EFFECT OF PROTEIN-ENERGY RESTRICTION, PREGNANCY AND LACTATION ON THE DEVELOPMENT OF LONG BONE IN GROWING SHEEP

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Summary

The effect of dietary protein-energy restriction (PER) on the growth of long bone were examined in sheep during growing period and then following a cycle of reproduction. Three months-old female sheep were offered protein-energy restricted feed for 6 months (growing period), thereafter changed to normal nutritional conditions for 8 months (reproduction period). A half of animals in each group took pregnancy, parturition and lactation. The nutritional restriction reduced the growth in bone diameter more than that in bone length. Compensatory growth resulting from the removal of the nutritional restraint strongly occurred in bone diameter, especially the bone cortical width, as compared to bone longitudinal growth. A cycle of reproduction severely decreased the growth in the bone cortical width relative to that in the bone width, and little effect was found on the growth in bone length and bone mass. The depression of bone development by pregnancy and lactation tended to exhibit severer in animals having received normal nutrition than in those having received PER. Bone mineral density was not affected by the nutritional restriction. A cycle of reproduction had an adverse effect on the mineral density between in animals having received normal nutrition and in those having received PER.

(Key Words: Protein Energy Restriction, Compensatory Growth, A Cycle of Reproduction, Bone Growth, Microdensitometry, Sheep)

Introduction

It is well known that dietary protein-energy restriction (PER) reduces the growth of bone in rats (Nakamoto and Miller, 1979a, b; Kuramitsu et al., 1985), man (Dickerson and John, 1969) and calf (Kanagawa et al., 1986a,b). Nakamoto et al. (1979a,b) showed that PER depressed the growth of bone length, bone volume and bone collagen synthesis, but calcium deposition in bone occurred in parallel with the formation of bone matrix in sucking rats. Kanagawa et al. (1986a,b) indicated that PER induced the inhibition of physical bone growth without affecting bone calcium content in ruminants similar to monogastric animals.

A number of studies showed that after the removal of nutritional restraint animals grew at a faster rate than normal, termed compensatory or catch-up growth (Wilson and Osbourn, 1960; Thomson et al., 1982). An increased efficiency of energy utilization, a reduced maintenance requirement and changes in the composition of body gain may account for the phenomenon of compensatory growth (Thomson et al., 1982; Blum et al., 1985). However, it is not clear whether the growth of bone completely recover from the effect of PER or not.

This study was conducted to determine the effect of PER during growing period on the growth of bone. The effects of a cycle of reproduction including pregnancy, parturition and lactation on the growth of long bone were examined for ewes fed diets differing in digestibility and nitrogen content.

Materials and Methods

Sixteen early weaned ewes of Suffolk were used. Experimental protocol is shown in figure 1. At the start of trial (month 0), 3-month-old animals were allocated to a control group and a protein-energy restricted (PER) group as shown in table 1 (growing period). The crude protein and

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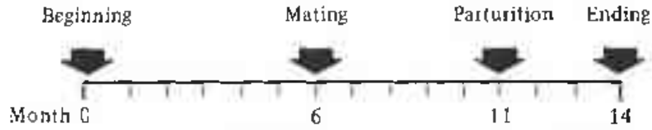
Received August 18, 1989
Accepted April 10, 1990



		Growing period	Reproduction period
Normal nutrition	Control (n=3)	Normal	Normal
	Reproduction (n=4)		Normal
Protein energy restriction (PER)	Control (n=5)	PER	Normal
	Reproduction (n=4)		Normal

Figure 1. Experimental design

metabolizable energy contents of PER diets were almost 70% and 76% as much as those of control diets, respectively. The growing period was 6 months and, then, 8 months of reproduction period was followed. At the start of reproduction period a half of animals in each group were naturally mated. Thereafter, all animals were offered the control diet (table 1). Each sheep was fed 0.5kg of the experimental diet at 9:00 and 16:00 from start to month 3 and fed 0.6kg of the diet until the end of study. Sheep in reproduction group was offered 0.3kg of additional diet daily in order to meet the increase in nutrient requirements for pregnancy and lactation. Pregnant animals had normal birth in month 11, and then suckled by their lambs until month 14.

TABLE 1. INGREDIENT AND CHEMICAL COMPOSITION OF DIETS (%)[a]

	Growing		Reproductive
	Normal	Restricted	Normal
Rice straw	0-25	0-25	0-19
Alfalfa haycube	25-36	25	25-48
Crushed corn	32-53	0	16-32
Corn cob	0	32-53	0
Wheat bran	14-32	14-32	9-32
Salt	0.25-0.38	0.25-0.38	0.25-0.33
Tricalcium phosphate	0.50-0.75	0.50-0.75	0.17-0.65
Vitamin mixture[b]	0.13-0.19	0.13-0.19	0-0.17
Mineral mixture[c]	0.13-0.19	0.13-0.19	0-0.17
DM	86.2-87.1	88.4-89.2	87.9-89.0
		DM basis	
CP	11.6-13.5	7.9-9.9	12.5-14.0
ME (Mcal/kg)	2.40-2.79	1.89-2.06	2.34-2.69
Ca	0.39-0.73	0.43-0.66	0.53-0.85
P	0.45-0.64	0.31-0.56	0.35-0.65

[a]Values are the range of given experimental diets. Chemical values of diets are calculated on the basis of standard tables of feed composition in Japan (1987) (National Research Council of Agriculture, Forestry and Fishry, 1987).
[b]One gram of vitamin mixture contains 10,000 IU of vitamin A, 2,000 IU of vitamin D and 10mg of dl-α-tocopherol acetate.
[c]One gram of mineral mixture contains 50mg of Mn, 200mg of Fe, 0.2mg of Co, 8mg of Cu, 50mg of Zn and 0.5mg of $Ca(IO_3)_2$.

Body weight and body measurements were recorded and left tibia and left metatarsus with a standard aluminium wedge were radiographed every 2 months. The bone length, width and cortical width in the middle of the bone were measured from the radiograph of left tibia. The radiograph of left metatarsus was analyzed to measure the width, the medullar width, the cortical width, the index of mass and the mineral density at 4cm from the distal terminal of the bone by using the microdensitometer to month 12 (Williams and Mason, 1962). The index of mass and the density of the metatarsus were expressed as aluminium equivalents. The relative growth coefficient was calculated by the allometry formula of by Huxley and Teissier (Yamagishi, 1987). The data were analyzed by least-squares analysis of variance and Student's t test using LSMLMW program written by Harvey(1985).

Results

Weight gain

Body weight changes are shown in figure 2. PER rapidly inhibited body weight gains and the gain in body weight of PER animals was only 44.3% of those under normal nutrition during a growing period. The change from PER to normal nutrition showed compensatory growth, although there were residual effects of the period inhibition of body weight gains detected at the end of study.

The growth of body size as body measurements are shown in table 2. PER apparently depressed the growth of whole body size. Although more

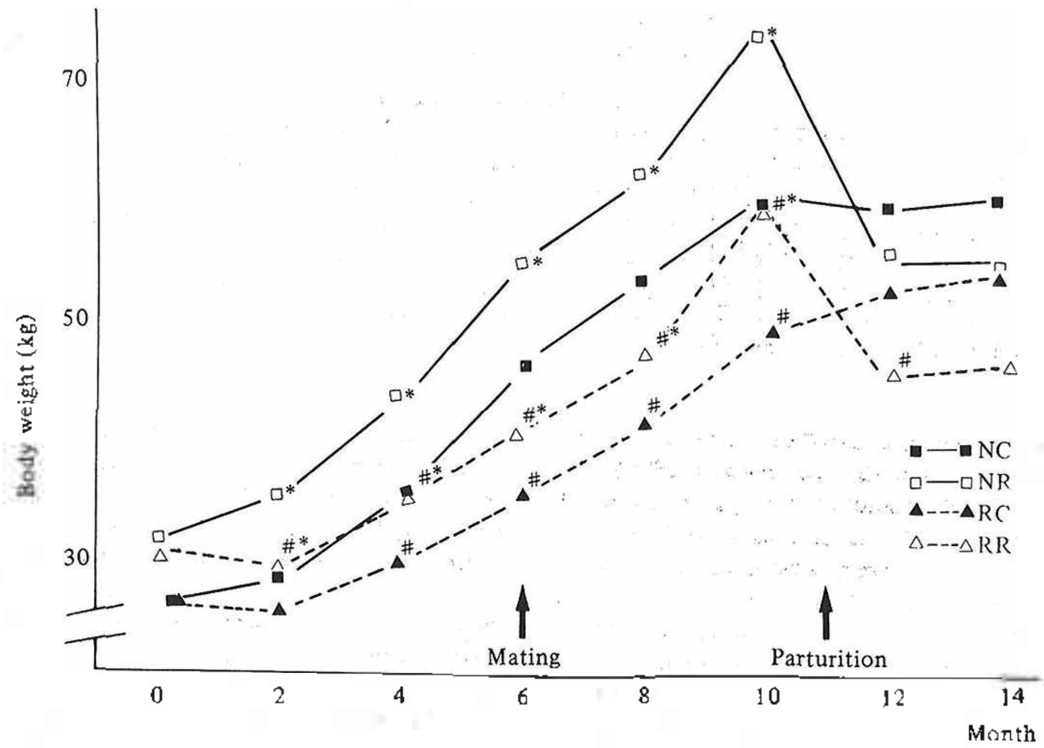


Figure 2. Effect of dietary protein-energy restriction and reproduction on body weight gains in sheep.
NC: normal nutrition and control group.
NR: normal nutrition and reproduction group.
RC: dietary protein-energy restriction and control group.
RR: dietary protein-energy restriction and reproduction group.
: Significant difference with the same group in normal nutrition, $p < 0.05$.
* : Significant difference with the control group in each nutrition, $p < 0.05$.

TABLE 2. THE GROWTH RATE OF THE BODY MEASUREMENTS DURING THE STUDY (CM)[a]

	Nutrition(N)		Reproduction(R)		Probability (p <)		
	Normal	Restricted	Control	Mate	N	R	NxR
No. of animals	7	9	8	8			
(Growing period)							
Withers height	10.0(0.7)	6.7(0.7)			0.005		
Body length	12.1(0.7)	8.3(0.6)			0.001		
Chest depth	6.9(0.4)	4.6(0.3)			0.001		
Rump length	5.1(0.2)	3.1(0.2)			0.001		
Hip width	4.9(0.3)	3.1(0.2)			0.001		
Thural width	2.7(0.3)	1.6(0.3)			0.02		
(Reproduction period)							
Withers height	4.8(0.3)	5.9(0.3)	6.5(0.3)	4.1(0.3)	0.02	0.001	0.77
Body length	9.5(0.7)	10.6(0.6)	11.1(0.7)	9.0(0.7)	0.28	0.05	0.37
Chest depth	4.7(0.2)	5.8(0.2)	6.2(0.2)	4.3(0.2)	0.003	0.001	0.07
Rump length	3.1(0.2)	4.1(0.2)	4.1(0.2)	3.0(0.2)	0.006	0.003	0.91
Hip width	3.5(0.4)	4.2(0.4)	4.0(0.4)	3.8(0.4)	0.27	0.70	0.27
Thural width	2.8(0.3)	3.0(0.3)	3.6(0.3)	2.3(0.3)	0.61	0.02	0.61
(Growing + Reproduction period)							
Withers height	14.6(0.6)	12.4(0.6)	14.8(0.6)	12.1(0.6)	0.03	0.009	0.02
Body length	9.5(0.7)	10.6(0.6)	11.1(0.7)	9.0(0.7)	0.28	0.05	0.37
Chest depth	4.7(0.2)	5.8(0.2)	6.2(0.2)	4.3(0.2)	0.003	0.001	0.07
Rump length	3.1(0.2)	4.1(0.2)	4.1(0.2)	3.0(0.2)	0.006	0.003	0.91
Hip width	3.5(0.4)	4.2(0.4)	4.0(0.4)	3.8(0.4)	0.27	0.70	0.27
Thural width	2.8(0.3)	3.0(0.3)	3.6(0.3)	2.3(0.3)	0.61	0.02	0.61

[a]Values are least squares means(SE).

growth of body size was observed by the removal of nutritional restraint, the effect of PER was sustained until the end of study; animals which had received PER remained smaller than those which received normal nutrition. A cycle of reproduction also reduced the growth of body size irrespective of the former nutritional state. The only dimension not so affected was the withers height where there was an interaction of previous nutrition and reproduction. The growth of the withers height was more strongly inhibited by the effects of reproduction in PER group than in normal nutrition group throughout the study; PER and no-reproduction was 15.0cm vs. PER and reproduction 9.8cm in contrast to normal and no-reproduction 14.7cm vs. normal and reproduction 14.5cm.

Bone growth during growing period

Similar to the body weight gains, PER depressed the growth of long bone (table 3). The growth in the length of tibia was inhibited by PER after month 4, although the width and cortical width of tibia were after month 2. The results of the microdensitometry of metatarsus also indicated that the nutritional restriction tended to affect not only the bone width, medullary width and cortical width but also the bone mass. However, the nutritional restriction had little effect on the bone mineral density (table 4). Table 5 shows the relative growth coefficient of tibia and metatarsus. PER tended to depress the growth of the bone width relative to that of the bone length.

Bone growth during reproduction period

The removal of nutritional restraint showed the compensatory growth of long bone except for

TABLE 3. THE GROWTH RATE OF TIBIA DURING THE STUDY (CM)[a]

	Nutrition(N)		Reproduction(R)		Probability(p <)		
	Normal	Restricted	Control	Mate	N	R	NxR
No. of animals	7	9	8	8			
(Growing period)							
Length	3.91(0.25)	2.23(0.22)			0.001		
Width	0.22(0.05)	0.12(0.04)			0.13		
Cortical width	0.11(0.03)	0.03(0.02)			0.06		
(Reproduction period)							
Length	1.68(0.23)	2.36(0.20)	2.12(0.22)	1.91(0.21)	0.05	0.50	0.66
Width	0.19(0.04)	0.20(0.03)	0.22(0.04)	0.16(0.04)	0.89	0.25	0.13
Cortical width	0.02(0.02)	0.04(0.02)	0.04(0.02)	0.02(0.02)	0.54	0.44	0.57
(Growing + Reproduction period)							
Length	5.60(0.28)	4.60(0.24)	5.23(0.26)	4.97(0.26)	0.02	0.50	0.98
Width	0.41(0.06)	0.31(0.06)	0.40(0.06)	0.32(0.06)	0.29	0.42	0.85
Cortical width	0.13(0.03)	0.06(0.02)	0.13(0.03)	0.06(0.03)	0.13	0.08	0.89

[a]Values are least squares means (SE).

TABLE 4. THE GROWTH RATE OF MATATARSUS DURING THE STUDY[a]

		Nutrition(N)		Reproduction(R)		Probability(p <)		
		Normal	Restricted	Control	Mate	N	R	NxR
No. of animals		7	9	8	8			
(Growing period)								
Width	(mm)	1.98(0.28)	1.20(0.23)			0.06		
Medullary width	(mm)	1.18(0.14)	0.73(0.11)			0.03		
Cortical width	(mm)	0.40(0.13)	0.23(0.11)			0.34		
Mass	(mm^2 Al)	37.80(3.84)	22.33(3.14)			0.009		
Density	(g/cm^3)	0.56(0.17)	0.32(0.14)			0.31		
(Reproduction period)								
Width	(mm)	0.87(0.36)	1.73(0.30)	1.62(0.32)	0.99(0.21)	0.10	0.22	0.74
Medullary width	(mm)	0.33(0.17)	0.40(0.14)	0.31(0.15)	0.42(0.16)	0.76	0.64	0.21
Cortical width	(mm)	0.27(0.18)	0.67(0.14)	0.65(0.16)	0.29(0.16)	0.12	0.15	0.34
Mass	(mm^2 Al)	16.24(5.88)	22.09(4.83)	19.18(5.26)	19.16(5.50)	0.46	1.00	0.66
Density	(g/cm^3)	0.08(0.23)	0.06(0.19)	0.02(0.20)	0.12(0.21)	0.95	0.74	0.15
(Growing + Reproduction period)								
Width	(mm)	2.95(0.40)	2.92(0.35)	3.19(0.38)	2.68(0.37)	0.95	0.36	0.63
Medullary width	(mm)	1.50(0.06)	1.13(0.06)	1.37(0.06)	1.26(0.06)	0.07	0.54	0.21
Cortical width	(mm)	0.73(0.20)	0.90(0.17)	0.91(0.19)	0.71(0.18)	0.54	0.47	0.97
Mass	(mm^2 Al)	53.13(4.94)	44.29(4.34)	45.03(4.73)	52.40(4.58)	0.21	0.29	0.07
Density	(g/cm^3)	0.53(0.21)	0.17(0.18)	0.16(0.20)	0.54(0.19)	0.23	0.20	0.32

[a]Values are least squares means (SE).

TABLE 5. THE RELATIVE GROWTH COEFFICIENT OF TIBIA AND METATARSUS DURING THE STUDY[a]

	Nutrition(N)		Reproduction(R)		Probability(p <)		
	Normal	Restricted	Control	Mate	N	R	NxR
No. of animals	7	9	8	8			
(Growing period)							
Width/Length	0.715(0.284)	0.484(0.250)			0.56		
Cortical/Length	1.184(0.432)	0.749(0.381)			0.47		
Cortical/Width	0.932(0.502)	1.556(0.410)			0.36		
Medullary/Width	1.019(0.274)	0.726(0.224)			0.43		
(Reproduction period)							
Width/Length	1.261(0.379)	1.259(0.333)	1.684(0.362)	0.837(0.351)	1.00	0.12	0.10
Cortical/Length	0.370(0.522)	0.661(0.459)	0.800(0.499)	0.232(0.484)	0.69	0.43	0.36
Cortical/Width	0.742(0.294)	1.987(0.241)	1.974(0.263)	0.754(0.275)	0.008	0.009	0.004
Medullary/Width	1.143(0.205)	0.406(0.168)	0.326(0.183)	1.223(0.192)	0.02	0.007	0.007
(Growing + Reproduction period)							
Width/Length	0.914(0.143)	0.897(0.126)	1.020(0.137)	0.791(0.133)	0.94	0.26	0.91
Cortical/Length	1.060(0.264)	0.695(0.232)	1.283(0.253)	0.472(0.245)	0.32	0.04	0.91
Cortical/Width	1.125(0.168)	1.515(0.148)	1.305(0.161)	1.335(0.156)	0.11	0.90	0.85
Medullary/Width	0.900(0.099)	0.685(0.087)	0.776(0.094)	0.808(0.091)	0.13	0.82	0.90

[a]Values are least squares means (SE).

the medullary width of metatarsus. However, despite evidence of compensatory growth, the length of tibia($p < 0.02$) did not recover as completely as did width, cortical width and mass of metatarsus; the latter indicated no effect of PER at the end of the study (table 3,4). Especially, the change from PER to normal nutrition stimulated the growth of the bone cortical width relative to that of the bone width and inhibited the growth of bone medullary width (table 5). These trends were observed throughout the study, i.e., animals having received the nutritional restriction thickened the bone cortical width and thinned the bone medullary width relative to the bone width.

A cycle of reproduction had a trend to depress the growth in the width and cortical width of metatarsus, irrespective of the nutritional state before reproduction period, although there was no effect on the growth in the length of tibia and the mass of metatarsus (table 3,4). Indeed, the growth of the bone width relative to that of the bone length was inhibited by reproduction in animals having received normal nutritional state. As the data of each group can be shown, the relative growth coefficient of the width relative to the length was 2.149 in ewes of normal nutrition and no-reproduction and 0.373 in ewes of normal nutrition and reproduction. In contrast to ewes of normal nutrition, the relative growth coefficient of the width relative to the length was 1.218 in ewes of PER and no-reproduction and 1.301 in ewes of PER and reproduction. Furthermore, the growth of the bone cortical width relative to that of the bone width was strongly depressed, while the growth of the bone medullary width was stimulated in animals having received the normal nutritional state.

A cycle of reproduction had a different effect on the mineral density of metatarsus depending upon the previous nutritional state. In animals having received normal nutritional state, pregnancy and lactation increased in the bone mineral density. When the effect of a cycle of reproduction on the mineral density in each group was considered, the change of the bone mineral density during reproduction period was −0.20 in ewes of normal nutrition and no-reproduction and 0.36 in ewes of normal nutrition and reproduction. On the contrary, in animals having received PER, the bone mineral density was decreased by pregnancy and lactation during reproduction period (−0.12) while animals without mating increased (0.24).

Discussion

Body weight gain and bone growth were clearly reduced by feeding the nutritional restricted diet. However, it could not be determined whether low metabolizable energy intake, low protein intake or these combinations were responsible for the reduction or retardation of growth in sheep, because both the crude protein and metabolizable energy contents of the experimental diets were restricted.

Protein-energy restriction reduced the growth in the length, width, cortical width and mass of long bone in sheep as well as rat (Nakamoto and Miller, 1979a,b; Kuramitsu et al., 1985), man (Dickerson and John, 1969) and calf (Kanagawa et al., 1986a,b). The inhibition of the growth in the bone width and cortical width preceded that in the bone length. PER also had a trend to depress to a great extent the growth of the bone width relative to that of the bone length. The result was in agreement with report by Kuramitsu et al. (1985). It is conceivable from these results that PER is likely to affect more strongly the growth in diameter of long bone rather than that in length.

Compensatory growth in the bone width and cortical width rapidly occurred upon removal of the nutritional restriction, leading to the complete recovery from the inhibition of bone growth by these criteria. However, the vertical growth did not catch up following the removal of nutritional restraint. Furthermore, a cycle of reproduction inhibited the growth in the bone width and especially cortical width relative to that in the bone length. The growth in diameter of long bone might be more sensitive to the protein-energy nutritional state when compared with the growth in length.

The growth in length of long bone is achieved on the basis of a process of proliferation of chondrocytes at the growth plate, while the growth in diameter results from the apposition of new bone by osteoblast on the periosteal surface (Vaughan, 1981). The difference in the bone growth pattern between the diameter and the length might be responsible for the difference in the response of the nutritional stress. It is well known that the alteration of nutritional state sharply changes endocrine function (Pimstone, 1976; Blum et al., 1985; Hart and Johnsson, 1986). Blum et al. (1985) reported that food restriction resulted in the decreases of plasma immunoreactive insulin (IRI), thyroxine(T_4) and triiodothyronine(T_3) concentrations and the increase of plasma growth hormone(GH) concentrations, while the removal of the restriction rapidly increased the plasma IRI, T_4 and T_3 and decreased the plasma GH.

It is possible that the changes of hormonal secretion and local growth factors involved in bone formation are responsible for the difference in the bone growth pattern (Canalis, 1983). Kanagawa et al.(1986a) indicated that PER decreased serum T_4, suggesting that it was associated with the depressed bone growth in calves. However, T_4 injection to weaned rats under PER failed to recover of the growth in the bone length and width (Kanagawa et al., 1987). Furthermore, T_3 injection to protein-depleted rats also had no effect on the growth in the bone width (Matsui et al., unpublished observation).

Shrader and Zeman (1973) showed that in rats, after prenatal protein-energy malnutrition postnatal normal diet supply resulted in compensatory growth but there was a residual effect of reduced growth in the bone width. Although PER inhibited body weight gains in this study, animals which received PER gained 9.7kg body weights during growing period. It was probable that PER in this study was quite mild, so that the removal of the nutritional stress permitted complete recovery of growth in bone width. The differences in the degree, time and period of nutritional restraint and animal species may be responsible for the discrepant results.

PER had no effect on the bone mineral density, similar to the results by Nakamoto and Miller (1979b) and Kanagawa et al.(1986a,b). Protein deprivation has been shown to decrease the intestinal calcium-binding protein activity, which is thought to be responsible for an active absorption of calcium, with a consequent decrease in the rates of intestinal calcium absorption and bone calcium accretion in rats (LeRoith and Pimstone, 1973; Kalk and Pimstone, 1974). Braithwaite (1978) also reported the depressed intestinal calcium absorption as well as in rats. Even if these changes occurred in this study, the bone mineral density might be unaffected by PER. PER was thought to affect the bone size rather than the bone mineral density.

The bone size was reduced by PER in this study. Sykes and Field(1972) suggested that protein restriction might result in impaired formation of bone matrix, which induced the decrease in the rates of accretion of calcium into bone. In rats, it was shown that protein deficiency inhibited the deposition of bone matrix (El-Maraghi et al., 1965) and the bone mineral content remained normal (Le Roith and Pimstone, 1973)

The effect of a cycle of reproduction on the growth in bone mineral density was quite different from that of nutritional restraint. Animals having received normal nutrition increased the bone density during reproduction period, although those having received PER decreased. This might be correlated with the growth in the bone cortical width. That is, the growth in the bone cortical width was more strongly reduced in normal nutrition group during a cycle of reproduction, little change was found in the growth of bone cortical width in animals having received PER. The little reduction of bone cortical width by a cycle of reproduction might lead to the reduction of bone density in animals having received PER. It may be concluded that the bone mineral density is unaffected by dietary protein energy restriction while the bone mineral density is reduced by a cycle of reproduction in animals having received the protein-energy restricted diet.

Acknowledgement

This study was supported by Grant-in-Aid for scientific research from the Japanese Ministry of Education, Science and Culture (No. 59480079).

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