0021-7557/08/84-03/264
Jornal de Pediatria

The effects of prenatal and postnatal malnutrition on the morphology, differentiation, and metabolism of skeletal striated muscle tissue in rats

Alessandra P. Alves,[1] Ana R. Dâmaso,[2] Vitalino Dal Pai[3†]

Abstract

Objective: To study the contractile properties, metabolism and morphological characteristics of muscles submitted to prenatal and postnatal protein malnutrition.

Methods: Animals were distributed into two groups: Control, normoprotein diet (CG; n = 15; 5/5/5), and Malnourished, hypoprotein diet (MG; n = 15; 5/5/5), and examined on the 7th, 14th, and 28th days of the experiment. Total body mass, weight, and the contractile properties and morphology of the anterior tibial muscle were assessed. Several 8 µm-thick tissue samples were taken from 7, 14, and 28 day old rats and stained with HE or subjected to NADH-TR or m-ATPase (pH = 4.4) techniques.

Results: Body and muscle weight were lower in the malnourished group. On the 7th day of malnutrition, muscle samples exhibited fibers with smaller diameter, higher polymorphism and higher endomysial conjunctive tissue content. Histochemical methods were unable to precisely determine the types of fiber present. On the 14th day, there were smaller muscle fibers, more polymorphism, many of them with central nuclei and moderate endomysial conjunctive tissue content. With reference to contractile properties, the m-ATPase reaction identified both slow and fast fibers. The NADH-TR reaction revealed the following types of fiber: slow oxidative (SO), fast oxidative glycolytic (FOG) and fast glycolytic (FG). On the 28th day smaller, bunched muscle fibers varying shapes. All three types of fiber exhibited unclear recognition limits with respect to contraction and metabolism.

Conclusion: Our experimental results suggest that, in addition to the reduction in numbers of fibers, malnutrition retards the differentiation of the morphological, metabolic, and contractile characteristics of skeletal muscle fibers in growing rats.

J Pediatr (Rio J). 2008;84(3):264-271: Malnutrition, skeletal muscle.

Introduction

Under normal nutritional conditions, uptake and utilization of nutrients are in equilibrium, which ensures growth, maturation and cell division. These phenomena have a distinct influence on the phases of gestation, lactation, young and adult.

Proteins are nutrients that are essential for cellular homeostasis and, if protein deficiency is accentuated and happens during pregnancy, known as prenatal malnutrition, then an imbalance will occur that causes changes to the tissues and structures of the organs,[1-3] such as low fetal weight and enzymatic and biochemical abnormalities. The effects of intrauterine malnutrition depend on the phase of development, and

1. Mestranda em Fisiologia do Exercício, Departamento de Ciências Fisiológicas, Universidade Federal de São Paulo – Escola Paulista de Medicina (UNIFESP-EPM), São Paulo, SP, Brazil.
2. Pós-doutorado em Pediatria. Docente, Departamento de Ciências da Saúde, UNIFESP-EPM, São Paulo, SP, Brazil.
3. Doutor, livre-docente. Docente, Departamento de Histologia, Universidade do Oeste Paulista (Unoeste), Presidente Prudente, SP, Brazil.

Equipment supplied by Universidade do Oeste Paulista (Unoeste), Presidente Prudente, SP, Brazil.

No conflicts of interest declared concerning the publication of this article.

Suggested citation: Alves AP, Dâmaso AR, Dal Pai V. The effects of prenatal and postnatal malnutrition on the morphology, differentiation, and metabolism of skeletal striated muscle tissue in rats. J Pediatr (Rio J). 2008;84(3):264-271.

Manuscript received Aug 28 2007, accepted for publication Jan 09 2008.

doi:10.2223/JPED.1769

will be more intense and longer-lasting the earlier that malnutrition occurs and the later that nutritional recovery takes place.[1]

Skeletal muscle tissue is susceptible to protein malnutrition because it is one of the body's protein stores. Therefore, when there is a dietary protein deficiency, these tissues become the target of depletion,[2,4,5] modifying growth phases and differentiation of muscle fibers.

Differentiation of fiber types is a gradual process, occurring during the prenatal and postnatal periods and varying from species to species.[6,7] During embryogenesis, primary and secondary myotubes are formed simultaneously, and are innervated differently in one phase of development, and for secondary myotubes, in addition to specific innervation, muscular activity is also necessary.[8]

At birth, the maturity of muscle fibers is independent of nutritional status, and energy malnutrition affects skeletal muscle if it occurs during the phase of myogenesis, altering the frequency of fiber types.[9]

Due to the high prevalence rate of malnutrition in developing countries, it is important to carry out experiments to evaluate the possible consequences for the process of growth of rats during the lactation-weaning transition phase. Based on this information, this study was carried out with the objective of evaluating the morphological characteristics of striated skeletal muscle and also oxidative and glycolytic metabolism and the contractile properties of slow and fast fibers from the offspring of rats subjected to protein malnutrition.

Methods

Experiment

For this experiment 24 Wistar rats (*Rattus norvegicus albinus*) were used, 18 nulliparous females and six males, at a proportion of 3:1. As soon as pregnancy was detected, the males were separated from the females, which were then split into two groups: a control group (CG), who were given a normoprotein diet (22% protein) and a malnourished group (MG), fed a hypoprotein diet (8% protein). Both groups received their experimental diets throughout gestation and lactation up to the 28th day of the experiment.[10] Animals were kept under a 12-12h light-dark regime at an ambient temperature of 23 to 25 ºC, with food and water *ad libitum*. After birth, the offspring were with or without intrauterine malnutrition were adjusted. Five male offspring were kept in each group with the lactating mothers up to the ages of 7, 14 and 28 days. The animals were then anesthetized with intramuscular acepromazine at 2%, and euthanized with intravenous sodium thiopental . The euthanasia protocol was approved by the Ethics Committee at the Universidade do Oeste Paulista (Unoeste), hearing number 025/02. Next, the anterior tibial muscle of the right pelvic limb was dissected. Next, tissue samples of approximately 1 cm in length and 0.5 cm in diameter were taken from the ventral area of the muscle. A period of 5 to 10 minutes was allowed to elapse for the material to acclimatize to room temperature, and then the samples were immersed in n-hexane that had been chilled to -70 ºC using liquid nitrogen.

Histochemical analyses

Several 8 µm-thick slices were taken from each specimen, using a cryostat microtome, at -22 ºC, and set on microscope slides for 30 minutes. After fixing in Baker's formol-calcium, some sections were stained with hematoxylin and eosin (HE), some underwent histochemical reaction with nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) and others were subjected to myofibrillar ATPase reaction (m-ATPase), after incubation in an acid medium (pH = 4.4).[11]

Several microscope fields were evaluated and the morphological characteristics of the tissues observed were described, as were the fibers' reactivity for oxidative and glycolytic metabolism and for fast and slow contractile properties. Muscle fibers were classified as slow oxidative (SO): slow contraction and oxidative metabolism; fast oxidative glycolytic (FOG): fast contraction and oxidative and glycolytic metabolism; or fast glycolytic (FG): fast contraction and glycolytic metabolism.

Statistical analysis

Data on body weight and muscle weight of the CG and MG underwent statistical analysis using Student's *t* test. The percentages of oxidative fibers (SO + FOG) and of glycolytic fibers (FG) underwent nonparametric analysis of variance, the Kruskal-Wallis test and Dunn's multiple comparisons test for rats aged 7, 14 and 28 days from the CG with the fibers from the MG. The level of significance was defined as $p < 0.05$.[12]

Results

Body weight and muscle weight

It was observed that both the overall body weights and the weights of the anterior tibial muscle were greater in CG animals than in MG animals, at 7, 14 and 28 days (Table 1).

Histochemical parameters: morphology, differentiation and metabolism

The sections stained with HE from the 7-day group, which had been cut perpendicular to the longest axis of the muscle, proved to be made up a large number of fibers with a variety of rounded and polygonal outlines, with mildly acidophilic cytoplasm and with one or more nuclei in peripheral positions. However, many fibers exhibited undifferentiated characteristics, more rounded outlines, nuclei in central positions

Table 1 - Means of body and muscle weight and medians of oxidative fibers (SO + FOG) and glycolytic fibers (FG) from offspring of rats in control and malnourished groups, assessed at days 7, 14 and 28

	Age					
	7 days		14 days		28 days	
Weight/fibers	Control	Malnourished	Control	Malnourished	Control	Malnourished
Body weight (g)	12.7±0.63	6.94±0.95*	18.1±1.30	14.6±2.26	50.6±2.30	17.1±1.48*
Muscle weight (mg)	17.4±1.47	4.98±1.75*	26.5±3.24	14.6±2.26*	96.5±4.51	23.9±2.47*
SO + FOG	89.0	90.0	96.0	91.0	91.0	99.0*
FG	31.0	30.0	24.0	29.0	29.0	21.0*

FG = fast glycolytic; FOG = fast oxidative glycolytic; SO = slow oxidative.
* $p < 0.05$.

and cytoplasm more alkaliphilic, in addition to a moderate proportion of endomysial conjunctive tissue. At this age, the fascicles exhibit an organization pattern that is not very defined. Some fibers had nuclei in central positions, being in the myotube phase, and there were also cells from the myogenic cell line (Figure 1A).

The histological sections that were processed using m-ATPase (pH = 4.4), revealed intense reactivity in all fibers, demonstrating that these fibers have slow contractile properties. Furthermore, in some of the fibers with greater diameter, this reactivity was even more intense. The conjunctive tissue between these fibers exhibited a negative reaction. Although it was not possible to delimit the different fiber types, reactivity was mildly more intense in some of the smaller fibers. These fibers are of type SO, slow contracting. The other fibers, of larger diameter, are of types FOG and FG, both fast contracting. At this age, distinctions between the fibers are not clear, with a high proportion of slow contracting myosin (Figure 1C).

At this stage of growth, reactivity of the muscle fibers to the NADH-TR enzyme, which reveals whether metabolism is oxidative or glycolytic, was more intense in the smaller diameter fibers, indicating SO fibers, and weaker in those of larger diameter, indicating fiber types FOG and FG, with oxidative/glycolytic metabolism and glycolytic metabolism, respectively. Furthermore, the intensity of the FOG fibers' reactivity was intermediate, between that of the SO and FG fibers (Figure 1E).

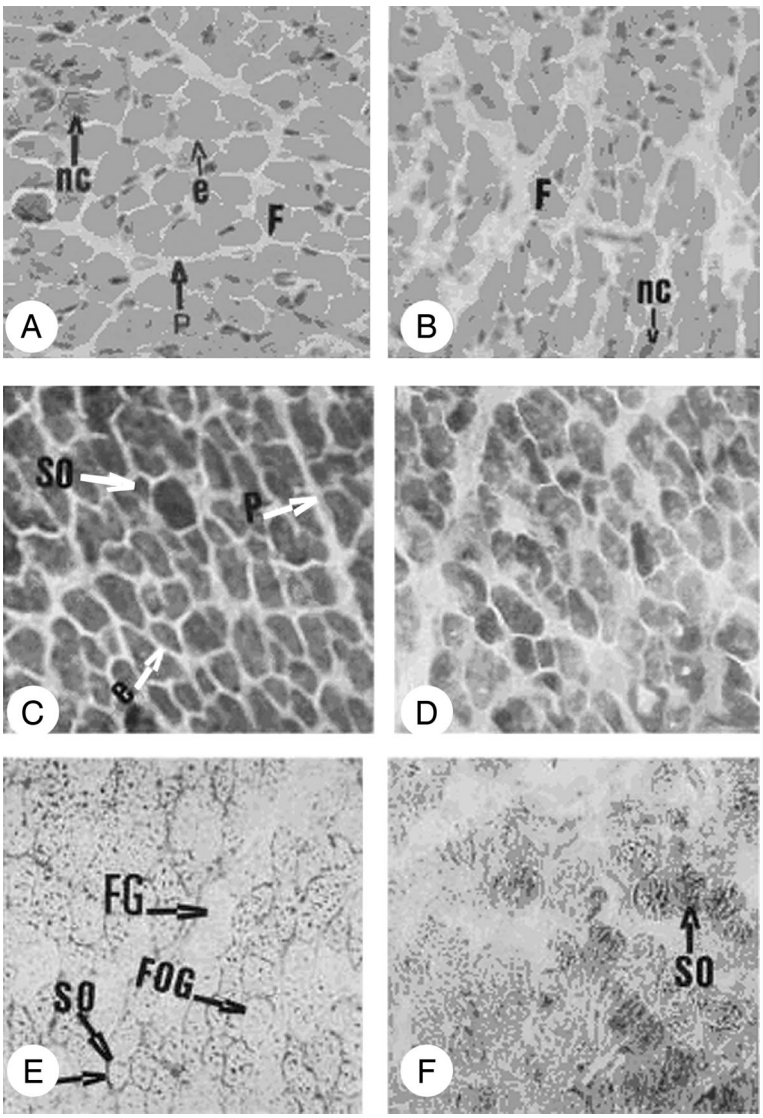
In contrast, in the muscle tissue of rats with 7 days' protein malnutrition, the fibers were smaller, with more variability of outline, less acidophilia and a high proportion of loose conjunctive tissue. In addition to this, the organization patter of the muscle fiber fascicles was disorganized. Likewise, there were a large number of fibers with central nuclei (Figure 1B).

Subsequent sections, when subjected to the m-ATPase (pH = 4.4) reaction, revealed a highly variable reaction intensity between different types of fibers. In some fibers, the reaction was intense, with a great deal of variation between the two extremes, varying from moderate to weak in the rest. Although the limits between fibers were difficult to identify, the fibers with intense reactivity were slow contracting fibers (SO), and the others were FOG and FG, both fast contracting (Figure 1D).

Still with relation to the group of rats with 7 days' malnutrition; the histological sections that underwent the reaction to reveal oxidative/glycolytic metabolism, the NADH-TR enzyme, reactivity was of variable intensity in different fibers, being discretely more intense than in the CG. However, recognition of the limits between the finer types was not very clear in this group of animals. Thus, smaller fibers were observed, with greater reactivity, which were the SO fibers, and fibers with less reactivity, belonging to the fiber subtypes FOG and FG (Figure 1F).

At 14 days postnatal, the anterior tibial muscle of the rats in the CG revealed, with HE staining, fibers bunched in fascicles with varying diameters, polygonal outlines and one or more nuclei in a peripheral position. Fibers with central nuclei were infrequent. With relation the 7-day rats, the fibers were larger and of a more differentiated appearance (Figure 2A). At this age, the m-ATPase (pH = 4.4) reaction identified three types of fibers: small with an intense reaction (SO), medium, with a moderate reaction intensity (FOG) and large, with weak reactivity (FG). At 14 days the FG make up the greater part of the fibers (Figure 2C). Histological sections, when subjected to the NADH-TR reaction, revealed intense reactivity in smaller fibers, indicating SO and FOG fibers, being weak in those of greater diameter (FG) (Figure 2E).

To a significant extent, at this age the anterior tibial muscle of the rats in the MG exhibited smaller fibers than that from the CG, with variable outlines, many with central nuclei, being compatible with myotubes. Between the fibers, a moderate proportion of conjunctive tissue was observed (Figure 2B). The m-ATPase (pH = 4.4) reaction demonstrated moderate reactivity in the majority of the fibers. In some of the fibers (SO), the reaction was more intense. At this phase of growth,

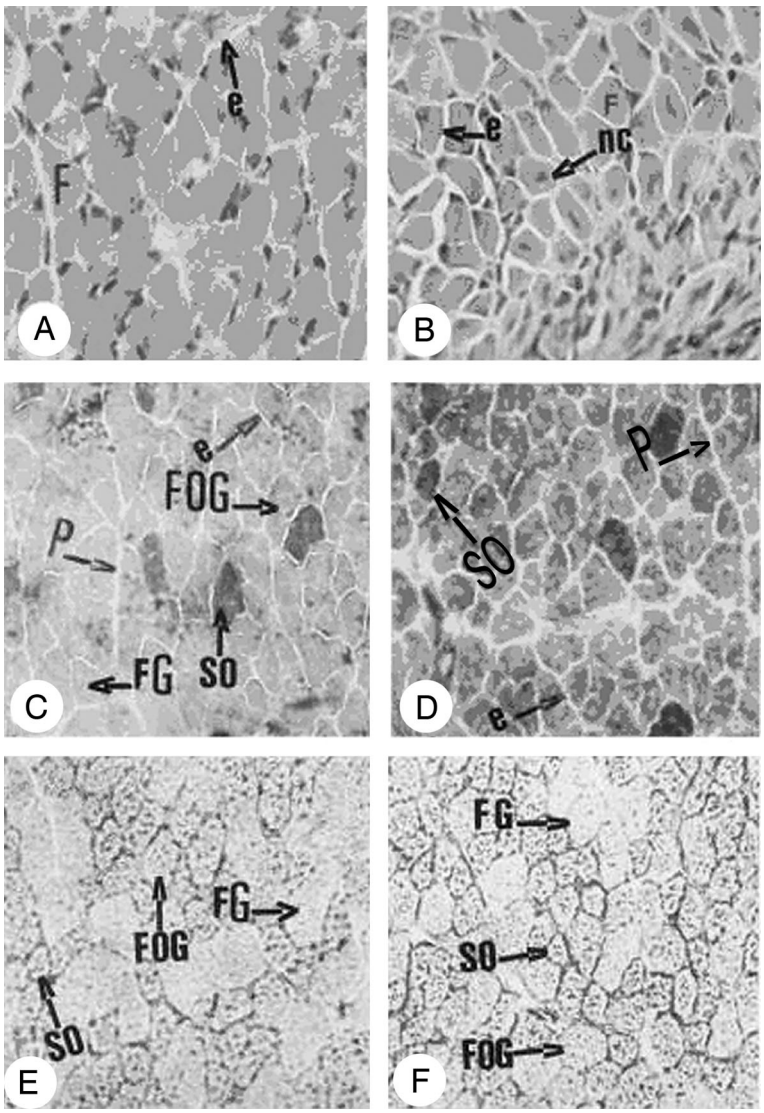


A) Control group: muscle fibers (F), perimysium (P), endomysium (e), central nucleus (nc); HE, 128x magnification. B) Malnourished group: smaller muscle fibers, more polymorphism (F), central nucleus (nc); HE, 128x magnification. C) Control group: slow contracting fibers and oxidative metabolism (SO), perimysium (P), small muscle fiber (B); m–ATPase (pH = 4.4), 128x magnification. D) Malnourished group: darker, intermediate and lighter fibers with diameters and outlines; m-ATPase (pH = 4.4), 128x magnification. E) Control group: slow contracting fibers and oxidative metabolism (SO), fast contracting and oxidative and glycolytic metabolism (FOG), and fast contracting and glycolytic metabolism (FG); NADH-TR, 128x magnification. F) Malnourished group: slow fibers and oxidative metabolism (SO), with intense reactivity, moderate and weak reactions in other fibers; NADH-TR, 128x magnification.

Figure 1 - Cross sections of anterior tibial muscle of Wistar rat: HE, m-ATPase (4.4), NADH-TR, 7 days (control and malnourished groups)

the distinction between the fiber types FOG and FG was difficult to discern (Figure 2D). The results of the NADH-TR reaction identified three basic types of fiber, being, the SO with an intense reaction, the FOG with moderate reactivity and the larger FG, with a weaker reaction (Figure 2F).

At 28 days, the tibial muscles of the rats in the CG were made up of fibers with significantly larger diameters, polygonal outlines and with peripheral nuclei, observed using HE staining. The cytoplasm of the smaller fibers was mildly alkaliphilic, compatible with SO type fibers. Some of the fibers still had central nuclei (Figure 3A). Reactivity to the m-ATPase enzyme identified the three basic types of fiber: SO, with very intense reactivity, through moderate in FOG and weak in FG (Figure 3C). Similarly, the reactivity to the NADH-TR enzyme revealed a patter of reactivity characteristic of an advanced degree of differentiation, where the SO fibers, with small diameter, exhibited an intense reactivity; while those of



A) Control group: muscle fibers (F), with larger diameter and with a more differentiated appearance, with relation to the 7-day group, endomysium (e), perimysium (P); HE, 128x magnification. B) Malnourished group: muscle fibers (F) smaller with higher proportion of conjunctive tissue, fibers with central nuclei (nc), endomysium (e), perimysium (P); HE, 128x magnification. C) Control group: fiber types (SO, FOG, FG), endomysium (e); m-ATPase (pH = 4.4), 128x magnification. D) Malnourished group: fibers with intense reactivity (SO), moderate reactivity in the majority of fibers, endomysium (e); m-ATPase (pH = 4.4), 128x magnification. E) Control group: smaller muscle fibers (SO, FOG) with greater reactivity and larger fibers (FG), with weak reactivity; NADH-TR, 128x magnification. F) Malnourished group: smaller fibers, with oxidative metabolism (SO + FOG), larger fibers (FG), with glycolytic metabolism; NADH-TR, 128x magnification.

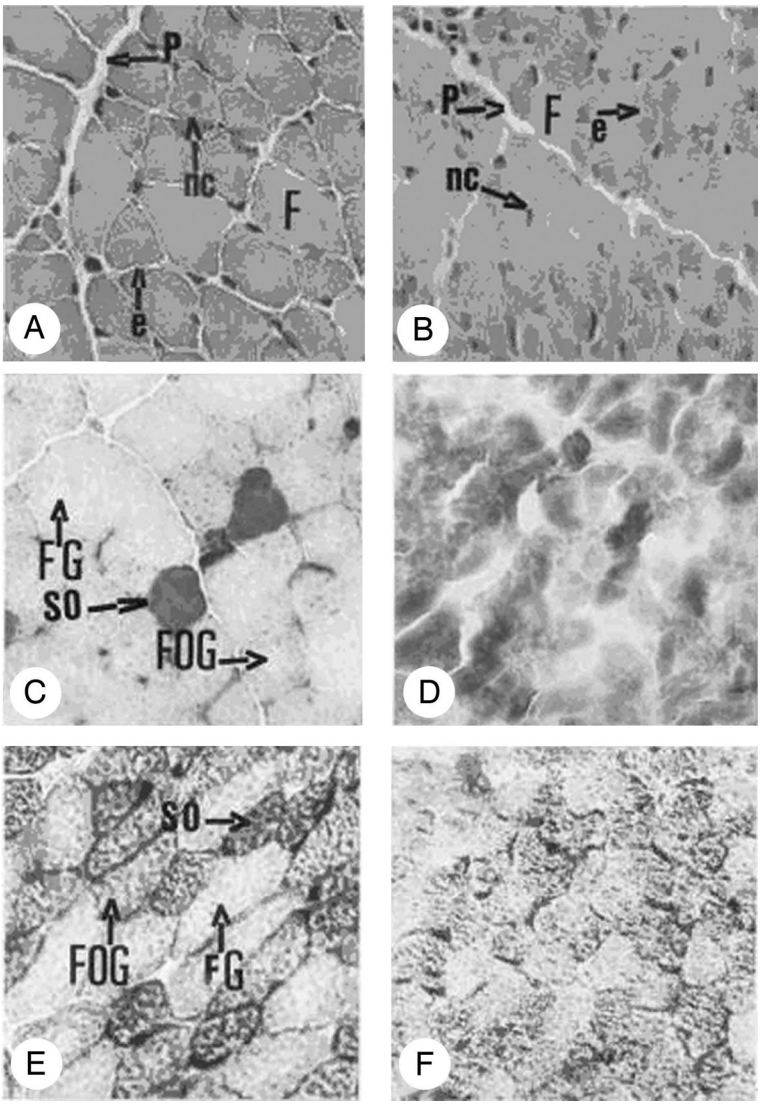
Figure 2 - Cross sections of anterior tibial muscle of Wistar rat: HE, m-ATPase (4.4), NADH-TR, 14 days (control and malnourished)

medium diameter (FOG) had medium reactivity and homogeneous distribution throughout the cytoplasm, and the FG, more hypertrophic, with weak reactivity (Figure 3E).

In the 28 days' malnutrition group, morphological, metabolic and contractile patterns of the fibers revealed the following changes: smaller fibers, that are elongated and in a compact arrangement (Figure 3B); fibers with central nuclei; reactivity to m-ATPase strong, medium and weak, with unclear limits between fiber types (Figure 3D); reactivity NADH-TR strong, medium and weak in SO fibers, FOG and FG, respectively (Figure 3F).

Discussion

According to the literature, nutritional restriction significantly affects growth and differentiation of tissues and cells. The earlier the malnutrition, the more severe the effects, resulting in greater severity and extent of damage to a range



A) Control group: muscle fibers (F), with differentiated appearance and varying diameters, perimysium (P), endomysium (e), central nucleus (nc); HE, 128x magnification. B) Malnourished group: fibers (F) with smaller diameter with relation to the control group, with variable diameters and outlines, perimysium (P), endomysium (e), central nucleus (nc). C) Control group: types of SO fibers, FOG, FG; m-ATPase (pH = 4.4), 128x magnification. D) Malnourished group: muscle fibers with strong, medium and weak reactivity with unclear limits; m-ATPase (pH = 4.4), 128x magnification. E) Control group: fibers with small diameters (SO), with intense reactivity, fibers of medium size, with moderate reaction (FOG), larger fibers, with weak reactivity (FG). F) Malnourished group: strong reactivity for oxidative metabolism in small, medium fibers, and weak reactivity in larger fibers with glycolytic metabolism; NADH-TR, 128x magnification.

Figure 3 - Cross sections of anterior tibial muscle of Wistar rat: HE, m-ATPase (4.4), NADH-TR, 28 days (control and malnourished)

of organs and systems.[4] These findings emphasize the importance of nutritional recovery at early phases of life, in order to achieve normal growth and development.[13-15]

Research demonstrates that maternal protein deficiency at the start or from the second half of gestation affects the body weight and number of muscle fibers in the offspring, observed in studies undertaken with low birth weight rats, dogs and monkeys.[2,5,16]

In our investigation, body weight was significantly lower in the MG than in the CG. This difference was approximately three times greater at 28 days' age, demonstrating that the deleterious effects on the offspring's growth are accentuated to the extent that the duration of malnutrition increases. Similar results have been reported by several other authors in their studies of prenatal malnutrition.[4] It has also been observed that protein malnutrition statistically reduces the muscle tissue weight. This is probably due to lost tissue proteins. Several authors have reported similar results.[2,5,17]

Studies of the size of the muscles of animals subjected to postnatal malnutrition have revealed that this reduction is primarily attributable to a reduction in the volume of the fibers (hypotrophy) rather than to a reduction in the total number of fibers in the tissue (hypoplasia).[17-19] With intrauterine malnutrition, however, there is evidence that the phenomenon of hypoplasia is what occurs. Therefore, in this study, the result of lower muscle weight among the malnourished animals appears to be the result of both processes, hypoplasia and hypotrophy, since they were subjected to malnutrition both during the intrauterine period and while newborn.

At birth, even in the normal rats, the muscle tissue had a high number of immature fibers. In addition to the format of the fibers, the increased cytoplasmic alkalinophilia and the occurrence of variable numbers of fibers with one or more nuclei in a central position are all characteristics indicative of the immaturity of the tissue.[20,21] As with the morphology, the metabolism and slow and fast contractile properties of the fibers revealed the greater immaturity at ages 7 and 14 days, although this was worse among the rats subjected to protein restriction.

A study involving quantitative aspects of the growth and differentiation of muscle fibers did not describe the occurrence of any increase in the interstitial space, although the number of fibers was reduced.[5] In our study, although the number of fibers was not analyzed, the increased proportion of the loose conjunctive tissue area between the fibers is indicative of hypoplasia.

Among mammals, it has been observed that tonically active striated red muscles have more oxidative activity than white ones.[2] According to the literature, there are two types of red fibers: those with slow contraction and oxidative metabolism (SO) and those with fast contracting and oxidative and glycolytic metabolism (FOG).[22] In this study, during the periods studied, the anterior tibial muscle proved to have an elevated proportion of red fibers, being greater in the MG.

In turn, the white fibers have rapid contractile properties and glycolytic metabolism (FG) and exhibit, during the ages studied, lower frequencies among the rats in the MG. The greater number of red fibers observed in the MG appears to be associated with the lesser degree of differentiation of the fibers, which is attributed to malnutrition.

The greater or lesser degree of the red coloration of a muscle or its regions is dependent on the types and frequency of its fibers.[23] For these authors, the deepest region of a muscle contains a greater frequency of fibers with aerobic metabolism (SO and FOG) and glycolytic fibers (FG) predominate in the superficial areas. Previous studies in this area of investigation have demonstrated that malnutrition significantly affects the growth and maturation of the central and peripheral nervous system.[23,24]

Some authors emphasize that differentiation between the different types of muscle fiber depends on the neuron that innervates it, and protein deficiency can result in hypoplasia.[25]

In the animals in the CG, and with greater intensity in the MG, the muscle tissue studied revealed the presence of fibers with central nuclei, compatible with fibers in an undifferentiated phase - myotubes. In the rat myotubes proliferation occurs from the 17th day of gestation on, continuing until the second week after birth.[25] In our results, we observed a greater number of myotubes in the weeks after birth, especially among the malnourished animals. These myotubes could still be detected in the 4-week-old animals. Based on the m-ATPase reactivity, which reveals the contractile properties of the fibers, slow or fast, at 7 days some fibers could be observed with larger diameter and greater reactivity for slow-contracting myosin than for other fibers. These appeared to be disperse and, from a histochemical point of view, are considered equivalent to the alkaliphilic B fibers, with the characteristics of myotonia described by Dubowitz.[26] In humans, these fibers account for 4 to 5% of fibers at the 18th week of gestation. Its myosin is of the slow contraction type,[27] and their number becomes greatly reduced at around the 30th week of gestation. At this phase of development, the slow contracting fibers are also present in the muscle in greater numbers, being variables and weak, indicating the presence of fast contracting myosin, characterized by its intense reactivity to m-ATPase, although only when the reaction is carried out without pre-incubation in acid pH.[28]

In the study groups, the reactivity pattern of the oxidative and glycolytic metabolism of the fibers was more differentiated than the contractile properties, even in the rats in the 7-day group, although this was more notable in the animals in the CG. Among these animals, both the morphology of the fibers and the endomysial conjunctive tissue was normal with a greater degree of differentiation.[27,29]

The results of this study suggest that intrauterine malnutrition and during the neonatal period provokes a reduction in the number of fibers, in addition to delaying the differentiation of morphological, metabolic and contractile characteristics of skeletal muscle fiber types in rats during growth, emphasizing the importance of good nutrition and of avoiding the deleterious effects becoming irreversible. However, further studies should be carried out with the objective of confirming this evidence.

Acknowledgements

We are indebted to Professor Dr. Vitalino Dal Pai (*in memoriam*), whose grandeur and constructive spirit are an example to us all, for his friendship and complete cooperation and assistance with the histochemical techniques.

References

1. Patrício FR, Nóbrega FJ, Tonete SS.Desnutrição intra-uterina em diferentes períodos de gestação em ratas: estudo do intestino delgado proximal ao nascimento e durante a recuperação nutricional. Rev Paul Ped. 1984;2:43-52.

2. Nascimento OJ, Madi K, Guedes e Silva JB, Soares Filho PJ, Hahn MD, Couto B, et al.Considerações sobre o músculo estriado na desnutrição protéica. Arq Neuropsiquiatr. 1990;48:395-402.

3. Trindade CE. Repercussões da nutrição da gestante sobre o recém-nascido. J Pediatr (Rio J). 1997;73:291-2.

4. Ihemelandu EC. Fibre number and sizes of mouse soleus muscle in early postnatal protein malnutrition. Acta Anat (Basel). 1985; 121:89-93.

5. Oliveira FL, Oliveira AS, Schimidt B, Amâncio OM. Desnutrição energética intra-uterina em ratos: alterações músculo-esqueléticas na 1ª e 2ª gerações. J Pediatr (Rio J). 1999; 75:350-6.

6. Dubowitz V. Enzyme histochemistry of skeletal muscle. J Neurol Neurosurg Psychiatry. 1965;28:516-24.

7. Dubowitz V. Developing and diseased muscle. A histochemical study. Spastics International Medical Publications Research Monograph 1968; 2:106.

8. Ross JJ, Duxson MJ, Harris AJ. Neural determination of muscle fibre numbers in embryonic rat lumbrical muscle. Development 1987;100: 359-409.

9. Marcondes E. Conceito e nomenclatura, classificação, etiopatogenia. In: Marcondes E, coordenador. Desnutrição. São Paulo: Sarvier, 1976. p. 3-28.

10. Sant'Ana DM. Estudo morfométrico e quantitativo do plexo mientérico do colo ascendente de ratos adultos normoalimentados e submetidos à desnutrição protéica. [Dissertação]. Maringá: Universidade Estadual de Maringá; 1996.

11. Peter JB, Bernard RJ, Edgerton VR, Gillespie CA, Stempel KE. Metabolic profiles of the three types of skeletal muscle fibers in guinea pigs and rabbits. Biochemistry. 1972;11:2627-33.

12. Vieira S. Bioestatística: tópicos avançados. Rio de Janeiro: Campus, 2003.

13. Gigante DP, Buchweitz M, Helbig E, Almeida AS, Neumann NA, Victora CG. Randomized clinical trial of the impact of a nutritional supplement "multimixture" on the nutritional status of children enrolled at preschool. J Pediatr (Rio J). 2007;83:363-9.

14. Fortes Filho JB, Barros CK, da Costa M, Procianoy RS. Results of a program for the prevention of blindness caused by retinopathy of prematurity in southern Brazil. J Pediatr (Rio J). 2007;83: 209-16.

15. Lermann VL, Fortes Filho JB, Procianoy RS. The prevalence of retinopathy of prematurity in very low birth weight newborn infants. J Pediatr (Rio J). 2006;82:27-32.

16. Nunes ML, Batista BB, Micheli F, Batistella V. Efeitos da desnutrição precoce e reabilitação nutricional em ratos. J Pediatr (Rio J) 2002;78:39-44.

17. Rowe RWD. Effect of low nutrition on size of striated muscle fibres in the mouse. J Exp Zool. 1968;167:353-8.

18. Howells KF, Mathews DR, Jordan TC. Effects of pre and perinatal malnutrition on muscle fibers from fast and slow rats muscle. Res Exp Med (Berl). 1978;173:35-40.

19. Stickland NC, Widdowson EM, Goldspink G. Effects of severe energy and protein deficiencies on the fibres and nuclei in skeletal muscle of pigs. Br J Nutr.1975;34:421-8.

20. Dall Pai V, Thomaz E, Curi PR. Postnatal growth of skeletal muscle of the rat. Gegenbaurs Morphol Jahrb. 1984;130: 827-34.

21. Sarnat HB. Developmental disorders of muscle. In: Mastaglia FL, Walton SJ, editors. Skeletal muscle pathology. New York: Churchill Livingstone; 1982. p. 140-60.

22. Henriksson-Larsen K, Fridén J, Wretling ML. Distribution of fiber sizes in human skeletal muscle. An enzyme histochemical stdy in m tibialis anterior. Acta Physiol Scand.1985;123:171-7.

23. Winick M, Rosso P. Brain DNA synthesis in protein-calorie malnutrition. In: Olson RE, editor. Protein-calorie malnutrition. New York: Academic Press; 1975. p. 94-101.

24. Chaves N, Linhares ED, Varela RM. Desnutrição calórico-protéica. In: De Angelis RC, editor. Fisiologia da nutrição. v. 2. São Paulo: Edart; 1977.

25. Mastaglia FL, Walton SJ. Skeletal muscle pathology. New York: Churchill Livingstone; 1982.

26. Dubowitz V, Brook MH, Neville HE. Muscle biopsy: a modern approach. London: W. B. Saunders; 1985.

27. Shafiq SA, Asiedu SA, Milhorat AT. Effect of neonatal neurectomy on differentiation of fiber types in rat skeletal muscle. Exp Neurol.1972;35:529-40.

28. Askanas V, Shafiq SA, Milhorat AT. Histochemistry of cultured aneural chick muscle. Morphological maturation without differentiation of fiber types. Exp Neurol. 1972;37:218-30.

29. Brameld JM. The influence of undernutrition on skeletal muscle development. Br J Nutr. 2004;91:327-8.

Correspondence:
Alessandra Pires Alves
Rua Moacir Colognesi, 2397
Jardim Petrópolis
CEP 87506-190 – Umuarama, PR – Brazil
Tel.: +55 (44) 3622-1254, +55 (44) 3624-5351
E-mail: gui-pires@unipar.br