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Impact of prenatal protein-calorie malnutrition on the odontogenesis of wistar rats

Impacto da desnutrição protéico-calórica pré-natal sobre a odontogênese de ratos wistar

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ABSTRACT

Objective: This study aimed to determine changes in odontogenesis arising from prenatal and postnatal protein-calorie malnutrition. **Material and Methods:** Twelve adult Wistar rats were selected; 8 females and 4 males. The females were divided into two groups, one of which received a normoproteic diet (NG) and the other received a hypoproteic diet (HG). After the birth of the litters, 24 pups were randomly separated from each group. The animals were sacrificed, 12 at five-days old and 12 at eight-days old, and their jaws were subjected to histological preparation to obtain cuts of tooth germs. Forty-eight slides were selected that presented the germs properly cut (24 from each group), which were analyzed by microscopy and measured by a calibrated examiner. The differences between means were verified by the nonparametric Mann-Whitney test. **Results:** The results showed that at 5 days the differences in thickness of enamel and dentin were statistically significant between NG (84.08 ± 28.9 and 141.51 ± 33.2; p = 0.026) and HG (47.26 ± 43.8 and 91.19 ± 54.7; p = 0.006). At 8 days of life there were no significant differences between the groups. **Conclusion:** The results showed evidence of the impact of malnutrition on the thickness of dental tissues. It is suggested that further work should be carried out in this line of research with more complex designs.

KEYWORDS

Protein-calorie malnutrition; Fetal nutrition disorders; Odontogenesis.

RESUMO

Objetivo: Este trabalho teve por objetivo determinar alterações na odontogênese decorrentes da desnutrição protéico-calórica pré e pós-natal. Foram selecionados 12 ratos Wistar adultos, 8 fêmeas e 4 machos. As fêmeas foram divididas em dois grupos, sendo que um deles recebeu dieta normoprotéica (G1) e o outro recebeu dieta hipoprotéica (G2). **Material e Métodos:** Após o nascimento das ninhadas, foram separados aleatoriamente 24 filhotes de cada grupo. Os animais foram sacrificados, 12 aos 5 dias de vida e 12 aos 8, e suas mandíbulas submetidas à preparação histológica para a obtenção de cortes dos germes dentários. Foram selecionadas 48 lâminas que apresentavam os germes adequadamente cortados (24 de cada grupo), as quais foram analisadas em microscópio e medidas por um examinador calibrado utilizando-se o software Image Pro Plus for Windows, versão 6.0. As diferenças entre as médias foram verificadas mediante o teste não paramétrico de Mann Whitney. **Resultados:** Os resultados obtidos demonstram que, aos 5 dias, as diferenças de espessura de esmalte e dentina foram estatisticamente significantes entre G1 (84,08 ± 28,9 e 141,51 ± 33,2; p = 0,026) e G2 (47,26 ± 43,8 e 91,19 ± 54,7; p = 0,006). Aos 8 dias de vida não foram identificadas diferenças significativas entre os grupos. **Conclusão:** Os resultados demonstraram indícios de impacto da desnutrição sobre a espessura dos tecidos dentários. Sugere-se o aprofundamento desta linha de investigação com delineamentos mais complexos.

PALAVRAS-CHAVE

Desnutrição protéico-calórica; Ttranstornos na nutrição fetal; Odontogênese.

INTRODUCTION

Child malnutrition is defined as an imbalance between the need for nutrients and consumption, resulting in an accumulated deficit of energy, protein and micronutrients that may adversely affect the growth and development of children [1]. It is among the greatest public health problems in the world, especially in developing countries, because every year 90% of births weighing less than 2.5 kg are in these countries [2].

The most severe form of malnutrition is protein-calorie malnutrition (PCM) [3], which is a pathological condition resulting from insufficient protein and dietary energy fuels not meeting the needs of the body [4]. When initiated during the intrauterine life, with maternal malnutrition, it may significantly affect fetal growth, causing low birth weight, neonatal mortality and subsequent infant malnutrition [5]. Moreover, malnutrition impairs the function of the immune system, causing increased susceptibility to infections [6] and it promotes the poor development of organs, which is the start/stimulus for the failure of most biological systems [2].

In dentistry, the involvement of PCM in the etiology of development of the craniofacial complex has been investigated [7]. Experimental studies in animals have shown that PCM interferes with bone [8] and occlusion [9] development, changes the salivary glands [10] and dental tissue [11], causes delay in the timing of eruption [9,12,13], as well as possible changes in soft tissues, such as a reduction in the taste buds of the tongue [14].

The dental structures of enamel and dentin may be affected in their chemical, cellular and structural composition. Studies of the effects of malnutrition in dental germs, using malnourished rat models, showed smaller size and number of cells [14], a lower amount of calcium [15], and delay in calcification [10]. Holloway et al. [12] and Shaw and Griffiths [13] found, in undernourished animals, changes in dental morphology as well as molars with some of the subsidiary cusps decreased in size or absent, a factor that was most frequent in the third molars.

Furthermore, there is evidence in the literature that malnutrition affects the thickness of dental tissues, but the authors of the present manuscript did not find any studies that reported the changes during the development of the tissues. Some studies have identified that undernourished rats, before and after birth, have marked reduction in teeth size, which has been attributed to a decrease in volume of dentin but not to the thickness of the enamel [12,16]. This may be related to changes in the secretion of collagen [11].

The objective of this study was to determine the alterations in odontogenesis arising from prenatal and postnatal (pre-eruptive) PCM in relation to the thickness of dental tissues in Wistar rats.

MATERIAL AND METHODS

This experimental study was random and blinded. The initial sample was composed of 12 randomly selected adult Wistar rats - 8 females and 4 males - 120 days-old and with average weight of 300 g. During the experiment, the animals were maintained on a 12 h photoperiodic cycle with light and 12 h in the dark at an ambient temperature controled between 23 and 26 °C. The females were divided into two groups, one of which received a hypoproteic diet 15 days before mating, with the aim of inducing malnutrition and the other received a normoproteic diet “ad libitum”, keeping the pre-established diet and with unrestricted water until the end of the experiment.

The diet was prepared based on casein at concentrations of 25% and 6%, following Mello’s methodology [17], with added vitamin and mineral mix and choline hydrochloride in accordance with the most recent recommendations for the preparation of diets for laboratory rodents from the American Institute of Nutrition - AIN-93 [18]. The average daily dose of food per animal was 15 g, administered at ladle adapted in the cage of the females because the consistency of the diet was oily powder.

The sample was composed of 48 rats, 24 from nourished mothers (control group - NG), and 24 from malnourished rats (experimental group - HG), which were sacrificed on the 5th and 8th day of life by decapitation, comprising a sample of 12 animals in each subgroup. The histological technique was determined in a previous pilot study. After sacrifice, the mandibles of the animals were collected and divided into hemimandibles, facilitating the processing of the material. The hemimandibles were fixed in Bouin solution and were descaled using EDTA (pH adjusted to 7.0) dehydration, paraffinization and embedding. For the preparation of the slides, the standard technique of the Laboratory of Histological Techniques at UEPG was used; the coloring technique used was hematoxylin-eosin. All solutions were manufactured by researchers.

The slides underwent a selection process using a binocular light microscope with coupled camera (Microscope Olympus ® BX41, Olympus, Tokyo, Japan) at 40x magnification. Only the first lower molars were examined, irrespective of side, with one slide per animal selected (48 in total) that met the following criteria: (a) the presence of molar tooth germs and (b) cutting height in the region in which three of the largest cusps were present. The selected tooth germs had their images captured. The thickness of enamel and dentin were then measured, using Image Pro Plus for Windows software (version 6.0, Media Cybernetics, Silver Spring, USA). For this study, the mean between the largest and lowest thicknesses of enamel and dentin obtained from each slide was considered as a measure of analysis. The measurements were performed in a blind fashion by a single observer as follows: the largest thickness, obtained from the arithmetic mean of the measurements obtained in the three regions of the slope of the cusps with the thicker enamel and dentin; and the thinnest thickness, obtained from the arithmetic mean of the measurements obtained in the three areas with the thinnest thickness of enamel and dentin, measured in the region where the least amount of odontoblast and ameloblast layers were visible . Before taking the measurements, the examiner was calibrated and 12 samples were measured twice, at an interval of 48 h. To assess the reproducibility of the measurements, the visual method of Bland Altman [19] was used, through GraphPadPrism software (version 5.0, GraphPad TM , San Diego, USA) with a confidence interval of 95%.

The results obtained after taking the measurements were analyzed using SPSS for Windows software (version 12.0.1, SPSS Inc., Chicago, USA) and were presented by descriptive statistics, calculating proportions, means and standard deviation, and with differences assessed by the nonparametric Mann-Whitney test.

All the offspring of the rats were weighed and measured before sacrifice and their mothers were weighed at the end of the experiment, using a semi-analytical scale (Marte Balanças e Aparelhos de Precisão Ltda, São Paulo, Brazil). The measurements were performed with the aid of a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) from the base of the tail to the snout of the animal. This study was approved by the Ethics in Research of UEPG, through the Ethics Committee on Animal Use (protocol SCEEA/COEP no. 03/2008).

RESULTS

At the end of the experiment the measurements of the weight of the mothers showed an average of 288 g for group NG and 150 g for group HG, indicating that the diet used was effective in inducing PCM. Figure 1 shows the differences between the measurements of weight and the length of each group of rats.

Figures 2 to 5 show the Bland Altman plots to determine the reproducibility of the measurements of the largest and lowest thickness of the enamel (Figures 2 and 3) and dentin (Figures 4 and 5). The results indicate adequate reproducibility.

Figure 6 shows the differences between the measurements of the thicknesses of enamel and dentin. It was observed that at 5 days, the differences in thickness of enamel and dentin were statistically significant between group NG and group HG. At 8 days no differences were found between the groups.

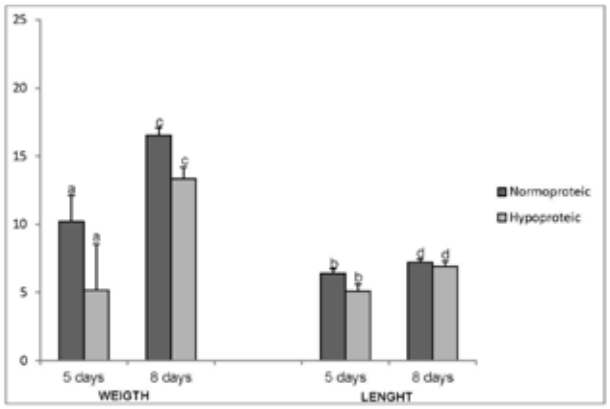

Figure 1 – Mean and standard deviation of weight and length for rats on the normoproteic and hypoproteic diets, according to age. Significant differences (Mann-Whitney test):
a) p < 0.001; b) p < 0.001; c) p = 0.030; d) p = 0.161.

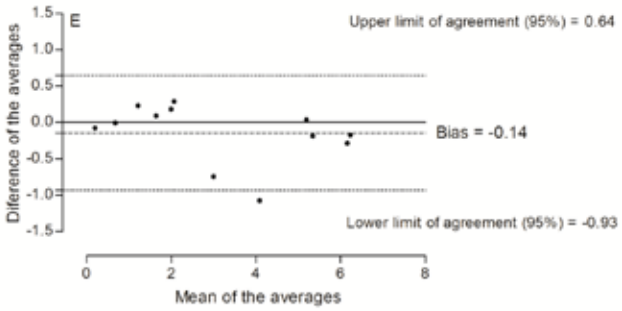

Figure 2 – Bland-Altman graph (intra-examiner reproducibility). Data relating to the measurements of largest enamel thickness, obtained on two occasions with an interval of 48 h. *p = 0.249. No significant differences in the hypothetical value (zero). T test for one sample.

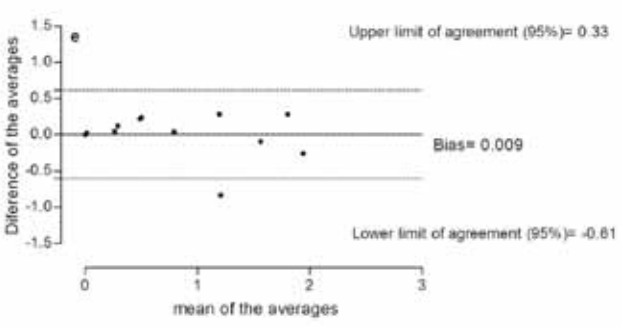

Figure 3 – Bland-Altman graph (intra-examiner reproducibility). Data relating to the measurements of smallest enamel thickness, obtained on two occasions with an interval of 48 h. *p = 0.920. No significant differences in the hypothetical value (zero). T test for one sample.

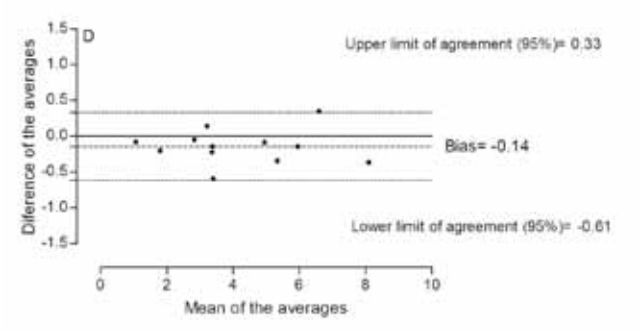

Figure 4 – Bland-Altman graph (intra-examiner reproducibility). Data relating to the measurements of largest dentin thickness, obtained on two occasions with an interval of 48 h. *p = 0.064 .No significant differences in the hypothetical value (zero). T test for one sample.

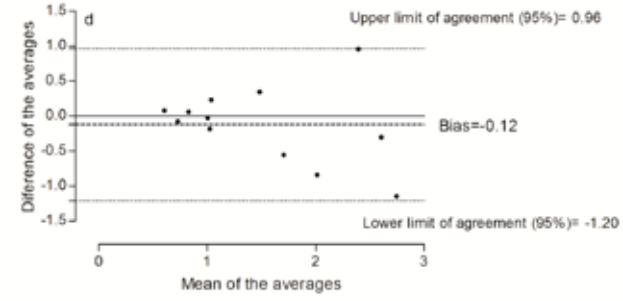

Figure 5 – Bland-Altman graph (intra-examiner reproducibility). Data relating to the measurements of smallest dentin thickness, obtained on two occasions with an interval of 48 h. *p = 0.466. No significant differences in the hypothetical value (zero). T test for one sample.

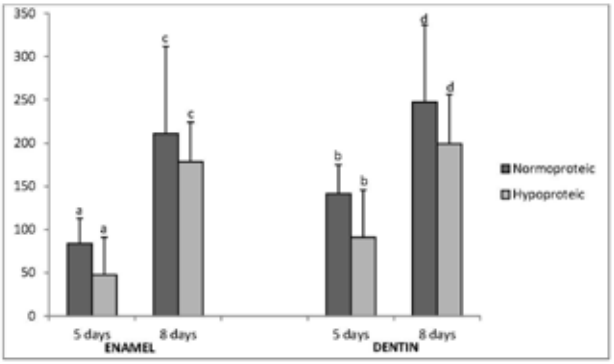

Figure 6 – Mean and standard deviation of the thickness of enamel and dentin for rats on the normal protein and low protein diet, according to age. Significant differences (Mann-Whitney test): a) p = 0.026; b) p = 0.006; c) p = 0.762; d) p = 0.186.

DISCUSSION

Even with decreasing levels of malnutrition, especially in developing countries, the condition is still prevalent and is considered to be a major problem in terms of public health [2]. Studies that seek to identify the impact of malnutrition on the development of dental structures have shown to be important in order to permit the adoption of measures to minimize it.

Human studies have shown that deficiencies in protein, minerals and vitamins in the perinatal period are related to changes in the development of the teeth (enamel hypoplasia and hypocalcification) and supporting structures, salivary glands, and delays in the chronology of eruption in children [20]. These conditions have been associated with an increased susceptibility of teeth to caries [21].

The use of rats as experimental biological models of nutritional deficiency is widespread and has demonstrated good results that, in the main, reflect those found in relation to the metabolism of malnourished humans [22]. To establish the methodology of this study, the period of early odontogenesis in rats described by Schour and Massler [23] was considered. According to these authors, in a normal development process the apposition of dentin of the first molar in rats begins between the 20th and 21st day of intrauterine life (IUL) and the crown is complete on the 11th day of life. Consequently, for this present research, so that maternal malnutrition would be maintained after the birth of the offspring, the animals were sacrificed on the 5th and 8th day of life (a period of apposition of enamel and dentin, and before the end of the formation of the crown) in order to preserve protein structure in the enamel.

Low birth weight in relation to gestational age, reflecting the conditions of intrauterine life, is indicative of a deficit in the development of the newborn [24]. Experimental studies in animals, in which prenatal and postnatal (or both) PCM is induced, demonstrates the impact of low birth weight on development. After inducing malnutrition in rats, Holloway et al. [12] found smaller, undernourished, lighter pups with retarded development (opening of eyes and outer ear). Nunes et al. [25] submitted rats to low PCM during their growth, resulting in animals with smaller size and lower weight than animals with a normal diet. Corroborating the findings of these authors, the present study found significant differences in the weight of the animals.

With respect to the length of the animals, it was observed that group HG (5 days) was statistically lower than group NG (see Figure 1). However, at the 8th day there was no statistically significant difference in length between the groups of animals. Some studies have found no differences between nourished and malnourished animals after the restoration of a normal diet. Shaw [26] studied the effects of malnutrition during the reproductive period of rats that were fed on diets with 24% and 6% casein. The malnourished animals during pregnancy were born smaller and lighter, but this difference was remedied over time after the administration of a normal diet which caused the malnourished animals to grow faster than the control group. These results demonstrate the resilience of the body in relation to the size of the animal. For the present study, it could be argued that the animals had sufficient amounts of protein for their lengths to be equivalent, from lactation onwards.

The results obtained for the differences in length of between 5 and 8 days were consistent with those observed regarding the thickness of dental tissues. At 5 days, differences were identified in the thicknesses of enamel and dentin that were significantly lower in group HG, with differences of 36% for enamel and 44% for dentin. However, at 8 days, the differences in enamel and dentin were not significant, falling as a percentage to 16% and 19.5%, respectively. With these results, it can be suggested that a hypoproteic diet administered during pregnancy can lead to lower thickness of dental tissues during a given period of development.

The results of this experiment also corroborate a research done by Huumonen and Larmas [16] who investigated the effects of protein deficiency and sucrose in the formation and mineralization of dentin in Wistar rat molars. Tetracycline was injected at the outset to mark the dentin formed up to that moment, and the average of 3 measurements made in the cleft of the primary molars was calculated. In line with the reduction of overall growth, rats in the undernourished group had smaller areas of dentin formed during the experiment in comparison with the nourished group.

However, the results of this study were different from those obtained by Gonçalves [11], who studied the first molars of rats fed on diets of 20% and 5% casein. At 21 days of life, the author found that the measurements of sagittal and transverse dentin showed a difference of 30% in dentin thickness; lower in the hypoproteic group. Glick and Rowe [10] administered a diet based on 1% and 18% lactalbumin for 10 weeks after birth. Abnormalities in the pigmentation and calcification of enamel were not found, only a reduction in the size of the tooth germ in the hypoproteic group with a decreased rate of dentin apposition of 32%. The thickness of predentin in the undernourished group was 135% higher than the control group, and this was related to low amounts of calcium.

The decrease in thickness of dental tissues found in this study may be related to changes in the composition and structure of enamel and dentin. Gonçalves [11] found changes in the thickness and the type of dentin collagen. Navia et al. [27] studied the effect of malnutrition in prenatal and postnatal rats and found areas of hypomineralization of the dental tissues that were more numerous in mothers that were fed a hypoproteic diet during lactation. The authors suggest that these hypomineralized areas might contribute to increased susceptibility to decay.

Although the results of the present study are consistent with reports in the literature, one must consider the limitations arising from the methodological choices that were adopted. In this study, we chose to perform only the analysis of tissues under light microscopy. The main difficulty that was identified was in obtaining histological sections, and it was attempted to overcome this problem by a comprehensive pilot study of histological techniques. It is suggested that further studies should be conducted with more sophisticated designs and techniques in order to confirm the findings of this experiment.

CONCLUSION

The impact of prenatal and postnatal protein-energy malnutrition was observed on the fifth day of life, and lessened at eight days. These results are consistent with the hypothesis that malnutrition can differently affect the various periods of tooth development.

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