Int. J. Morphol.,
31(2):584-589, 2013.

How Does Protein Malnutrition or Food Deprivation Interfere with the Growth of the Epiphyseal Plate in Animals?

¿Cómo Interfieren la Desnutrición Protéica o la Privación Alimentaria en el Crecimientos de la Placa Epifisaria de Animales?

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DA SILVA, K. C.; SILVA, C. R. S.; COSTA, R. C. S. & DE MORAES, S. R. A. How does protein malnutrition or food deprivation interfere with the growth of the epiphyseal plate in animals? ***Int. J. Morphol., 31(2)***:584-589, 2013.

SUMMARY: the aim of this study was to conduct a literature review of impacts of protein malnutrition and/or early food deprivation on the formation of the growth plate in young animals. A search was performed using the databases PubMed/MEDLINE, LILACS, SciELO and the Cochrane Library, without language restrictions or any limit as to year. Initially, 150 articles were identified, however, after application of the inclusion and exclusion criteria only five remained. In three studies the nutritional stress was due to protein malnutrition, another due to fasting and the fifth was due to food restriction. All the studies had some methodological omissions. The studies included in this review-demonstrated corroboration in the results obtained, regardless of whether the animals had been subjected to protein malnutrition, fasting or food restriction. The findings uncovered were reduction in height of the epiphyseal plate, in the number of proliferative and hypertrophic chondrocytes, in the bone growth rate and in the longitudinal length of the bone in animals subjected to nutritional stress. In this systematic review, it was possible to observe the susceptibility of the epiphyseal plate in the first place, and secondarily, of the long bones to the effects of nutritional stress by means of protein malnutrition or food deprivation applied in young animals.

KEY WORDS: Epiphyseal plate; Bone growth; Protein malnutrition; Food deprivation.

INTRODUCTION

Several studies have attempted to explain the possible disorders that may lead to insufficiency or failure of bone growth (Heinrichs *et al.*, 1997; Farnum *et al.*, 2003). As a general rule, the secondary effect of growth disorders is the result of a defect that did not start primarily in bone tissue (Boersma & Wit, 1997) such as, for example: endocrine disorders, specific disorders of organic systems (Boersma & Wit) and malnutrition (Boersma & Wit; De Luca, 2006). With respect to nutritional state, inadequate feeding in the early stages of life can lead the individual to malnutrition (Hsueh *et al*., 1974; Ferreira & Ott, 1988; Guzmán *et al*., 2006) as a result of physiopathological changes translated initially into functional impairment and, later, biochemical and physical damage (Rol De Lama *et al*., 2000; Gurmini *et al.*, 2005) such as weight loss (Winick & Noble, 1966; Oliveira *et al*., 2003; Gurmini *et al*.), and inhibition of longitudinal bone growth (Heinrichs *et al*.).

Failure in longitudinal bone growth is mainly due to the low rate of bone growth, which, in turn, depends essentially on the rate of chondrocyte genesis (De Luca) as well as the coordinated activity of chondrocytes within the growth plate. Therefore, the rate at which this process occurs is reflected in changes in the synthesis and development of chondrocytes (Hunziker & Schenk, 1989).

As a specialized structure with the exclusive function of providing longitudinal bone growth, the epiphyseal plate is sensitive to physiological, biochemical and pathological changes that occur in the body as a whole (Oliveira *et al*., 1993). Therefore, protein malnutrition (Moraes, 2006) or even a short period of fasting (Heinrichs *et al*.; Farnum *et al*.) has deleterious effects on the morphology of the growth plate (Heinrichs *et al*.; Farnum *et al*.; Moraes) represented by a reduction in the total height of the plate and in the

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number of chondrocytes (Heinrichs *et al*.; Farnum *et al*.), the presence of short chondrocyte columns with a tendency for disorganization at the extremities, change in shape and size of the chondrocytes, formation of chondrocyte nest and areas of injury in the matrix (Moraes). This means that the growth of the epiphyseal plate responds to any serious period of nutritional stress (Heinrichs *et al*.; Farnum *et al*.; Moraes) In this way, it bears pointing out the significant effect that the nutritional state has on the development (Hsueh *et al*.; Bonjour, 2005) and rates of bone growth (Schultze, 1954; Hsueh *et al*.; Heinrichs *et al*.; Rol De Lama *et al*.; Farnum *et al*.).

Thus, the aim of this study was to conduct a literature review of impacts of protein malnutrition and/or early food deprivation on the formation of the growth plate in young animals.

MATERIAL AND METHOD

In the realization of this review, conducted in the period from November 2011 to January 2012 and without language restrictions, published articles were selected from journals indexed in LILACS, SciELO, PubMed / MEDLINE and the Cochrane Library.

The descriptors used were based on the list of MeSH, and the following terms were chosen: growth plate, malnutrition, fasting. Key words on the topic were also chosen for the search: long-bone growth and nutrition-induced. For the Decs list the descriptors selected were: lâmina de crescimento, combined with the terms desnutrição, jejum.

All these combinations were used for all databases. The references of the selected articles were also checked to identify other studies that might have been omitted from the electronic search.

The titles and abstracts identified through searches were reviewed independently by three reviewers (KCS, CRSS, RCSC) on the computer screen to select potentially relevant studies. Cases of disagreement, when present, were solved by a fourth evaluator (SRAM).

For selection of the study the following inclusion criteria were considered: studies with animals submitted to protein malnutrition or food deprivation, studies which used morphometric and/or morphological parameters as a means of assessing the development of the growth plate, studies of longitudinal bone growth and studies involving experimental and control groups.

Studies in which the nutritional stress was due to deficiency of nutrients other than protein malnutrition, fasting or food restriction, articles in which the animals tested were not subjected to early nutritional stress (a critical period of development) and studies in which the nutritional state was related to other types of interventions were excluded. Changes in the growth plate were considered as a primary outcome and as a secondary outcome, the length of the longitudinal bone.

RESULTS

Initially, 150 articles were identified through the electronic search: 144 in PubMed/Medline, 1 in LILACS, 0 in SciELO and 5 in the Cochrane Library. After the implementation of inclusion and exclusion criteria, only 5 articles were included in the systematic review: Heinrichs *et al*.; Nakamoto & Miller (1979); Even-Zohar *et al*. (2008); Kuramitsu *et al*. (1985), and Kanagawa *et al*. (1987), (Table I).

The study of Nakamoto & Miller aimed to assess whether protein malnutrition would affect the activity of acid and alkaline phosphatase and consequently if such biochemical changes would bring about changes in the long bones of rats. Heinrichs *et al.,* evaluated the mechanisms responsible for suppression of growth plate function in rabbits subjected to fasting. Even-Zohar *et al*., studied the mechanisms that govern the recuperative growth of the epiphyseal plate during food restriction and immediately after nutritional replacement. Kuramitsu *et al.*,verified the effects of protein and/or energy deficiency on the growth of long bones and Kanagawa *et al*., aimed to clarify whether malnutrition affects bone growth through reduced thyroid function.

For the evaluation of these parameters, Nakamoto & Miller used 16 mice randomly distributed in a control group and a malnourished group. Kanagawa *et al.,* used 12 male Wistar rats randomly assigned to a control group and a malnourished group each of which was subdivided into saline and thyroxine groups. The other three studies did not specify if sample randomization was done. Heinrichs *et al.,* used 16 New Zealand rabbits, aged 5 weeks, separated into a control group (free access to feed) and a fasting group (fasting of 48 h), each group with 8 animals. Even-Zohar *et al.,* used Sprague Dawley rats to compose three groups: *ad libitum*, food restriction and catch-up. Kuramitsu *et al*., made use of 84 male Wistar rats weighing 100 g and separated into the following groups: normal protein and energy; low energy; low protein and low energy and protein. Only the studies of

conditions of keeping the animals: light/dark cycle of 12/12h in the study of Even-Zohar *et al.*, and animals kept in conditions of constant temperature (21-24°C) with 14 h of light and 10 h of darkness in the study of Kanagawa *et al*., (Table I).

The nutritional stress in the studies by Nakamoto & Miller, Kuramitsu *et al*., and Kanagawa *et al*., was the result of protein malnutrition by offering feed containing 6% protein. On the other hand, Heinrichs *et al.*, and Even-Zohar *et al*., subjected animals to food deprivation: Heinrichs *et al*., through a fasting period of 48 h and Even-Zohar *et al*., through 40% food restriction based on a previous study in which the animals were kept individually and the daily consumption of feed per animal was assessed (Table I).

The age at which the nutritional stress was applied in the study of Nakamoto & Miller was from birth until the age of 10, 15 or 20 days of life. Heinrichs *et al*., applied the fasting in 5-week-old rabbits. In the study by Even-Zohar *et al.*, food restriction began at 24 days of life with a duration of 10 days. Kanagawa *et al*., applied malnutrition from birth until the age of 15 days. The study of Kuramitsu *et al*., does not mention the age of induction of malnutrition, it only states they used mice weighing 100 g and that malnutrition lasted the 15, 30 or 45 days of the experiment (Table I).

Among the studies selected, four used as an evaluation parameter the longitudinal length of the bone. Nakamoto & Miller measured the length of the femur with the aid of a caliper from the fovea of the head of the femur to the medial condyle. The studies by Even-Zohar *et al*., Kuramitsu *et al*., and Kanagawa *et al*., do not describe the tools used for the measurement of bone length nor the anatomical reference applied.

The histology of the epiphyseal plate was assessed in the studies by Heinrichs *et al*., Even-Zohar *et al*., Kuramitsu *et al*., and Kanagawa *et al*., In the study by Heinrichs *et al*., growth rate was evaluated by inserting metal pins percutaneously into the tibial metaphysis and then undergoing radiography before fasting. The x-rays were examined using a dissecting microscope and a micrometer. The examiner was unaware of the nutritional manipulation. After 48 hours of the experiment the animals were sacrificed. Histological sections of 6 μm of the proximal epiphyseal plate of the tibia were stained with Masson's Trichrome. In the study by Even-Zohar *et al.*, both tibial plates were isolated and stored at -20°C. For the histological analysis, 5 μm-thick sections were made and stained with Hematoxylin-Eosin and Alcian blue. Morphometric analysis was performed using the Olympus DP-soft software (Olympus Optical Co.). The measurement of the thickness of the epiphyseal plate was represented as the average of 6 measurements of two histological sections per animal. The count of the number of proliferative and hypertrophic chondrocytes was made in 4 columns per animal, in two histological sections. In the study by Kuramitsu *et al.*, and Kanagawa *et al*., the femurs were fixed with 10% formalin and decalcified with 10% formic acid. Sections of the distal growth plate of the femur were stained with Hematoxylin-Eosin for the measurement of the thickness of the growth plate. In addition, the study by Kanagawa *et al.*, also conducted a count of the number of chondrocytes in the proliferative and hypertrophic zones in one column in the center of the plate.

With respect to the longitudinal bone length it was found that protein malnutrition or food restriction caused deleterious effects on bone tissue, as evidenced by the shorter bone length in animals subjected to nutritional stress in the studies by Nakamoto & Miller, Even-Zohar *et al.*, Kuramitsu *et al*., and Kanagawa *et al*. Histological analysis of the epiphyseal plate demonstrated in the study by Heinrichs *et al*., a reduction in the number of chondrocytes per column in the hypertrophic and proliferative zones, a decrease in the total height of the plate and in the height of the terminal hypertrophic chondrocytes in animals subjected to fasting, moreover, the rate of growth of the tibia was smaller in animals subjected to fasting than in the nourished group. In the study by Even-Zohar *et al.*, the thickness of the epiphyseal plate remained constant for the *ad libitum* group, but was significantly reduced in the food-restricted group. The food-restricted group presented a reduction in the number of chondrocytes. In the study by Kuramitsu *et al.*, malnutrition resulted in a thinner epiphyseal plate and Kanagawa *et al*., found a reduction in the growth plate thickness and in the number of hypertrophic chondrocytes in the malnourished animals compared to the nourished animals, however, malnutrition did not change the number of proliferative chondrocytes. Heinrichs *et al*., concluded that, in rabbit, fasting causes a rapid decrease of growth plate chondrocytes.

DISCUSSION

Some methodological flaws were observed in the studies included. Heinrichs *et al*., Nakamoto & Miller and Kuramitsu *et al*., do not report the environmental conditions under which animals were kept. This is an important interference factor, as unfavorable environmental conditions become stress factors that can influence the

Table I. Description of the studies included in the quality evaluation of the articles.

Author	Model animal	Total size of the sample	Type of nutritional stress	Age of the animal when stress applied	Duration of the nutritional stress	Type of analysis for obtaining results	Randomization	Analytical masking of the results
Heinrichs *et al.* (1997)	Male *New Zealand* rabbits	16	Fasting	5 weeks	48 h	Histological analysis of the epiphyseal plate	Not reported	Yes
Nakamoto & Miller (1979)	Rats (breed and sex not reported)	16	Protein malnutrition (6%)	Birth	10, 15 and 20 days	Measurement of the length of the bone	Yes	Not reported
Even-Zohar *et al.* (2008)	Male *Sprague Dawley* rats	Exact sample size not reported. Authors stated that they used 5 to 6 animals per group	Food restriction (40%)	24 days	10 days	Histological analysis and measurement of the length of the bone	Not reported	Not reported
Kuramitsu *et al.* (1985)	Male *Wistar* rats	84	Protein malnutrition (6%)	Birth	15 days	Histological analysis and measurement of the length of the bone	Yes	Not reported
Kanagawa *et al.* (1987)	Male *Wistar* rats	12	Protein malnutrition (6%)	Not reported	15, 30 and 45 days	Histological analysis and measurement of the length of the bone	Not reported	Not reported

behavior and food consumption of animals, significantly altering the results. The studies by Even-Zohar *et al*., Kuramtsu *et al.*, and Kanagawa *et al*., do not describe the tools used for the measurement of bone, nor the anatomical reference used, which can make it difficult to compare their results with other works, besides making the reproduction of the their methodology of this parameter impossible. In only two studies (Nakamoto & Miller; Kanagawa *et al*.), were the random choice of groups described, based on another study. All of these studies used control groups, which leads one to presume that they were randomized, controlled studies. In the study by Kanagawa *et al.*, only the saline subgroups (control) were used as a means of comparison of the nourished and malnourished groups.

Malnutrition or food deprivation employed in the studies included in this review took place in the postnatal period, a critical period of development of the long bones (Nakamoto & Miller) that is characterized as being a fundamental stage for the acquisition of bone mass (Pettersson *et al.*, 2000) and in which a nutritional deficit can compromise the structure of the skeleton (Bonjour). In the studies by Nakamoto & Miller and Kanagawa *et al.*, malnutrition was applied in the period of breastfeeding. In the studies by Heinrichs *et al*., and Even-Zohar *et al.*, malnutrition was applied in the post-weaning period. However, the study by Kuramitsu *et al*., does not specify the age of induction of malnutrition.

The results of the effects of nutritional stress on the length of the long bones found in the studies by Nakamoto & Miller, Even-Zohar *et al*., Kuramitsu *et al*., and Kanagawa et al. are consistent and show that protein malnutrition (Nakamoto & Miller; Kuramitsu *et al*.; Kanagawa *et al*.), and food restriction (Even-Zohar *et al*.), lead to shorter lengths of longitudinal bone in animals subjected to nutritional stress. According to Nakamoto & Miller protein malnutrition affects the linear dimensions of long bones with damage to

the processes of formation and calcification of bone tissue. According to Kuramitsu *et al*., the changes found are possibly due to a disorder in the secretion of hormones that stimulate growth. These results suggest that protein deficiency reduces proliferation of cells in the growth plate, which induces slower longitudinal growth of long bones.

Similarly, the histological analysis of the epiphyseal plate in the studies by Heinrichs *et al*., Even-Zohar *et al*., Kuramitsu *et al*., and Kanagawa *et al*., demonstrated similarity in their findings: a reduction in the height of the epiphyseal plate (Heinrichs *et al*., Even-Zohar *et al*.; Kuramitsu *et al*.; Kanagawa *et al*.), and in the number of proliferative (Heinrichs *et al*., Even-Zohar *et al*.), and hypertrophic (Heinrichs *et al*., Even-Zohar *et al*.; Kanagawa *et al*.), chondrocytes as a result of protein malnutrition (Kuramitsu *et al*.; Kanagawa *et al*.), fasting (Heinrichs *et al*.,) or food restriction (Even-Zohar *et al*.). However, Kanagawa *et al*., found no difference in number of proliferative chondrocytes between the animals in the nourished and malnourished groups. The choice of the type of chondrocytes for the histological evaluation of the epiphyseal plate and its relationship with bone growth is consistent with the literature that demonstrates that the activity of the proliferative (Roach *et al.*, 2003) and hypertrophic (Hunziker & Schenk; Roach *et al*.), chondrocytes are what regulates the growth of longitudinal bone.

In conclusion, it has been demonstrated with this systematic review that there are few studies that evaluate the effects of nutritional stress by means of protein malnutrition or food deprivation applied in young animals on the growth of longitudinal bone. The studies included in this review demonstrated corroboration in the results obtained, although they did not present a standardized model in performing the nutritional stress (protein malnutrition, fasting or food restriction) nor in the period chosen for assessment of bone tissue subjected to this stress. With regard to the methodological aspect of the studies, there is also a deficiency in relation to criteria adopted for obtaining the sample (sample calculation and randomization).

Despite the different methodologies used for this type of evaluation, an analysis of the studies included in this systematic review demonstrates that there is susceptibility, primarily, of the epiphyseal plate, and secondarily, of the long bones to the effects of nutritional stress caused by protein malnutrition or food deprivation when applied during the postnatal period.

The need was also demonstrated for studies that evaluate the effects of protein malnutrition or food deprivation in the development and/or growth of the epiphyseal plate during the critical period of development and growth of bone tissue that apply similar methodologies and comply with the criteria that avoid as much as possible any bias of the work.

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RESUMEN: El objetivo fue realizar una revisión de la literatura sobre el impacto de la desnutrición proteica y/o privación de alimentos en la temprana formación de la placa de crecimiento en animales jóvenes. La búsqueda fue realizada en las bases de datos PubMed/MEDLINE, LILACS, SciELO y Biblioteca Cochrane sin restricción de lengua o límite de año. Fueron identificados inicialmente ciento cincuenta artículos que, posterior a la aplicación de los criterios de inclusión y exclusión, solo quedaron cinco. En tres estudios el estrés nutricional fue causado por la desnutrición proteica, otro al ayuno y el tercero fue por restricción alimentaria. Todos los estudios presentaban algunas omisiones metodológicas. Las investigaciones incluidas en esta revisión demostraron concordancia en sus resultados obtenidos, independientemente que los animales hayan sido sometidos a desnutrición proteica, ayuno o restricción alimentaria. Los datos obtenidos mostraron que los animales sometidos al estrés nutricional presentaron una reducción en la altura de la placa epifisaria, en el número de condrocitos proliferativos e hipertróficos, en la tasa de crecimiento óseo y en la longitud ósea. En esta revisión sistemática fue posible observar que los efectos del estrés nutricional, a través, de desnutrición proteica o de la privación alimentaria aplicada en animales jóvenes presentan, en primer lugar, una susceptibilidad de la placa epifisaria y de los huesos largos de manera secundaria.

PALABRAS CLAVE: Placa epifisaria; Crecimiento óseo; Desnutrición proteica; Privación alimenticia.

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Received: 07-10-2012
Accepted: 18-02-2013