

# Effects of physical exercise during pregnancy and protein malnutrition during pregnancy and lactation on the development and growth of the offspring's femur

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## Abstract

**Objective:** To evaluate the effects of physical training of mother rats during pregnancy associated with a low-protein diet offered during pregnancy and lactation on the development and growth of the femur of their offspring.

**Methods:** Forty 90-day old male Wistar rats were divided into four groups: pups of sedentary nourished mothers, pups of sedentary malnourished mothers, pups of trained nourished mothers, and pups of trained malnourished mothers; all groups included 10 rats. Physical training on a treadmill for 8 weeks, 5 weeks before conception and 3 weeks in the gestational period for mother rats of pups of trained nourished mothers and pups of trained malnourished mothers. Induction of low-protein diet to the mother rats during pregnancy and lactation for the groups of pups of sedentary malnourished mothers and trained malnourished mothers. After the pups were sacrificed, on the 90th day of life, we analyzed weight, length, and femoral bone mineral content.

**Results:** Decreased body weight, femur weight, and femur length ( $p < 0.05$ ) were observed for the groups of pups of sedentary malnourished mothers and trained malnourished mothers in comparison with to the groups of pups of sedentary nourished mothers and trained nourished mothers, respectively. There was no difference in bone mineral content of the femur in either of the groups.

**Conclusion:** Mild physical training on the treadmill during pregnancy does not interfere with bone development and growth of the offspring. However, protein malnutrition during this period and during lactation promotes permanent damage to the bone structure of the offspring.

*J Pediatr (Rio J). 2010;86(3):233-238: Physical training, protein malnutrition, pregnancy, post-natal, femur.*

## Introduction

During pregnancy, the practice of moderate and appropriate physical activity brings several benefits to different systems of the maternal body.<sup>1</sup> On the other hand, physical exercises performed in an inadequate manner by pregnant women may impair fetal development, since this practice may interfere with the distribution of blood

flow to the placenta, damaging the supply of oxygen and nutrients.<sup>1</sup>

It is natural for pregnant women to need a higher carbohydrate intake, both at rest and during exercise, than non-pregnant women.<sup>2,3</sup> After the 13th week of pregnancy, about extra 300 kcal per day are needed to meet the

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metabolic needs of a pregnant woman.<sup>1,4</sup> This extra energy requirement is increased when the daily energy expenditure is increased due to exercises.

On their turn, proteins are also nutrients necessary for cell homeostasis, and when a pregnant woman has a protein deficit, known as prenatal malnutrition, the course of the pregnancy will be affected, causing changes in the tissues and organs of the fetus.<sup>5</sup> Bone tissue is sensitive to protein malnutrition due to the fact that its non-mineral bone composition is mostly constituted by protein; therefore, it is evident that there is a relationship between proper protein absorption and bone metabolism<sup>6,7</sup> and that prenatal and neonatal malnutrition will lead to disorders in bone growth.<sup>8</sup> However, not only the period in which it occurs, but also the duration of the nutritional deficit is an important factor to estimate the effects on skeletal growth.<sup>9</sup>

Due to the large increase in physical activity practice by pregnant women and high prevalence of malnutrition in developing countries, it is important to conduct experimental studies to assess possible consequences of both factors: effect of physical exercise and malnutrition induced in pregnant rats on the process of bone growth of the offspring. Based on this information, the objective of the present study was to evaluate the effects of physical training induced in pregnant rats associated with low-protein diet induced during pregnancy and lactation on the bone tissue growth of pups.

## Methods

### *Experimental design*

Before conception, twenty-eight adult female albino rats (Wistar) were randomly divided into two groups: sedentary mother (Sm) and trained mother (Tm). In order to adapt to the physical training, the rats of Tm group performed a low intensity run (0.3 km/h<sup>-1</sup> during 10 min) on a treadmill (motorized treadmill INSIGHT®, 1380 x 600 x 400 mm - L x W x H) for 3 consecutive days. Next, the rats accomplished a moderate physical training program in accordance with the protocol suggested by Fidalgo.<sup>10</sup> The intensity used was approximately 50% of the maximum speed reached during the performance test and 70% of VO<sub>2max</sub> (maximal oxygen uptake). During this period, the animals in the Sm group remained in their cages.

After 5 weeks of physical training, the rats in both groups were mated (one male rat to three female rats). After conception was confirmed using a vaginal smear test,<sup>11</sup> the training protocol was changed for the rats of the Tm group. The speed and duration of the stages were progressively reduced, featuring a mild training, with approximately 25% of maximum speed reached during the maximum performance test and 30% of VO<sub>2max</sub>.<sup>10</sup>

Furthermore, after pregnancy was confirmed, half of the mother rats in each group (Sm and Tm) were offered

low-protein diet (8% casein protein) and the remaining rats received a normal protein diet (17% casein protein) during the entire period of gestation and lactation of pups. One day after birth, the litter was standardized in six male pups per mother. This number seems to provide higher lactotrophic potential (Committee on Laboratory Animal Diets).

After lactation, on the 22nd day of life of the pups, the mothers were separated from their offspring and all animals received the same standard diet in guinea pig cages (LABINA, Purina do Brasil). Pups were kept in an experimental animal facility, at temperature of 23 °C ± 1, inverted light-dark cycle of 12/12 hours and free access to water and food.

Four experimental groups were defined comprising 40 male pups: pups of sedentary nourished mothers (pSNm, n = 10), pups of sedentary malnourished mothers (pSMm, n = 10), pups of trained nourished mothers (pTNm, n = 10), and pups of trained malnourished mothers (pTMm, n = 10).

The animals' body weight was weighed daily from birth until 90 days of life (using a scale Marte®, model S-4000, with a sensitivity of 0.1 g). In the 90th day of life, the animals were sacrificed by decapitation and an incision was made from the right lower abdominal region to the knee on the same side for disarticulation and femur removal. After dissection, femurs were fixed in buffered formalin (10 mL formalin at 37% and 27 mL of 0.1 M phosphate buffer and pH = 7.0) at a volume 50 times higher than the sample and stored in containers. The procedures performed in the present study were approved by the Research Ethics Committee on Animal Experiments (CEEAA) of Universidade Federal de Pernambuco (UFPE) and complied with the standards suggested by the Brazilian Committee of Animal Experiments (COBEA).

### *Morphometric analysis*

The weight of femurs was measured using a digital hydrostatic balance (sensitivity for density 0.001 g; maximum capacity of 500 g, and minimum capacity of 0.02 g) (Marte®). The total bone length was determined using a pachymeter (Western, 0.02 mm). Finally, after decalcification carried out using nitric acid solution at 10% for 2.5 hours for the pSNm and pTNm groups and 2 hours for the pSMm and pTMm groups, bone mineral content was calculated using the formula suggested by Gomes et al.<sup>12</sup>

### *Statistical treatment*

The data on body weight, femur weight, femur length, and femur mineral content of the pSNm, pSMm, pTNm and pTMm groups of the animals studied were statistically analyzed using the Student's *t* test. Data are presented as mean ± standard deviation in a table, and p-value was < 0.05.

## Results

### Weight evolution

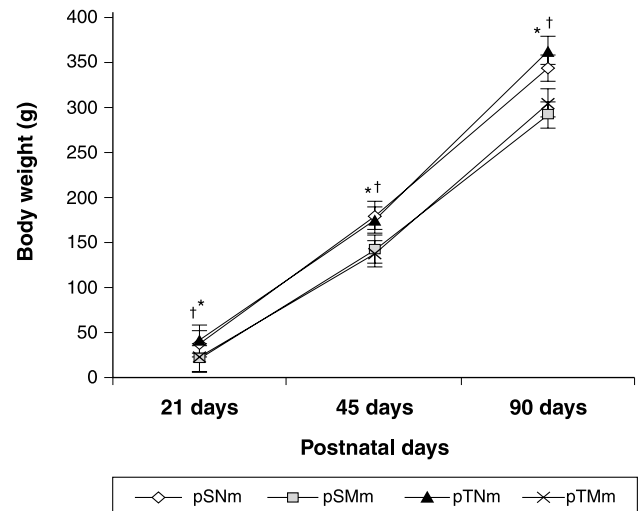
At 21 days of life, weaning phase of the animals, pups of the pSNm group ( $38.33 \pm 6.4$  g) showed difference in body weight in relation to the pSMm group ( $21.77 \pm 3.89$  g). Likewise, pups of the pTNm group ( $40.5 \pm 2.88$  g) showed a difference in body weight at 21 days of life compared to the pups in the pTMm group ( $22.25 \pm 2.75$  g). However, we did not observe any difference in body weight between the animals of the pSNm and pTNm groups, as well as no difference in body weight at 21 days of life between the animals of the pSMm and pTMm groups. Therefore, only malnutrition caused changes in body weight, since only the malnourished animals showed a difference in body weight at 21 days of life compared to the malnourished groups (Figure 1).

At 45 days of life, the body weight of the animals in the pSNm group ( $174.57 \pm 8.67$  g) showed a difference in body weight in relation to the pSMm group ( $143 \pm 6.48$  g). The same happened with the pups of the pTNm group ( $173 \pm 17.34$  g), which showed a difference in body weight when compared to the pups in the pTMm group ( $133.6 \pm 16.74$  g). On the other hand, we did not find any difference in body weight between the animals of the pSNm and pTNm groups, as well as no difference in body weight at 21 days of life between the animals of the pSMm and pTMm groups. The difference in body weight persisted in the group of malnourished animals, which occurred at 21 days of life, since malnutrition was the only factor of change in the absolute weight of the animal (Figure 1).

In the present study, we found that the malnutrition induced during pregnancy and lactation of mother rats had an influence on the body weight of pups, since the weight of the animals in the pSNm group ( $340.33 \pm 16.24$  g), measured at 90 days of life, was higher in comparison with the values found for the pSMm group ( $249.88 \pm 21.86$  g). However, physical training of nourished pregnant rats did not cause changes in the body weight of their offspring, since there was no difference in the body weight of the pups in the pSNm and pTNm groups ( $363.3 \pm 30.14$  g). In both groups of pups of malnourished mothers, physical exercise induced in the malnourished rats also did not affect the body weight of their offspring, since the body weight of the pups in the pSMm group remained equivalent when compared to the body weight of the offspring of the pTMm group ( $305 \pm 16.80$  g). Animals in the group pTNm, on their turn, had higher body weight compared to the body weight of the offspring in the pTMm group after 90 days (Figure 1).

### Femur weight

At 90 days of life, the femur weight of the offspring in the pSNm group ( $0.892 \pm 0.0661$  g) proved to be greater than the femur weight of the offspring in the pSMm group



pSMm = pups of sedentary malnourished mothers; pSNm = pups of sedentary nourished mothers; pTMm = pups of trained malnourished mothers; pTNm = pups of trained nourished mothers.

\* Relationship between SN and SM (21 days  $p < 0.0001$ , 45 days  $p < 0.0001$ , 90 days  $p < 0.0001$ )

† Relationship between TN and TM (21 days  $p < 0.0001$ , 45 days  $p < 0.0001$ , 90 days  $p < 0.0001$ )

Relationship between SN and TN (21 days  $p < 0.0573$ , 45 days  $p < 0.542$ , 90 days  $p < 0.1143$ )

Relationship between SM and TM (21 days  $p < 0.6855$ , 45 days  $p < 0.2969$ , 90 days  $p < 0.1572$ )

**Figure 1** - Weight evolution, including comparative analysis of body weight (g) between the groups of pups whose mothers were malnourished and nourished and those whose mothers were trained nourished and malnourished at 21 days, 45 days, and 90 days of life, statistically analyzed using the Student's *t* test, with  $p < 0.05$

( $0.81 \pm 0.058$  g), which had malnutrition induced during gestation and lactation. However, the femur weight of the animals in the pSNm group did not show any difference in relation to the femur weight of the animals in the pTNm group ( $0.9 \pm 0.0528$  g), whose nourished mothers practiced physical activity during pregnancy. The same also occurred regarding the femur weight of the animals in the pSMm group when compared to the femur weight of the animals in the pTMm group ( $0.813 \pm 0.0334$  g). In the same period, the femur weight of the pups in the pTNm group was higher than the femur weight of the pups in the pTMm group, in which the mother rats in both groups practiced physical activity during pregnancy (Table 1).

### Femur length

At 90 days of life, the femur length, which indicates the longitudinal growth of the bone, in the pSNm group ( $35.787 \pm 0.99$  mm) was higher than the length of the femur of the pups in the pSMm during gestation and lactation ( $34.46 \pm 0.37$  mm). The same group pSNm, on its turn, showed that the femur length of its pups was equivalent to the femur length of the pups in the pTNm group ( $35.599 \pm 0.46$  mm),

as well as the femur length of the offspring in the pSMm group was also equivalent to the femur length of the offspring in the pTMm group ( $34.302 \pm 0.718$  mm) in the same period. The animals in group pTNm showed higher values of femoral length when compared to the values of the pTMm group (Table 1).

### Bone mineral content

Neither the physical training during pregnancy of mother rats nor the low-protein diet induced in mother rats during pregnancy and lactation affected the mineral content of the femur of their pups because there was no difference in mineral content of the femur of the pups of the pSNm ( $30.386 \pm 5.776$  mg/cm<sup>2</sup>), pSMm ( $25.012 \pm 6.297$  mg/cm<sup>2</sup>), pTNm ( $30.18 \pm 6.16$  mg/cm<sup>2</sup>) and pTMm groups ( $23.671 \pm 7.775$  mg/cm<sup>2</sup>) at 90 days of life (Table 1).

### Discussion

The individual's growth is conditioned not only by genetic background but also by environmental stimuli, such as, for example, adequate supply of nutrients and practice of physical activity.<sup>1</sup> A low-protein diet during critical periods of development severely affects all tissues, causing harmful effects on the growth of several organs, mainly the bone tissue in patients undergoing such aggression.<sup>13,14</sup>

In this study, protein malnutrition imposed to the mother rats during pregnancy and lactation periods resulted in a deficit observed until the end of the study regarding the body weight of the offspring. Similar to these findings, Golstein & Bond<sup>15</sup> observed that mice that underwent protein deprivation during lactation weighed less than the control animals, which persisted even with nutritional supplementation. This phase is considered a period of physiological stress for the newborn, during which it goes

through several adjustments leading to a high energy expenditure.<sup>16</sup> Thus, the infant's weight gain rate is high, and inadequate intake regarding the quality or quantity of food may influence the reduction in body weight.<sup>17</sup>

The physical training on a treadmill carried out by the nurturing mothers during pregnancy, on its turn, did not cause changes in the pups' weight. Unlike our findings, a study conducted with pregnant women who exercised during pregnancy showed that their children were born weighing more than the children of sedentary mothers.<sup>18</sup> Likewise, Clapp et al.<sup>19</sup> highlighted that adequate physical exercise throughout the whole human pregnancy, or part of it, may contribute to the increase in fat percentage, weight, and head circumference of children. However, the physical training protocol used in the study by Clapp et al.<sup>19</sup> differs from that used in the present study: the maximum aerobic capacity corresponded from 55 to 60%, the physical training lasted for 20 min, from three to five times a week, and the physical exercise included the use ladder or stool.

The femur weight of the offspring was assessed at the age of 90 days of life and showed a behavior similar to that presented by body weight, with decreased weight of the animals whose mothers were malnourished during the critical period of development. Similarly, Boyer et al.<sup>20</sup> demonstrated that the femur weight was also reduced according to the nutritional deficit. Malnutrition during these important phases of development causes failure to complete bone recovery at least until 100 days of life in rates<sup>21</sup> and up to 6 months of life in human beings.<sup>22</sup>

Physical training during pregnancy, on its turn, did not change the femur weight of the pups. There are no reports in the literature demonstrating the influence of physical training during pregnancy on the bone tissue of the offspring, making it impossible to make any comparison with the findings of the present study.

**Table 1** - Mean values and standard deviation of the femur weight, femur length, and femur mineral content of the offspring in the groups whose sedentary and malnourished mothers and nourished and malnourished mothers trained, assessed at 90 days, analyzed by the Student's *t* test, with  $p < 0.05$

	pSNm	pSMm	pTNm	pTMm
Femur weight (g)	$0.892 \pm 0.0661$	$0.81 \pm 0.058^*$	$0.9 \pm 0.0528$	$0.813 \pm 0.0334^†$
Femur length (mm)	$35.787 \pm 0.99$	$34.46 \pm 0.37^‡$	$35.599 \pm 0.46$	$34.302 \pm 0.718^§$
Mineral content (mg/cm <sup>2</sup> )**	$30.386 \pm 5.776$	$25.012 \pm 6.297$	$30.18 \pm 6.16$	$23.671 \pm 7.775$

pSMm = pups of sedentary malnourished mothers; pSNm = pups of sedentary nourished mothers; pTMm = pups of trained malnourished mothers; pTNm = pups of trained nourished mothers.

\* pSNm > pSMm ( $p = 0.008$ ); pSNm = pTNm ( $p = 0.765$ ); pSMm = pTMm ( $p = 0.831$ ).

† pTNm > pTMm ( $p < 0.001$ ).

‡ pSNm > pSMm ( $p = 0.001$ ); pSNm = pTNm ( $p = 0.765$ ); pSMm = pTMm ( $p = 0.831$ ).

§ pTNm > pTMm ( $p < 0.001$ ).

\*\*pSNm = pSMm ( $p = 0.062$ ); pSNm = pTNm ( $p = 0.939$ ); pSMm = pTMm ( $p = 0.677$ ); pTNm = pTMm, ( $p = 0.053$ ).

Some studies have reported a decrease in the femur length after induced malnutrition both in the early period (postnatal)<sup>23,24</sup> in late (after the postnatal period). In the present study, the femur length of the pups of malnourished mother rats was reduced at 90 days of life. The reduction in the length of long bones, such as the femur, due to a protein deficiency has often been attributed to a decrease in bone formation<sup>25</sup> and a lower rate of appositional bone growth.<sup>26</sup> Physical training during the gestational phase, similarly to the data of body weight and femur weight, did not change the femur length of the pups. We expected that the stress of training was harmful, aggravating the damage caused by the nutritional aggression against the bone, since physical activity occurs during the formation of bone tissue and also uses extra energy expenditure in addition to the required energy intake during the gestational period for the development of the fetus.

In our study, we found that the time of decalcification of malnourished femurs was reduced in 30 min with respect to the nourished femurs. The suppression of longitudinal growth of long bones of animals caused by protein malnutrition during lactation has been described by several studies, and most likely occurs as a result of a quantitative decrease in bone formed than due to a change in the mineral content.<sup>27,28</sup> Our findings support this hypothesis, since there was no difference in the mineral bone content among the groups, even when being influenced by the treadmill training during pregnancy and/or protein malnutrition of the mother during pregnancy and lactation.

The concept of "fetal programming" suggests that the fetus can be programmed during intrauterine development for developing diseases in adulthood, and it has been discussed as a key factor to understand the origin of some disorders observed during adulthood.<sup>29</sup> The basic hypothesis suggests that the fetus is programmed in the uterus to develop many diseases in adulthood, including mainly metabolic and cardiovascular diseases as a result of some attacks, which permanently change physiological and metabolic processes. The specific nature of these attacks is unclear, however, there seem be involvement of fetal growth retardation at a specific stage of development.<sup>30</sup>

The model of experimental protein malnutrition of our study was induced at the time of maternal depletion of reserves, which is common during pregnancy and lactation and could have been exacerbated by increased nutritional demand during physical training. Based on these findings, there is stronger evidence that protein malnutrition imposed during critical developmental periods leads to a stunted growth of the animal and that the severity of this deficit is related to stage of life during which it was induced, which are crucial periods for bone formation and type of impairment.<sup>23,25</sup>

Protein malnutrition during pregnancy and lactation promotes permanent damage to the bone structure of

the pups. However, the practice of mild physical activity performed by female rats during pregnancy does not intensify the changes in the bone structure of the malnourished offspring or promotes changes in the bones of nourished offspring.

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## References

1. Clapp JF. Exercise in pregnancy: a brief clinical review. *Fetal Med Rev.* 1990;161:1464-9.
2. Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D. Growth in infancy and bone mass in later life. *Ann Rheum Dis.* 1997;56:17-21.
3. Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C. Intrauterine programming of adult body composition. *J Clin Endocrinol Metab.* 2001;86:267-72.
4. Ilich JZ, Skugor M, Hangartner T, Baoshe A, Matkovic V. Relation of nutrition, body composition and physical activity to skeletal development: a cross-sectional study in preadolescent females. *J Am Coll Nutr.* 1998;17:136-47.
5. Trindade CE. Repercussões da nutrição da gestante sobre o recém-nascido. *J Pediatr (Rio J).* 1997;73:291-2.
6. Rizzoli R, Bonjour JP. Determinants of peak bone mass and mechanisms of bone loss. *Osteoporos Int.* 1999;9:S17-23.
7. Yoshimura T, Tohya T, Onoda C, Okamura H. Poor nutrition in prepubertal Japanese children at the end of World War II suppressed bone development. *Maturitas.* 2005;52:32-4.
8. Gudehithlu KP, Ramakrishnan CV. Effect of undernutrition on the chemical composition and the activity of alkaline phosphatase in soluble and particulate fractions of the rat calvarium and femur. II: effect of preweaning undernutrition in the suckling rat. *Calcif Tissue Int.* 1990;46:378-83.
9. Hughes PC. Catch-up growth in the limbs of rats undernourished for different lengths of time during suckling. *Acta Anat (Basel).* 1986;125:50-8.
10. Amorim MA. Desnutrição experimental e treinamento físico: estudo de parâmetros murinométricos de ratas gestantes e dos conceptos [dissertação]. Recife: Universidade Federal de Pernambuco; 2006.
11. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. *Braz J Biol.* 2002;62:609-14.
12. Gomes RJ, de Mello MA, Caetano FH, Sibuya CY, Anaruma CA, Rogatto GP, et al. Effects of swimming training on bone mass and the GH/IGF-1 axis in diabetic rats. *Growth Horm IGF Res.* 2006;16:326-31.
13. Miller JP, German RZ. Protein malnutrition affects the growth trajectories of the craniofacial skeleton in rats. *J Nutr.* 1999;129:2061-9.
14. Reichling TD, German RZ. Bones, muscles and organs of protein malnourished rats (*Rattus norvegicus*) grow more slowly but for longer durations to reach normal final size. *J Nutr.* 2000;130:2326-32.
15. Golstein RS, Hook JB, Bond JT. The effects of maternal protein deprivation on renal development and function in neonatal rats. *J Nutr.* 1979;109:949-57.

16. Boxwell J, Ayson P, Ramenofsky M. [Growth and metabolic parameters in pups of undernourished lactating rats](#). *Physiol Behav*. 1995;57:469-75.
17. Resnick O. [Development protein malnutrition: influences on the central nervous system of the rat](#). *Neurosci Biobehav Rev*. 1979;3:233-46.
18. Hatch MC, Shu XO, McLean DE, Levin B, Begg M, Reuss L, et al. [Maternal exercise during pregnancy, physical fitness, and fetal growth](#). *Am J Epidemiol*. 1993;137:1105-14.
19. Clapp JF 3rd, Kim H, Burciu B, Lopez B. [Beginning regular exercise in early pregnancy: effect on fetoplacental growth](#). *Am J Obstet Gynecol*. 2000;183:1484-8.
20. Boyer PM, Compagnucci GE, Olivera MI, Bozzini C, Roig MC, Compagnucci CV, et al. [Bone status in an animal model of chronic sub-optimal nutrition: a morphometric, densitometric and mechanical study](#). *Br J Nutr*. 2005;93:663-9.
21. Houdijk EC, Engelbregt MJ, Popp-Snijders C, Deleamarre-Vd Waal HA. [Endocrine regulation and extended follow up of longitudinal growth in intrauterine growth-retarded rats](#). *J Endocrinol*. 2000;166:599-608.
22. Engelbregt MJ, van Weissenbruch MM, Lips P, van Lingen A, Roos JC, Deleamarre-Vd Waal HA. [Body composition and bone measurements in intra-uterine growth retarded and early postnatally undernourished male and female rats at the age of 6 months: comparison with puberty](#). *Bone*. 2004;34:180-6.
23. Alippi RM, Meta MD, Olivera MI, Bozzini C, Schneider P, Meta IF, et al. [Effect of protein-energy malnutrition in early life on the dimensions and bone quality of the adult rat mandible](#). *Arch Oral Biol*. 2002;47:47-53.
24. Fortman JK 2nd, Reichling T, German RZ. [The impact of maternal protein malnutrition on pre-weaning skeletal and visceral organ growth in neonatal offspring of \*Rattus norvegicus\*](#). *Growth Dev Aging*. 2005;69:39-52.
25. Platt BS, Stewart RJ. [Transverse trabeculae and osteoporosis in bones in experimental protein-calorie deficiency](#). *Br J Nutr*. 1962;16:483-95.
26. Lee M. [Skeletal growth and bone size of rats during protein-energy malnutrition and during rehabilitation](#). *Nut Rep Int*. 1976;13:527-33.
27. Glick PL, Rowe DJ. [Effects of chronic protein deficiency on skeletal development of young rats](#). *Calcif Tissue Int*. 1981;33:223-31.
28. Sturman JA, Devine E, Resnick O, Morgane PJ. [Maternal protein malnutrition in the rat: effect on protein and two enzymes in the milk](#). *Nutr Res*. 1986;6:437-42.
29. Langley-Evans SC, Gardner DS, Welham SJ. [Intrauterine programming of cardiovascular disease by maternal nutritional status](#). *Nutrition*. 1998;14:39-47.
30. Fall CH, Osmond C, Barker DJ, Clark PM, Hales CN, Stirling Y, et al. [Fetal and infant growth and cardiovascular risk factors in women](#). *BMJ*. 1995;310:428-32.

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