EFFECT OF VITAMIN D SUPPLEMENTATION IN LACTATING RATS ON THE NEONATAL GROWTH

RAMESH K. MARYA*, AJIT S. SAINI, HARBANS LAL AND KIRAN CHUGH

Departments of Physiology and *Biochemistry,*
Medical College, Rohtak - 124 001

Abstract : In lactating rats consuming a commercial diet adequate in calcium, phosphorus and vitamin D, the effect of supplementation of 3000 IU and 7,500 IU of vitamin D_3 on the lactational performance of the dams and soft tissue and skeletal growth in the pups has been investigated. On 28th day of age, the pups in the supplemented groups were significantly heavier than in the control group. Study of the indices of cellular growth in the liver and gastrocnemius muscle revealed that the increase in the soft tissue weight was due to a significant increase in protein, RNA and DNA contents (cellular hyperplasia) without any change in protein/DNA ratio (cell size). In the tibia, compared to controls, the dry bone weight and ash weight were more in the supplemented groups, but ash weight/dry bone weight ratio was not altered. The improvement in the neonatal growth was most probably due to the greater milk yield observed in the dams in supplemented groups and not due to any anabolic effect in the pups since direct administration of 500 IU or 1,000 IU of vitamin D_3 in 10 day old pups did not increase their body weight.

Key words :   vitamin D_3 rat pups   DNA RNA   liver   gastrocnemius muscle   tibia

INTRODUCTION

Recent studies in vitamin D deficient rats have revealed that the neonatal growth failure in the pups of vitamin D- deficient mothers is produced primarily by a defect in the mother, rather than in the tissues of neonate (1, 2). Vitamin D- deficient mothers were found to produce reduced quantities of milk (3). The possibility of a role of vitamin D in the production of milk is further suggested by the observations of receptors sites for 1, 25-dihydroxy vitamin D_3 in the mamary gland (4), and in the prolactin producing acidophil cells of the anterior pituitary (5). However malnutrition in lactating animals per se can also reduce the milk production (6, 7). Therefore, the reduction of lactational performance of vitamin D deficient animals may not be due directly to the vitamin D deficiency since it could be an indirect effect of maternal malnutrition produced by concurrent anorexia present in such animals. To eliminate this extraneous variable, the role of vitamin D in growth or lactation may be better investigated by supplementation rather than deprivation of the vitamin (8). In this work an effort has been made to in-

vestigate the effect of vitamin D supplementation on the lactational performance and neonatal growth in the rat.

METHODS

15-17 days pregnant Wistar rats weighing 225-250 g were obtained from Haryana Agricultural University, Hisar, and put on a commercial diet (Lipton India Ltd), containing 1% calcium, 0.6% phosphorus and 1800 IU vitamin D_3/kg diet. The rats were kept away from direct sun light in an animal room maintained at $27 \pm 1°C$. The feed and tap water were available to the animals *ad libitum*. The rats were allowed to deliver and on the third day of lactation (d3) the number of pups in each litter was adjusted to 8. Two groups of rats were administered 3,000 IU (group II) and 7,500 IU (group III) of vitamin D_3 (Dupharinteferan) in 100 μl of arachis oil as a single intramuscualar injection. The control lactating rats (group I) received 100 μl of the vehicle (n=8 for each group).

On the 14-16th day of lactation food intake and

*Corresponding Author

the milk yield of each rat was estimated. After an overnight separation the pups were allowed to suckle the dam between 8-10 a.m. Each litter was weighed before and after the feeding session and the difference was taken as the milk yield (9,10).

The dam and the pups were weighed 24 h after birth and subsequently on the 10th, 20th and 28th day of lactation/age. On d 28, three pups were selected from each litter using a table of random numbers and sacrificed by decapitation (n=24 in each group). The liver and both the gastrocnemius muscles were removed, blotted and weighed. Ten percent homogenate of each tissue was prepared in ice-cold phosphate buffered sucrose (0.25 M Sucrose in 0.05M phosphate buffer; pH 7.0) using a potter Elvehjem homogenizer fitted with a teflon pestle. Protein was estimated by biuret method (11). Nucleic acids extraction and estimations were done according to Schneider (12). The cell size (Protein/DNA ratio) and protein synthetic capacity (RNA/DNA ratio) were calculated.

For estimation of the skeletal growth, both the tibial bones were removed from each animal, cleaned of extraneous tissues, frozen and stored at-20°C. Later, the bones were thawed, thoroughly cleaned and placed in distilled water for 6 h for removal of any adherent soft tissue. The bones were then made fat-free by extracting first with 100% ethanol and then with 100% diethylether for 24 h

each using a Soxhlet extractor. After drying at 95°-100°C, for 48 h, the bones were weighed to obtain the dry weight. The bones were then ashed in muffle furnace at 550-600°C for 24 h. The ash was weighed (13).

In another series of experiments, 10 female rats (on commercial diet) were allowed to deliver and suckle their young ones. On d3, the number of pups in each litter was adjusted to 8. On d 10, 4 pups in each litter were randomly selected and administered 500 IU (Group A) or 1000 IU (Group B) of vitamin D_3 in 100 μl of arachis oil as a single intramuscular injection. The other four pups in each litter received 100 μl of the vehicle only and served as control. Both the control and vitamin D treated pups (in each litter) continued to suckle their mother. Body weights of the two groups of pups (n=20 in each group) were compared at d 10, d 20 and d 28 of age.

The data were analysed by Student's 't' test.

RESULTS

The mean body weights of the dams were similar in the three groups from d 1 to d 28 of lactation. At d 1 and d 10 mean body weights of the pups were also similar in the three groups but at d 20 and d 28, groups II and III pups were significantly heavier (P<0.05) than group I pups (Table I). The increase in the total body weight of the pups at d 28 (about

TABLE I : Effect of administration of vitamin D_3 in lactating rats on the weight of dams and their pups at different stages of lactation. Values are mean ± (g).

Lactation day	Control (Group I)	Vitamin D administration	
		3,000 IU (Group II)	7,500 IU (Group III)
Dams			
d 1	188.2 ± 10.5	195.4 ± 7.6	191.8 ± 9.1
d 10	194.3 ± 7.2	196.8 ± 8.1	193.6 ± 10.0
d 20	195.3 ± 6.1	205.2 ± 8.1	193.5 ± 9.8
d 28	189.2 ± 6.9	193.0 ± 6.1	190.8 ± 7.2
Pups			
d 1	5.9 ± 0.4	6.0 ± 0.2	5.9 ± 0.3
d 10	12.9 ± 1.0	14.1 ± 0.7	14.2 ± 0.5
d 20	23.0 ± 1.4	27.1 ± 1.9*	27.5 ± 1.9*
d 28	36.9 ± 2.0	43.0 ± 2.1*	42.8 ± 2.0*

*P < 0.05,　　　n = 8 each group

TABLE II : Effect of administration of vitamin D_3 in lactating rats on the indices of soft tissue and skeletal growth of the pups at d 28. Values are mean ± SE.

| | Control | Vitamin D administration | |
| | | 3,000 IU | 7,500 IU |
	(Group I)	(Group II)	(Group III)
Liver			
Weight, g	1.7 ± 0.1	2.0 ± 0.1*	2.1 ± 0.1*
Protein, mg	341.2 ± 18.2	430.3 ± 17.1***	438.0 ± 19.7***
RNA, mg	31.5 ± 1.7	41.1 ± 1.9***	40.9 ± 2.5***
DNA, mg	4.4 ± 0.1	5.4 ± 0.2**	5.49 ± 0.3**
Protein/DNA	76.6 ± 2.5	80.3 ± 2.5	79.78 ± 2.1
RNA/DNA	7.1 ± 0.3	7.7 ± 0.3	7.45 ± 0.3
Gastrocnemius muscle			
Weight, mg	400.0 ± 20.2	460.7 ± 10.7*	470.0 ± 20.0*
Protein, mg	36.9 ± 1.3	41.5 ± 1.1**	40.4 ± 1.0*
RNA, mg	3.3 ± 0.2	3.9 ± 0.2*	3.9 ± 0.2*
DNA, mg	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Protein/DNA	103.3 ± 3.7	91.7 ± 6.1	101.4 ± 4.1
RNA/DNA	9.3 ± 0.4	9.2 ± 0.5	9.0 ± 0.5
Tibia			
Dry bone weight, mg	64.8 ± 2.9	77.5 ± 3.1**	74.4 ± 3.5*
Ash weight, mg	37.1 ± 1.8	42.9 ± 1.9*	43.1 ± 2.1*
Ash/bone weight	0.6 ± 0.01	0.55 ± 0.01	0.58 ± 0.01

*P<0.05,   **P<0.01,   ***P<0.001,   n = 24 in each group

TABLE III : Effect of administration of vitamin D_3 during lactation on the daily food intake and milk yield of the dams on d 14-16 of lactation (Values are mean ± SE).

| | Control | Vitamin D administration | |
| | | 3,000 IU | 7,500 IU |
	(Group I)	(Group II)	(Group III)
Food intake g/day	52.1 ± 1.0	56.9 ± 1.2*	57.2 ± 1.8*
Milk yield, g	15.6 ± 1.4	21.8 ± 1.8**	21.0 ± 1.7*

*P<0.05,   **P<0.02, n = 8 in each group

TABLE IV : Effect of administration of 500 IU (Group A) and 1000 IU (Group B) of vitamin D_3 to pups at d 10 on their body weight. Values are mean ± SE (g).

| Age | Group A | | Group B | |
	Control	Vitamin D_3 500 IU	Control	Vitamin D_3 1000 IU
d 10	13.3 ± 0.8	13.7 ± 0.8	13.5 ± 0.9	14.1 ± 0.6
d 20	24.3 ± 0.5	23.2 ± 0.9	24.0 ± 0.7	22.2 ± 0.8
d 28	36.6 ± 1.0	36.1 ± 1.2	37.5 ± 1.2	36.7 ± 1.4

n = 20 in each group

16%) was reflected by an almost similar increase in the weight of the soft tissues as well as the tibia. In the soft tissues, study of the indices of cellular growth revealed a significant increase in protein, RNA and DNA contents of the liver and gastrocnemius muscle of the pups in the supplemented

groups. However, protein/DNA ratio and RNA/DNA ratio were similar to the control values (Table II). In the tibia, the dry bone weight and ash weight/dry bone weight ratio was similar to that of group I. Comparison of the data of groups II and III revealed that administration of 3,000 IU or 7,500 IU of vitamin D_3 produced similar improvement in the neonatal growth.

On 14-16th day of lactation, food intake and milk yield in groups II and III were significantly higher than in group I (Table III).

Administration of 5,00 IU or 1,000 IU of vitamin D_3 directly to the pups at d 10 did not produce any improvement in their body weights measured at d 20 and d 28 (Table IV).

DISCUSSION

The study has shown a significant increase in the neonatal growth of the pups whose mothers received moderate supplements of vitamin D_3 in the early stage of lactation (Table I). The accelerated growth involved the soft tissues as well as the bone (Table II). In the soft tissues, analyses of the pattern of cellular growth revealed evidence of cellular hyperplasia (significant increase in DNA content) without any difference in the cell size (protein/DNA ratio). In the bone significantly greater osteogenesis involved both the organic as well as inorganic components since dry bone weight and ash weight of the tibia in the supplemented groups were significantly greater than controls but the ash weight/dry bone weight ratio was not affected. The generalized acceleration of body growth in the pups of supplemented groups, suggested by these results, could be either due to greater lactational performance of the mothers or an anabolic effect in the pups due to improvement in their vitamin D status.

Measurement of milk yield on d 14-16 of lactation revealed significantly better ($P<0.05$) lactational performance of vitamin D supplemented dams (Table III). An additional evidence of improved lactational peformance of the supplemented groups was the fact that inspite of significantly greater food in-

take during lactation (Table III) groups II and III dams had mean body weights similar to that of controls at d 1 and d 28.

Between d 10 and d 20, the increase in total body weight of the pups was about 77% in group I and 93% in groups II and III. On the other hand, between d 20 and d 28, the increase in the body weight of the pups was similar (about 60%) in all the groups (Table I). In view of the fact that in rat, milk yield declines after d 21 postpartum, the data suggest that improved lactational performance of the mothers was the chief cause of growth acceleration in the pups in the supplemented groups.

In an earlier study, improved lactational performance and neonatal growth was observed by us on administration of similar doses of vitamin D on 10-12th day of pregnancy (8). However, in that study significant improvement in the body weight of the pups could be observed from d 10 onwards, whereas in this study the supplemented groups of pups were heavier than controls only at d 20 and d 28. The difference in the results of these two studies could be due to the difference in the duration of action of vitamin D_3.

The present work supports the view that mammary gland is one of the target tissues for vitamin D metabolites (14). Receptor sites for $1,25\ (OH)_2\ D_3$ have been reported in not only lactating and non-lactating mammary tissues (15) but also anterior pituitary cells involved in secretion of growth hormone (16) and prolactin (5). Moreover, human breast tumour cells in tissue culture have been shown to respond to addition of vitamin D metabolites in the medium by greater DNA synthesis (17, 18). Besides mammary glands, proliferation and differentiation of many other tissues seems to be modulated by vitamin D_3. It is believed that, like other steroid hormones, $1,\ 25\ (OH)_2\ D_3$ modulates the genomic events which might result in the anabolic effect. Secondary alterations in intracellular Ca^{++} may also be involved in the process (19).

Besides the evidences cited above, the anabolic effect of vitamin D was suggested long ago by the

clinical observation that while a daily dose of 100 IU of vitamin D was sufficient to prevent rickets, optimum growth of the infants occurred with administration of 400-600 IU of the vitamin (20). In view of the transfer of vitamin D and its metabolites in the milk (21), the improved neonatal growth of pups in the supplemented groups might have been due to an improvement in their vitamin D status. However, such a possiblility was ruled out by the observation that administration aof vitamin D_3 supplements (500 IU or 1,000 IU) directly to the pups did not improve the neonatal growth (Table IV).

REFERENCES

1. Brommage R, Neuman WF. Growth failure in vitamin D deficient rat pups. *Calcif Tissue Int* 1981: 277-280.

2. Brommage R, De Luca HF. A maternal defact is responsible for growth failure in vitamin D-deficient rat pups. *Am J Physiol* 1984; 246: E 216-220.

3. Brommage R, De HF. Vitamin D- deficient rats produce reduced quantities of a nutritionally adequate milk. *Am J Physiol* 1984; 246: E 221-226.

4. Narbiatz R, Sar M, Stumpf WE, Huang S, De Luce HF. 1, 25-dihydroxy- vitamin D_3 target cells in rat mammary gland. *Horm Res* 1981; 15: 263-269.

5. Wark JD, Tashjian AH. Vitamin D stimulates prolactin synthesis by $GH_4 C_1$ cells incubated in chemically defined medium. *Endocrinology* 1982; 111: 1755-1757.

6. Mueller AJ, Cox WM. The effect of changes in diet in the volume and composition of rat milk. *J Nutr* 1946; 31: 249-259.

7. Crnic LS, Chase HP. Models of infantile undernutrition in rats: Effect of milk. *J Nutr* 1978; 108: 1755-1760.

8. Marya RK, Lal H, Chugh K, Saini AS. Effect of vitamin D administration during pregnancy on neonantal growth in the rat. *Ann Nutr Metan* 1989; 33: 261-265.

9. Naismith DJ, Richardson DP, Pritchard AE. The utilization of protein and energy during lactation in the rat with particular regard to the use of fat accumulated in pregnancy *Br J Nutr*; 1982; 48: 433-441.

10. Chugh K, Lal H, Saini AS. Effect of feeding excess leucine diet on nursing performance an mammary gland development in rats. *Nutr Res* 1989; 9: 233-236.

11. Gornall AG, Bardawill CJ, David MN. Determination of serum protein by means of the biuret reaction. *J Biol Chem* 1949; 117: 751-766.

12. Schneider WC. Determination of nucleic acids in tissues by pentose analysis, *Meth Enzymol* 1957; 3: 680-684.

13. Marya RK, Saini AS, Jaswal TS. Effect of vitamin D supplementation during pregnancy on the neonatal skeletal growth in the rat. *Ann Nutr Metab* (In press).

14. Hollis BW, Lambert PW, Horst RL, In: Perinatal Calcium and Phosphorus Metabolism, *Amsterdam, Elsevier*, 1983; p 166.

15. Reinhardt TA, Conrad HR. Specific binding protein for 1, 25-dihydroxyvitamin D_3 in bovine mammary tissue. *Arch Biochem Biophys* 1980; 203: 108-116.

16. Haussler MR, Manolagas SC, Debtos LJ. Receptors for 1, 25-dihydroxyvitamin D_3 in GH_3 pituitary cells. *J Steroid Biochem* 1982; 16: 15-18.

17. Freake HC, Marcocci C, Iwasaki J, MacIntyre I. 1, 25- Dihydroxy-vitamin D_3 specifically binds to a human breast cancer cell line (T 47D) and stimulates growth. *Biochem Biophys Res Commun* 1981 101: 1131-1133.

18. Iwasaki Y, Iwasaki J, Freake HC. Growth inhibition of human breast cancer cells induced by calcitonin. *Biochem Biophys Res Commun* 1983: 110: 235-237.

19. Haussler MR. Vitamin D receptors: Nature and function. *Annu Rev Nutr* 1986; 6: 557-562.

20. Jeans PC, Stearns CJ. Effect of vitamin D on linear growth infancy. *J Pediatr* 1938; 13: 730-740.

21. Oh YJ, Horst RL. Vitamin D metabolites in colostrum and milk from normal, vitamin D_3 and 1, 25-dihydroxyvitamin D_3- treated cows. *Fed. Proc.* 1981; 40: 895-902.