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Undernutrition in the last period of lactation may have adverse effects on skeletal development

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Abstract

Objectives: Malnutrition in childhood causes permanent damage. Studies report that there are different developmental mechanisms at different stages of breastfeeding. Our study aims to observe the effects of undernutrition in the first and last weeks of lactation on body weight and skeletal development after rehabilitation until puberty, and ultimately to reveal which period of lactation is more critical.

Methods: Lactating rats were undernourished by receiving half the diet consumed by control mothers during the first (0–7th day, U1 group) or third (14–21st day, U2 group) week of lactation. Rats were weighed each week and radiographs were taken on the 21st and 49th days. All measurements were taken directly on the radiographs.

Results: On day 49, the body weight and body length of the two undernourished groups were lower than those of the controls. U2 was behind in all measurements except head, pelvic, iliac, and ischial lengths. U2 also lagged behind U1 in body, tail, spine, upper limb, and tibia lengths. While U1 did not differ from controls in many measurements, femur length, bi-iliac, bi-acetabular, and ischial width were less than controls.

Conclusion: Undernutrition in the last week of lactation affected body weight and skeletal development more than malnutrition in the first postnatal week.

Keywords: lactation; radiograph; rat; skeletal development; undernutrition

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Introduction

The growing world population brings with it the problem of malnutrition. The global nutrition crisis we faced even before Covid-19[1] has become far worse, with worrying trends across every form of malnutrition, from hunger to obesity. People affected by hunger leaped by 150 million since the Covid-19 outbreak, from 618 million in 2019 to 768 million in 2021, while those unable to afford a healthy diet[2] rose by 112 million to 3.1 billion in 2020 alone.[3] Almost a third (29.3%) of the world's population, 2.3 billion people, were moderately or severely food insecure[4] in 2021, up from 25.4% before the pandemic.[5] At the same time, what we eat across the world continues to fall short of the minimum standards for healthy and sustainable diets[6] with resulting obesity and diet-related non-communicable diseases (NCDs) on the rise and at epidemic levels – around 40% of all adults and 20% of all children are now overweight or obese.[7] Policy interventions to date are failing to reverse these trends, while conflict around the world and the impacts of climate change, which are key drivers of increases in malnutrition, continue unabated.[8]

Many studies have investigated the effects of malnutrition on body weight of rats at different ages. Most of these studies found that undernutrition of rats before weaning can lead to permanent defects in body weight.[9–12] However, body weight alone is not a sufficient parameter to indicate growth; additional indicators are needed, such as body length and skeletal growth. Using skeletal growth to monitor the effects of nutritional intervention provides a more reliable method of measuring growth retardation.[13] Several studies have examined the effects of malnutrition on skeletal development. Some of these studies examined the effects of undernutrition on skeletal development after weaning and others before weaning. However, some studies include both periods.[14–16] Studies have shown that growth and development occur through different mechanisms at different stages of lactation.[17,18]

Previous studies do not provide detailed comparative information on the effects of undernutrition during the first and last weeks of lactation on skeletal development and body weight in rats. In this study, we aimed to observe skeletal development and body weight of rats exposed to undernutrition during the first and last week of lactation and rehabilitation until puberty, and finally to find out which period of lactation is more critical for skeletal development.

Materials and Methods

Eight female Wistar albino rats weighing approximately 210 g were used for this study. The animals were kept under standard conditions (21°C, 12h light/dark cycle) and had ad libitum access to food and water. After overnight mating with male rats, females were classified as pregnant with sperm in the vaginal smear. Pregnant females gave birth to pups approximately 21 days after mating. Pups born on the same day were divided according to sex, and male pups were distributed in groups of 8 to three different mothers. Birth weights (P0) were weighed using a precision balance (310M, Precisa, Dietikon, Switzerland). This procedure was repeated every week until day 49 (P49).

The experimental groups were undernourished at different time points during the 21-day breastfeeding period. The first group (U1) was undernourished between P0 and P7, and the second group was undernourished between P14 and P21. The undernutrition protocol was implemented by giving the experimental groups half of the food that the control group (C) ate on the same days (Oğuzlar Tarim Urunleri Sanayi ve Ticaret A.S. Eskisehir-Türkiye, for the content of the food, see **Table 1**). According to this, the U1 mother was undernourished with 10 g of food at P0 to P3 and 15 g at P4 to P7. In contrast, the U2 mother received 20 g of feed at P14 and 25 g at P15 to P21. On the other days of lactation, the mothers had access to food ad libitum.

At the end of day 21, all pups were separated from their mothers and anesthetized with an intramuscular injection of 50 mg/kg ketamine (Ketalar, Zentiva, Luleburgaz, Türkiye). Rats were placed ventrally on an X-ray cassette according to the study by Hughes and Tanner (1970) (**Figure 1**).[19] Radiographs were taken with a Philips Diagnostic PCS 2000 X-Ray machine

Table 1
Composition of the rat chow.

Essential Nutrients Included (%)	
Dry matter	88 min.
Protein	16 min.
Cellulose	14 min.
Ash	9 max.
Calcium	0.8-1.5 min.-max.
Phosphorus	0.5 min.
Sodium	0.2-0.4 min.-max.
NaCl	1.00 max.
Energy (Kcal/kg)	2400 min.
Vitamins (IU/kg)	
Vitamin A	5000 min.
Vitamin D3	1000 min.
Vitamin E	30 min.

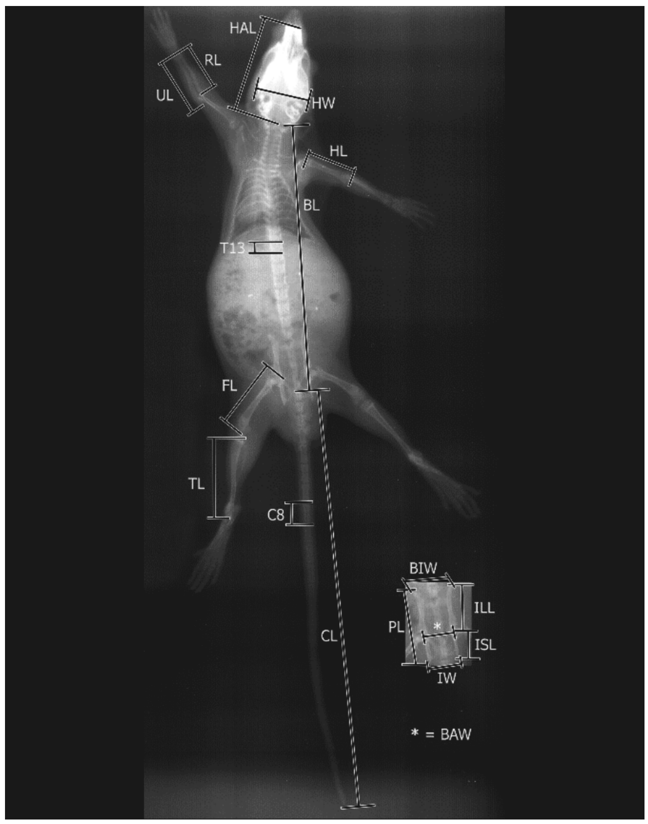


Figure 1. Measurements made on radiographs. The radiograph was taken on 21st day (P21). **BAW:** bi-acetabular width; **BIW:** bi-iliac width; **BL:** body length; **C8:** length of the 8th caudal vertebra; **CL:** tail length; **FL:** length of the left femur; **HW:** head width; **HAL:** head length; **HL:** length of the right humerus; **ILL:** iliac length; **ISL:** ischial length; **IW:** ischial width; **PL:** pelvic length; **RL:** (length of the left Radius), **T13:** length of the 13th thoracic vertebra; **TL:** length of the left tibia; **UL:** length of the left ulna.

from a distance of 120 cm with a dose of 41 kV-2 mA. On day 49, second radiographs were taken in the same manner.

All measurements were made by the same observer directly on radiographs using a caliper with an accuracy of 0.05 mm (Izeltas, Izmir, Türkiye). Body and tail length were measured with a thread shaped according to the shape of the structures. All measurements were taken three times and the average of these measurements was taken into account. The dimensions of the skeleton were measured in previous studies and certain relevant landmarks were considered in all measurements.[13,19–23]

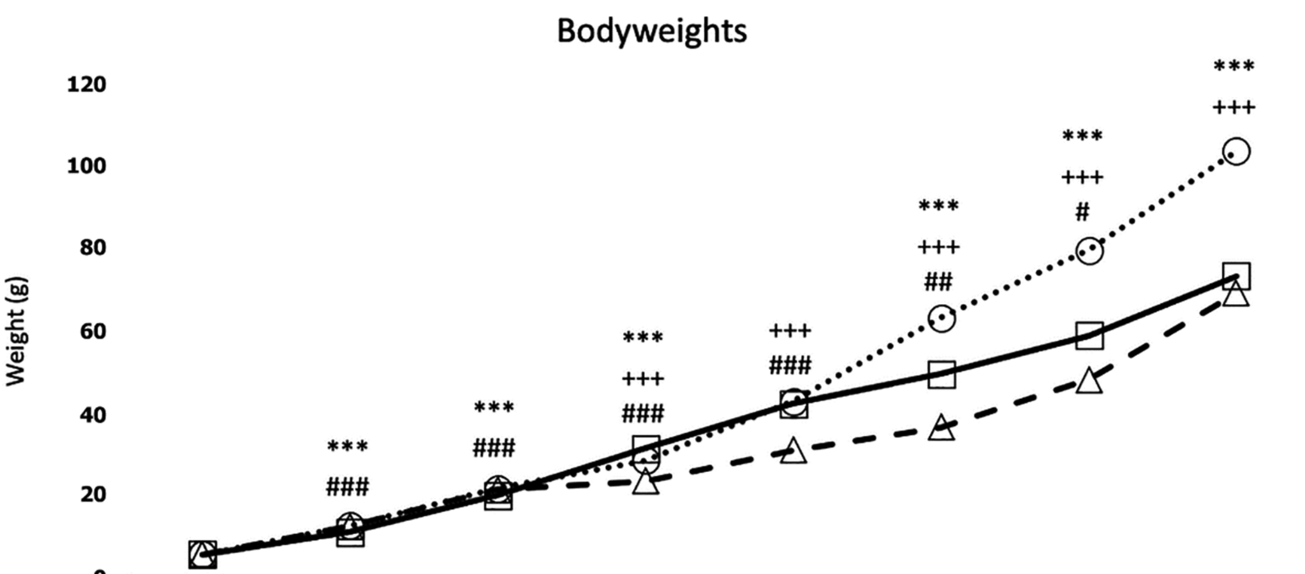
Data sets were analyzed for normality using the Kolmogorov-Smirnov test and compared with ANOVA (Tukey posthoc tests) using JAMOVI 2.3.28 software.

Results

Body weights

There was no significant difference between the birth weights of the groups at P0. At P7, it was observed that the body weight of U1 was lower than that of the normally fed groups. Although all groups were fed normally between P7 and P14, the body weight of nutritionally damaged U1 remained lower than that of the other groups. At P21, the body weight of undernourished U2 was lower than that of the other two groups; meanwhile, the body weight of U1 had risen above that of C. At P28, the body weight of U2 was still lower than that of the other two groups. At P35, C's body weight was higher than that of the experimental groups, while U2's body weight was also lower than that of U1 on that day. At P42, the body weight of the experimental groups remained lower than that of C, but the body weight of U1 remained higher than that of U2. The final weights measured at P49 showed that the body weights of the experimental groups were still lower than that of C, but the difference between the experimental groups disappeared (Figure 2).

In terms of growth rates, it can be seen that the growth rate of U1 between P0–7 was 78% of C and the growth rate of U2 was 98%. The growth rates of the



	P0	P7	P14	P21	P28	P35	P42	P49
C	5.5±0.6	12.5±0.5	21.6±0.4	28.5±0.4	42.9±6.5	63.2±9.6	79.6±12.3	103.8±14.8
U1	5.5±0.5	11.0±0.4	19.9±0.5	31.4±0.9	42.3±2.3	49.5±3.6	58.9±5.5	73.4±1.3
U2	5.5±0.7	12.4±0.5	21.3±0.4	23.4±0.6	30.9±1.8	36.6±3.6	48.2±5.5	69.2±3.4

Days

Figure 2. Body weights weighed from day 0 (P0) to day 49 (P49) (±standard deviation), n=8. **C:** control group; **U1:** the first group that was undernourished between day 0 (P0) and day 7 (P7); **U2:** the second group that was undernourished between day 14 (P14) and day 21 (P21); sig: significancy; *: comparison of C with U1; +: comparison of C with U2; #: comparison of U1 with U2. One symbol means p<0.05; two means p<0.01; three means p<0.001.

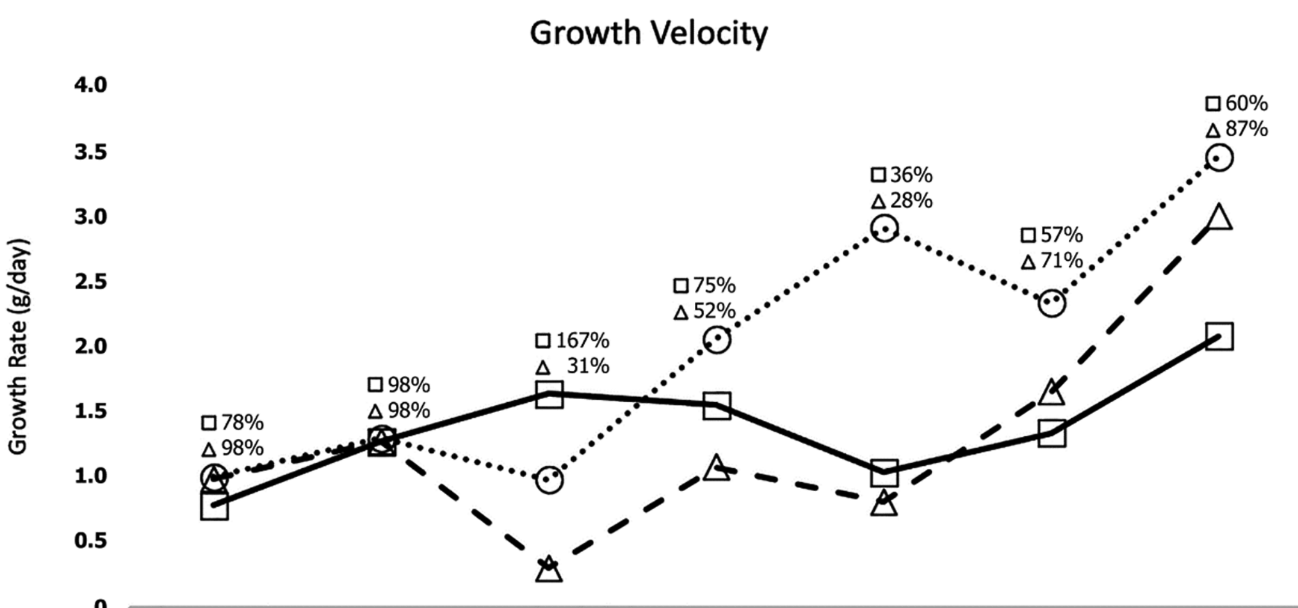
groups between P7–14 were similar (98%). Between P14–21, the growth rate of malnourished U2 was very low (31%), while the growth rate of U2 increased dramatically (167%). As the growth rate of C also increased between P21 and P28, the growth rates of the malnourished groups lagged behind those of this group. While the growth rate of U1 was 57% to 60% of C between P35–49, the growth rate of U2 was higher than that of U1, being 71% and 87% of C (**Figure 3**).

Measurements made on P21

Rats that were malnourished between postnatal day 0 and 7 showed significantly higher values in body length, head length, 8th caudal vertebra length, left femur length, left tibia length, and ischium length compared to control rats at postnatal day 21. However, rats that were malnourished between postnatal day 14 and 21 showed significantly lower values in body length, left femur length, pelvic length, ischial length, bi-iliac width and bi-acetabular width compared to control rats. On the other hand, right humerus length and ischial width at postnatal day 21 showed no significance between all groups. When comparing the two experimental groups, all skeletal measures except right humeral length and ischial width were significantly lower in the rats malnourished in the last week of lactation than in the other group (**Table 2**).

Measurements made on P49

At the end of the 49th day, only body length, length of the left femur, bi-iliac width, bi-acetabular width, and ischial width in rats undernourished between 0–7th postnatal days showed significant deficits compared to control rats. However, rats undernourished between 14–21st postnatal days showed significant deficits in the value of body length, tail length, head width, 13th thoracic vertebra length, 8th caudal vertebra length, right humerus length, left radius length, left ulna length, left femur length, left tibia length, bi-iliac width, bi-acetabular width and ischial width compared to control rats. On the other hand, body length, tail length, 13th thoracic vertebra length, 8th caudal vertebra length, right humerus length, left radius length, left ulna length, left tibia length of rats undernourished between 14–21st postnatal



	P0-7	P7-14	P14-21	P21-28	P28-35	P35-42	P42-49
C	1.0	1.3	1.0	2.1	2.9	2.3	3.5
U1	0.8	1.3	1.6	1.6	1.0	1.3	2.1
U2	1.0	1.3	0.3	1.1	0.8	1.7	3.0

Days

Figure 3. Calculated growth rates (g/day) of the groups from day 0 (P0) to day 49 (P49), n=8. The percentages of the groups relative to the controls are given. **C:** control group; **U1:** the first group that was undernourished between day 0 (P0) and day 7 (P7); **U2:** the second group was that undernourished between day 14 (P14) and day 21 (P21).

Table 2

Measurements made from X-rays on 21st day (P21) (±standard deviation), n=8.

Measurements on P21 (cm)	C	U1	U2	Sig.
Body length	5.77±0.09	6.30±0.21	5.53±0.18	***, +, ###
Tail length	7.39±0.43	7.67±0.15	7.23±0.17	#
Head length	2.31±0.05	2.57±0.13	2.23±0.07	***, ###
Head width	1.57±0.04	1.63±0.04	1.52±0.09	##
Length of the 13. th. vertebra	0.20±0.01	0.21±0.02	0.19±0.02	#
Length of the 8. caud. vertebra	0.24±0.03	0.27±0.01	0.23±0.01	**, ###
Length of the right humerus	1.37±0.07	1.34±0.03	1.32±0.06	n.s.
Length of the left radius	1.22±0.05	1.25±0.02	1.20±0.01	#
Length of the left ulna	1.39±0.07	1.43±0.02	1.37±0.02	#
Length of the left femur	1.36±0.01	1.41±0.04	1.32±0.01	***, +, ###
Length of the left tibia	1.53±0.02	1.59±0.04	1.51±0.04	**, ###
Pelvic length	1.56±0.07	1.63±0.05	1.37±0.14	++, ###
Iliac length	0.91±0.03	0.94±0.03	0.85±0.08	##
Ischial length	0.61±0.02	0.72±0.06	0.52±0.07	**, ++, ###
Bi-iliac width	0.92±0.03	0.92±0.03	0.83±0.03	+++, ###
Bi-acetabular width	0.71±0.04	0.74±0.04	0.64±0.03	+++, ###
Ischial width	0.81±0.04	0.83±0.07	0.79±0.03	n.s.

C: control group; **U1:** the first group that was undernourished between day 0 (P0) and day 7 (P7); **U2:** the second group that was undernourished between day 14 (P14) and day 21 (P21); **sig:** significancy; *: comparison of C with U1; +: comparison of C with U2; #: comparison of U1 with U2. One symbol means p<0.05; two means p<0.01; three means p<0.001; **n.s.:** not significant.

days were shorter than pups undernourished in the first week of lactation. Finally, no significant difference was observed between the groups in terms of head length, pelvic length, iliac length, and ischial length measurements (**Table 3**).

Discussion

In the present study, it was observed that on the 21st postnatal day, the body weight of the pups undernourished in the last week of lactation was lower than that of the control animals, but the body weight of the pups undernourished in the first week of lactation was higher than that of the control animals due to catch-up growth. However, at the final measurements on postnatal day 49, the offspring of both undernourished groups were found to be lighter than the control animals.

Studies have shown that undernutrition in the first postnatal week reduces the body weight of infants. Winick et al.[24] undernourished their animals from P0 to P9, while Williams and Hughes[23] malnourished their rat pups from P0 to P8. Both studies reported that the body weight of these offspring was statistically lower than that of their control animals, and the statistical difference between them disappeared before the end of lactation (P0–P21).[23,24] Recent literature highlights also the significant impact of maternal undernutrition during the first week of lactation on the body weight and overall development of offspring in rat models. This critical period is essential for growth, and any nutritional deficits can lead to long-lasting effects on the progeny. Studies indicate that undernutrition during lactation can lead to stunted growth in pups. For instance, research shows that pups from mothers experiencing protein and energy restrictions during lactation exhibit minimal weight gain, contrasting sharply with those from well-nourished mothers who demonstrate rapid growth during this same period.[25,26] Specifically, one study noted that pups subjected to maternal malnutrition maintained consistent body weight during the initial week of lactation, while control groups showed significant weight increases. This discrepancy suggests that nutritional status directly influences growth trajectories in young rats.[27,28] The undernutrition period of U1 is consistent with previous studies, and the body weight of this group lagged behind that of the other groups at P7. However,

Table 3
Measurements made from X-rays on 49th day (P49) (±standard deviation), n=8.

Measurements on P49 (cm)	C	U1	U2	Sig.
Body length	9.22±0.10	9.05±0.09	8.65±0.09	**, +++, ###
Tail length	14.30±0.11	14.33±0.08	13.96±0.17	+++, ###
Head length	2.80±0.09	2.86±0.10	2.92±0.14	n.s.
Head width	1.87±0.07	1.83±0.03	1.76±0.06	+
Length of the 13th vertebra	0.30±0.01	0.29±0.02	0.26±0.01	+++, #
Length of the 8th caudal vertebra	0.63±0.02	0.66±0.06	0.54±0.04	++, ###
Length of the right humerus	1.77±0.03	1.77±0.04	1.62±0.07	+++, ###
Length of the left radius	1.60±0.07	1.58±0.04	1.44±0.05	+++, ###
Length of the left ulna	1.98±0.06	1.93±0.02	1.78±0.04	+++, ###
Length of the left femur	2.28±0.13	2.03±0.06	2.02±0.09	***, +++
Length of the left tibia	2.49±0.14	2.41±0.05	2.23±0.07	+++, ##
Pelvic length	2.40±0.28	2.49±0.12	2.36±0.12	n.s.
Iliac length	1.47±0.18	1.46±0.07	1.42±0.07	n.s.
Ischial length	0.93±0.13	1.03±0.07	0.94±0.09	n.s.
Bi-iliac width	1.48±0.10	1.34±0.03	1.28±0.04	***, +++
Bi-acetabular width	1.03±0.06	0.92±0.04	0.91±0.03	***, +++
Ischial width	1.15±0.09	1.05±0.04	0.98±0.03	*, +++

C: control group; **U1:** the first group that was undernourished between day 0 (P0) and day 7 (P7); **U2:** the second group that was undernourished between day 14 (P14) and day 21 (P21); **sig:** significancy; ***:** comparison of C with U1; **+:** comparison of C with U2; **#:** comparison of U1 with U2. One symbol means $p<0.05$; two means $p<0.01$; three means $p<0.001$; **n.s.:** not significant.

unlike the above studies, U1 did not catch up between P7 and P14. Instead, U1 showed this period of accelerated growth between P14 and P21, exceeding the growth rate of C by 67%. This late catch-up period may be due to insufficient maternal milk production immediately after malnutrition. However, strain differences could also be a factor in the occurrence of differences in this recovery phase. Also, the composition of maternal milk is crucial for pup development. Research indicates that malnourished mothers may produce milk with lower macronutrient levels, which could further exacerbate growth issues in their offspring.[29,30] Although some studies did not find significant differences in protein or fat content between milk from malnourished and control dams, there is evidence suggesting that bioactive factors in milk may play a role in modulating growth outcomes.[29] The catch-up growth period observed in other studies peaked at P21[23,31,32] and it can be seen that the growth rate of U1 slowed down after seven days, so that the body weight of U1 at P28 is no longer different from that of C.

In our study, the U2 pups did not show a steep catch-up process like the pups from U1. But after P28, a steady accelerated growth was observed compared to U1. There are no studies that we can directly compare to the period of undernutrition of U2. In contrast to our observations some Studies indicate that pups from mothers experiencing undernutrition during the third week of lactation exhibit accelerated weight gain compared to control groups. This catch-up growth is observed as pups begin consuming solid food in addition to suckling maternal milk.[29,33] But a study on early weaning could lead to similar results. Pietrobon et al.[34] pharmacologically or physically inhibited offspring milk intake during the last three days of breastfeeding. Body weight of malnourished males still lagged behind that of the control group at P45 (although there was no statistical difference). However, at P150, the body weight of the experimental groups exceeded that of the control group. The authors concluded that early weaning caused metabolic changes in the experimental groups.[34] While the data from this study up to P45 are consistent with our study, we do not know if the results at P150 are the same for us. However, the argument made by the authors for the cause of growth retardation in the subjects could also apply to our subjects.

The body weights and growth rates of our experimental groups decreased from P28 to P35. This suggests that the offspring in the experimental groups have not completed their development and therefore cannot feed sufficiently independently. Ferraz-Perreira et al.[35] suggested that malnutrition reduces masticatory efficiency by slowing, weakening and delaying the maturation of masticatory muscles. However, in another study, perinatal undernutrition was observed to reduce the number of taste buds in rats.[36] Both arguments could explain why undernourished rats develop less than control subjects immediately after weaning.

The final body weights measured at puberty showed that the weights of the experimental groups were still lower than those of the control group, but the difference between the experimental groups was closed. Williams et al.[32] reported that the weight losses of malnourished rats continued after P120.[32] However, it was observed that although the malnourished rats consumed 30% more food than their control counterparts after the nutritional insult. These animals ate more irregularly and less food than the control animals in the following days.[22] Williams and Hughes[23] found in their study that the effects of malnutrition were greater in the late stages of lactation. However, the animals in this study were undernourished in three different groups from birth to P8, P14 and P21. The offspring that were malnourished from P0 to P8 reached the control group at P22. In contrast, the other two groups already had lower body weights at P120.[23] The body weights of the undernourished pups during lactation were lower at P21 and remained lower at P150.[37] Recent studies indicate that maternal undernutrition can alter hormonal profiles and neuropeptide expressions related to appetite regulation in offspring. This alteration may lead to increased caloric intake but decreased fat storage efficiency, resulting in abnormal growth patterns as seen in various studies.[25,30] Despite the nutritional restoration through adequate calories later in life, the capacity for catch-up growth is diminished in pups subjected to maternal undernutrition during the third week of lactation. This suggests that early-life nutritional deficits can have lasting effects on growth trajectories.[38] Since the results of our study showed that the body weight of U1 was higher than that of U2 at P28, P35 and P42, this suggests that the 3rd week of lactation is a more critical postnatal developmental period.

Our measurements immediately after weaning showed that although the measurements of U1 were higher than those of the other groups due to catch-up growth, the size of the thoracic vertebrae was not affected. However, measurements at P49 showed that both the thoracic and caudal vertebrae of U2 were less developed than the other two groups, suggesting that malnutrition in the last week of lactation affects vertebral size in the long term. This suggestion is also supported by the shorter tail length of U2. Research supports our observations and states that undernutrition during lactation can lead to significant reductions in vertebral size and integrity. Studies have shown that malnutrition during lactation can lead to a significant reduction in the size and integrity of the spine. Puppies from malnourished mothers have smaller vertebrae compared to those from well-nourished mothers, probably due to insufficient calcium and protein intake.[39] A more recent study confirms this opinion by finding that puppies from malnourished mothers have smaller vertebrae than those from well-nourished mothers. And that this reduction is often due to inadequate calcium and protein intake, which are essential for bone growth and density.[40] Based on our observations on U2, we can assume that the last week of lactation is a critical period for vertebral development. Several studies indicate that pups from mothers experiencing undernutrition during lactation exhibit reduced body length compared to control groups. This effect is observed throughout the lactation period and into adulthood.[29,41] Despite the nutritional restoration through adequate calories after weaning, the capacity for catch-up growth in body length is often diminished in pups subjected to maternal undernutrition during lactation. Even when body weight normalizes, linear growth deficits may persist.[41] In a study of protein restriction during pregnancy, short spines were reported in protein-restricted rats.[42] In a more recent study, Nemoto and Kakinuma[43] fed dams a calorie-restricted diet during pregnancy and reported that the pups failed to catch up and resulted in short body length.[43]

Dahinten and Pucciarelli[44] observed in their study of rats that prenatal malnutrition affects many cranial parameters. However, this study concludes that postnatal malnutrition has a greater negative effect on these parameters.[44] This may be attributed to the fact that 85% of cortex neurogenesis in the rat occurs after birth.[45] Although U1 had a rapid growth curve before P21, the cranial width of U1 was not different from that of C at P49. On postnatal day 21, the skull length of pups malnourished between 0–7 days of the lactation period was greater than that of the control group; however, this difference disappeared on postnatal day 49. In contrast to the U1 pups, the U2 pups had a smaller head width at postnatal day 49. In a previous study, the deficit in this parameter persisted until 200 days of age in rats that were malnourished from gestation day 18 to 100 days of age.[22] Recent studies are in line with our observations. They indicate that pups from mothers experiencing undernutrition during lactation exhibit reduced skull size compared to control groups. This effect is observed in various cranial measurements, including skull length, width, and height.[46–48] It is also emphasized that maternal undernutrition during lactation can lead to changes in the shape of the skull. One study found that the skulls of pups from undernourished mothers were smaller and thinner than those of the control group in several parameters.[48]

At 21 days of age, the body length, the length of the 8th caudal vertebra, the length of the left femur, the length of the left tibia, and the length of ischial bones were longer in U1 than in the control animals, while the pups of the U2 group had smaller skeletons than the control animals. At 49 days of age, the significant differences in some features of the skeleton between U1 and control rats disappeared except for femur length. However, some features of the pelvic bones of the U1 rats differed from those of the control animals. In contrast to the U1 rats, the U2 rats had smaller skeletal measurements than the control rats at 49 days of age, except for pelvic bone lengths and head length.

Research has demonstrated that undernutrition during lactation leads to significant reductions in pelvic size and dimensions in offspring. For instance, pups from undernourished mothers exhibit smaller pelvic widths and lengths compared to those from well-nourished controls. This reduction can have implications for reproductive health and overall mobility in adulthood.[38,49] It was found that the rat pelvis develops rapidly from P0 to P80, that ossification of the acetabular complex begins at P70, and that synostosis of the three pelvic components begins at P100.[50] Therefore, the effects of nutrition can be observed directly on the rat pelvis because the pelvic components are not fully developed on the days of the measurements. Studies of malnutrition have shown that pelvic dimensions do not recover despite a prolonged period of rehabilitation.[29,33,51] Although the measurements we performed on P49 confirm this study, they emphasize that undernutrition even in only a part of lactation affects the measurements of pelvic widths of pups and may cause permanent damage.

Our study shows that undernutrition in either the first or last week of the lactation period affects long bone development. In particular, U2 measurements are similar to limb measurements of offspring malnourished prenatally or throughout the lactation period. This observation suggests that especially the last week of lactation is a critical period for long bone development.

Nakamoto and Miller[52] have reported that long bones are more prone to malnutrition.[52] According to Kimura et al.,[53] prenatal malnutrition impairs postnatal growth of the tibia.[53] It was observed that postnatally malnourished rats showed catch-up growth after weaning, but their tibial length remained shorter than that of the control group even at week 15.[54] Numerous studies have reported that undernutrition during lactation results in significantly reduced length and density of long bones, such as the femur and tibia. For example, research by Babinski et al.[38] demonstrated that pups from mothers on low-protein diets exhibited stunted growth in femoral length and altered bone density compared to those from well-nourished mothers. This reduction is attributed to inadequate intake of essential nutrients, particularly protein and calcium, which are vital for bone development.[38] The timing and rate of bone growth are also affected by maternal nutrition. A study by Ortiz-Valladares et al.[49] indicated that while long bone measurements may not show immediate deficits, the growth trajectories are altered, resulting in delayed skeletal maturation in pups subjected to undernutrition. This delayed growth can have long-term implications, as it may affect the overall structural integrity of the bones as the rats reach adulthood.[49] The effects of maternal undernutrition during lactation can persist into adulthood, with previously undernourished rats exhibiting altered long bone morphology. Studies indicate that even after nutritional rehabilitation post-weaning, these rats may continue to show reduced bone length and density, potentially increasing their risk for fractures and other skeletal issues later in life.[38]

Conclusion

The results of our study showed that undernutrition in the first or last week of lactation can cause long-term damage to body weight and pelvic measurements of rats. However, most skeletal measurements of rats exposed to undernutrition in the last week of lactation were lower than those of the control group and the group exposed to undernutrition in the first week of lactation. These results suggest that the last week of lactation is more critical for skeletal development.

Conflict of Interest

The authors declare that they have no known conflicts of interest.

Author Contributions

HA: investigation, data curation, software, writing- original draft; FY: methodology, resources, supervision, writing - review & editing.

Ethics Approval

All animal applications in this study were approved by the local ethics committee of Eskişehir Osmangazi University (protocol number 155/850).

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