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 Crecimiento de la Placa Epifisaria de Animales?

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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 (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: lámina 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.

<table>
<thead>
<tr>
<th>Author</th>
<th>Model animal</th>
<th>Total size of the sample</th>
<th>Type of nutritional stress</th>
<th>Age of the animal when stress applied</th>
<th>Duration of the nutritional stress</th>
<th>Type of analysis for obtaining results</th>
<th>Randomization</th>
<th>Analytical masking of the results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heinrichs et al. (1997)</td>
<td>Male New Zealand rabbits</td>
<td>16</td>
<td>Fasting</td>
<td>5 weeks</td>
<td>48 h</td>
<td>Histological analysis of the epiphyseal plate</td>
<td>Not reported</td>
<td>Yes</td>
</tr>
<tr>
<td>Nakamoto &amp; Miller (1979)</td>
<td>Rats (breed and sex not reported)</td>
<td>16</td>
<td>Protein malnutrition (6%)</td>
<td>Birth</td>
<td>10, 15 and 20 days</td>
<td>Measurement of the length of the bone</td>
<td>Yes</td>
<td>Not reported</td>
</tr>
<tr>
<td>Even-Zohar et al. (2008)</td>
<td>Male Sprague Dawley rats</td>
<td>Exact sample size not reported</td>
<td>Food restriction (40%)</td>
<td>24 days</td>
<td>10 days</td>
<td>Histological analysis and measurement of the length of the bone</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Kunamitsu et al. (1985)</td>
<td>Male Wistar rats</td>
<td>Exact sample size not reported</td>
<td>Protein malnutrition (6%)</td>
<td>Birth</td>
<td>15 days</td>
<td>Histological analysis and measurement of the length of the bone</td>
<td>Yes</td>
<td>Not reported</td>
</tr>
<tr>
<td>Kanagawa et al. (1987)</td>
<td>Male Wistar rats</td>
<td>12</td>
<td>Protein malnutrition (6%)</td>
<td>Not reported</td>
<td>15, 30 and 45 days</td>
<td>Histological analysis and measurement of the length of the bone</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

The results of the effects of nutritional stress on the length of the long bones found in the studies by Nakamoto & Miller, and Kunamitsu et al., and Kanagawa et al., indicate that protein malnutrition in animals subjected to nutritional stress, lead to shorter lengths of the long bones. According to Nakamoto & Miller, and Kunamitsu et al., food restriction and protein malnutrition in animals subjected to nutritional stress, lead to shorter lengths of the long bones. In the studies by Heinrichs et al., malnutrition was applied in the post-weaning period. However, the study by Kunamitsu et al., does not specify the age of induction of malnutrition. 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; Kuramitsu et al.; Kanagawa et al.), 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 Kunamitsu et al., does not specify the age of induction of malnutrition. 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; Kuramitsu et al.; Kanagawa et al.), 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 Kunamitsu et al., does not specify the age of induction of malnutrition.
¿Cómo interfieren la desnutrición protéica o la privación alimentaria en el crecimiento de la placa epifisaria de animales?

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 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, se reflejan en el crecimiento de la placa epifisaria y de los huesos largos de manera secundaria.

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

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