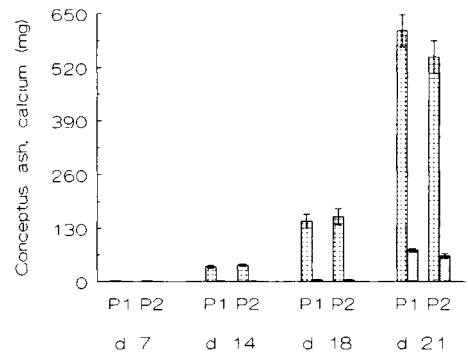

Fig 2. Influence of food restriction on conceptus mineral composition in rats. (▥) Ashes, (▨) calcium. Values represented are means with their SEM for 4 animals per group, except for 5 and 8 animals in P1 and P2 respectively on d 21.

Table VII. Bone parameters in non-pregnant and pregnant rats fed a diet *ad libitum* and in pregnant rats submitted to moderate food restriction.

	Non-pregnant (NP)				*Pregnant*							
					P1				*P2*			
Day of gestation	*7* (n = 5)	*14* (n = 5)	*18* (n = 4)	*21* (n = 8)	*7* (n = 4)	*14* (n = 5)	*18* (n = 4)	*21* (n = 6)	*7* (n = 6)	*14* (n = 8)	*18* (n = 4)	*21* (n = 12)
Weight (g)												
Mean	0.539^{a}	0.542^{a}	0.555^{ab}	0.592^{b}	0.545^{a}	0.554^{ab}	0.558^{ab}	0.605^{b}	0.516^{a}	0.541^{a}	0.577^{ab}	0.595^{b}
SEM	0.011	0.005	0.013	0.010	0.013	0.060	0.016	0.015	0.020	0.011	0.016	0.016
Volume (ml)												
Mean	0.33^{a}	0.33^{a}	0.36^{a}	0.38^{b}	0.34^{a}	0.32^{a}	0.37^{ab}	0.40^{b}	0.30^{a}	0.29^{a}	0.39^{ab}	0.40^{b}
SEM	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.03	0.01	0.03	0.02
Ashes (mg)												
Mean	194^{a}	199^{a}	209^{ab}	217^{b}	192^{a}	206^{ab}	218^{b}	220^{b}	193^{a}	199^{a}	214^{ab}	216^{b}
SEM	5	3	6	4	6	5	5	4	4	4	9	3
Ca (mg)												
Mean	60.1^{a}	67.9^{b}	68.1^{b}	77.0^{c}	65.9^{a*}	70.5^{ab}	73.0^{b}	79.0^{c}	70.0^{a*}	71.0^{a}	75.9^{a}	78.0^{ab}
SEM	1.3	1.7	1.8	1.0	1.8	1.5	1.9	1.0	2.0	2.2	5.0	2.0
Ashes/wt (mg)												
Mean	36.0	36.7	37.7	36.7	35.2^{a}	37.2^{ab}	39.1^{b}	36.4^{a}	37.4	36.8	37.1	36.3
SEM	0.8	0.4	0.9	0.5	0.8	0.9	0.8	0.7	0.9	0.5	1.0	0.7
Apparent density												
Mean	1.63	1.64	1.54	1.56	1.60^{a}	1.73^{b}	1.51^{a}	1.51^{a}	1.72^{a}	$1.87^{a*,**}$	1.48^{b}	1.49^{b}
SEM	0.04	0.02	0.04	0.03	0.03	0.04	0.03	0.03	0.09	0.04	0.05	0.05

Within each group, means without a common superscript letter are significantly different ($p < 0.05$); * significantly different from the corresponding NP value ($p < 0.05$); ** significantly different from the corresponding P1 value ($p < 0.05$).

Table VIII. Influence of food restriction on uterus–placental complex composition in rats (mean values and SEM.

	P1				*P2*			
Day of lactation	*18 (n = 4)*		*21 (n = 5)*		*18 (n = 4)*		*21 (n = 8)*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Weight (g)	6.5	0.2	9.4	0.6	6.6	0.1	8.6	0.4
Ashes (mg)	64.5	2.5	80.7	5.6	61.1	1.0	75.5	3.8
Ca (mg)	0.55	0.33	1.11	0.88	0.70	0.03	0.96	0.14

The differences between groups were not significant.

Table IX. Influence of moderate food restriction on fetus calcium content at d21 of pregnancy in rats (mean values with their SEM).

	P1 (n = 10)		*P2 (n = 16)*	
	Mean	*SEM*	*Mean*	*SEM*
Litter size	9.8	0.3	10.0	0.3
Fetal wt (g)	3.8	0.1	3.2*	0.1
Fetal ashes (mg)	53.7	1.1	48.4*	2.5
Fetal Ca				
(mg)	7.24	0.23	6.13*	0.36
(mg/wt)	1.90	0.05	1.87	0.04

* $P < 0.05$

organism decreased in weight in favor of the conceptus (Rasmussen and Fellows, 1985).

The decrease in serum calcium which is typical of pregnancy (Thomas *et al,* 1981; Bruns *et al,* 1987) was more pronounced under a restricted diet. Under this regimen a simultaneous reduction in Ca^{2+} levels was observed. Although values for the control pregnancy are not available, this result was similar to that for total calcium and agrees with findings in humans of a relationship between low dietary calcium and decreased Ca^{2+} concentrations (Salinas *et al,* 1987).

The elevation of $1,25(OH)_2D_3$ found in our experiments agrees with the observations of others (Toverud and Boass, 1979; Halloran and DeLuca, 1980b), and according to Toverud and Boass (1979) may be related to the decline in serum calcium during late pregnancy. A correspondence between the levels of $1,25(OH)_2D_3$ and the calcium absorbed appeared in the 3rd wk and only in the group of pregnant rats fed freely.

Nevertheless, the calcium absorption rate did not change with respect to the non-pregnant controls either in this group or in the restricted pregnant group.

The final third of gestation is usually considered to be associated with an intensified absorption of calcium and other minerals but such an effect has not always been demonstrated in rats (Graves and Wolinsky, 1980). In the present study the

higher absorption of calcium in the rats which consumed freely resulted from an increase in the amount of calcium ingested rather than from a specific enhancement of calcium absorption.

Halloran and DeLuca (1980b) found that the inflow of calcium in the duodenum of rats increased on d 20 of pregnancy, when the serum concentrations of $1,25(OH)_2D_3$ attained a level 4-fold higher than the basal values. It is possible that the balance technique used in our experiments failed to detect such small increases in intestinal calcium absorption. On the other hand, another interesting feature observed in several investigations on pregnant and lactating animals is that the absorption of calcium may be accomplished without vitamin D and that physiologic adjustments may offset some dietary deficiences.

Femur calcium content tended to increase in all animals during the experimental period; however, on d 7 it was higher in both groups of pregnant rats than in the non-pregnant group. This could be related to the increase in the absorption of this element during the 1–3-d interval mentioned previously. Nevertheless, the changes that apparent bone density underwent reflect some osseus accretion during mid-pregnancy followed by a decline. This was noticed in both pregnant groups and is in accordance with Miller *et al* (1986). The ratio ashes: weight in the femurs varied only in the normal pregnant rats, showing an evolution similar to that of density.

It seems therefore that the production of serum $1,25(OH)_2D_3$ was enhanced during the 3rd wk of pregnancy, independently of dietary intake and with no close relationship to calcium absorption. The levels of this vitamin D metabolite in turn show a strong correlation with the accretion of calcium in the conceptus, in agreement with the observations of Danan *et al* (1982). There is evidence that a significant quantity of $1,25(OH)_2D_3$ is produced in the placenta by the 18th d of pregnancy (Garel, 1987), when fetal mineralization starts taking place (Thomas *et al,* 1981). In our experience, the peak of $1,25(OH)_2D_3$ was simultaneous with an increase in ashes and calcium accretion of the conceptus (fig 2).

The absorption of calcium during the first few days of pregnancy was high both in absolute and relative terms irrespective of the dietary regimen, and must therefore be related to the animals' physiological status. This agrees with Pitkin's (1985) hypothesis that changes in calcium absorption might occur shortly after conception as a consequence of hormonal adjustments. Unfortunately, in our experiments serum calcium and $1,25(OH)_2D_3$ concentrations were not measured until the 7th d when no significant difference was found. Other observations indicate that serum calcium increases during the first 3 d of pregnancy (Thomas *et al,* 1981) and that circulating levels of $1,25(OH)_2D_3$ are high during the first trimester of human pregnancy (Kumar, 1980; Reddy *et al,* 1983); but the involvement of other hormones should not be ruled out.

The general nutritional deficit induced by the dietary treatment in pregnant rats led to a reduced maternal body weight. Conceptus weight, ashes and calcium content were apparently not affected, but a slight placental growth retardation, although not significant, may have contributed to a reduction in fetal growth. Limiting the animals' calcium supply did not significantly modify the efficiency of calcium absorption and urine and osseus calcium levels. Consequently the fetuses of undernourished dams exhibited at term a low weight and a smaller calcium amount than those of the untreated mothers, although the calcium concentration in their body was normal. Yet, since bone mineralization of the rat fetus takes place during late pregnancy and lactation (Stevenson,

1983), continued food restriction throughout lactation might have further deleterious effects on the progeny.

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