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Differential effect of chronic undernutrition on the fiber type composition of fascicles in the extensor digitorum longus muscles of the rat

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ABSTRACT

Several studies have shown that chronic low food consumption alters the composition and metabolism of the *extensor digitorum longus* muscle (EDLm) fiber types. EDLm is constituted by four independent fascicles (F2–F5) of different sizes; their constitution and metabolism, however, as well as how chronic undernourishment affects these is virtually unknown. Thus, the aim of this study is to evaluate the relative fiber type composition and metabolism of each independent fascicle in the EDLm, using control and chronically undernourished young male rats by using the alkaline ATPase and NADH-TR histochemical techniques. Our results indicate that all control fascicles showed a higher percentage of intermediate fibers ($P < 0.001$), except for F5, which had a higher percentage of fast fibers ($P < 0.001$). After chronic undernutrition, the proportion of intermediate fibers decreased in F4 ($P < 0.05$) and increased in F5 ($P < 0.001$), whereas fast fibers decreased in F3 ($P < 0.05$) and F5 ($P < 0.001$). When we investigated fiber metabolism we found that F3 and F4 had a similar composition (mainly glycolytic), whereas F2 and F5 predominantly contained oxidative fibers. All fascicles of chronic undernourished rats showed a general decrease in oxidative fibers ($P > 0.05$), except for F3, in which oxidative fibers increased ($P < 0.05$). After determining the possible predominant metabolism expressed in intermediate fibers, we propose that chronic undernutrition induces the transformation of fast-glycolytic to intermediate-oxidative/glycolytic fibers, mainly in F3 and F5. Our observations confirm that chronic undernourishment differentially affects the fiber types of each fascicle in the EDLm, which could alter their individual physiological contractile properties.

1. Introduction

Nutrition is a key factor in the life of animals, especially during their fetal development, because it determines the epigenetic control throughout their growth (fetal programming) (Wu et al., 2004). Good nutrition involves an adequate amount, quality and balance of nutrients obtained from food (Castrogiovanni et al., 2014; Musumeci et al., 2015b); in the absence of good nutrition, tissues like the skeletal muscle can be physiological affected (Lennmarken et al., 1984; Young et al., 1990). Chronic undernutrition has become a severe worldwide health issue (WHO, 2016). Due its protein composition and high metabolic activity, skeletal muscle is seriously affected by low quality and/or quantity of food, especially during myogenesis (during embryonic development), which is a highly controlled process (see Musumeci et al., 2015a). The effects of undernourishment are likely to alter the number and types of fibers, as well as their contractile properties (Pereyra-Venegas et al., 2015; Toscano et al., 2008). In the long term, muscles will undergo visible physiological alterations. Altogether, these observations suggest that changes in skeletal muscle mass and functional properties associated with undernourishment are the result of a complex process of cell-adaptive responses.

The fiber type composition of muscles varies from one muscle to

Abbreviations: ATPase, adenosine triphosphatase; CSA, cross sectional area; EDLm, Extensor Digitorum Longus muscle; F2, fascicle 2; F3, fascicle 3; F4, fascicle 4; F5, fascicle 5; F, fast; G, glycolytic; I, intermediate; i. p., intraperitoneal; NADH-TR, nicotinamide adenine dinucleotide tetrazolium reductase; NBT, nitro-blue tetrazolium; O, oxidative; S, slow; v/v, volume per volume; w/v, weight per volume

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another (Schiaffino and Reggiani, 2011) and the relative proportion of fibers changes during an animal's lifetime (Lehnert et al., 2007; Ruíz-Rosado et al., 2013b) in response to intrinsic and/or extrinsic factors such as exercise (Röckl et al., 2007), hormonal fluctuations and nutritional status, among others (Matsakas and Patel, 2009). Several studies have demonstrated that muscles which are mostly composed of fast fiber types are usually more affected by low food intake than those which are primarily composed of slow fibers (Howells et al., 1978; Wareham et al., 1982; Dwyer et al., 1994; Ruíz-Rosado et al., 2013b). Recently, we have shown that chronic undernutrition decreased the relative proportion of type IIb fibers (fast, glycolytic, fatiguing) and proportionally increased the IIa fiber type (intermediate, oxidative-glycolytic, fatigue resistant), but it practically does not influence the proportion of type I fibers (slow, oxidative and fatigue resistant) in *extensor digitorum longus* muscles (EDLm) of rats (Ruíz-Rosado et al., 2013a,b). Such results disagree with what has been reported by Pereyra-Venegas et al. (2015), who found that undernutrition increases the percentage of fast fibers. These discrepancies may arise because the muscle's sampling region (proximal, medial, distal) may include a high fiber type distribution heterogeneity or because of differences in the histological techniques used (Ruíz-Rosado et al., 2013a,b, used the basic ATPase technique, while Pereyra-Venegas et al., 2015 employed a modified acid ATPase technique; see also Schiaffino and Reggiani, 2011).

A typical fast muscle that is frequently used in physiological and histological studies is the EDLm. Although this muscle comprises four well-defined independent fascicles (F2–F5; Balice-Gordon and Thompson, 1988) which participate in the extension of phalanges 2–5 and the dorsiflexion of the ankle, **is** considered as a single muscle in most studies. In transversal sections, the complete EDLm is mainly composed of type IIa and IIb fibers (~95%; Bloemberg and Quadrilatero, 2012) which are not homogeneously distributed (Wang and Kernell, 2001). High proportions of type IIa and type I fibers have been found in deeper regions of the muscle, as well as a predominance of type IIb fibers in superficial and lateral regions of the muscle (Pullen, 1977), indicating the occurrence of specific regional distributions of fiber types at different levels of muscles. In addition, the EDLm contains a mixture of oxidative and glycolytic fibers, not homogeneously distributed along the muscle's cross-sectioned area (Kissane et al., 2016). However, the regionalization of fiber types is probably related to differences in the fiber type composition of each fascicle within the EDLm. Recently, it has been shown that fascicles 2 and 5 exhibit different percentages of fibers types (F2: Type IIb 41%, Type IIa 49% and Type I 10%, while F5: Type IIb 95% and Type I 5%; Kissane et al., 2016). Because of the contrasting findings on the effects of chronic undernutrition over the EDLm, and given the absence experiments designed to disclose the alterations caused by undernutrition on the different fascicles of the EDLm, here we investigate the effects of chronic undernutrition on the composition and metabolism of fiber types situated in middle regions of each fascicle (F2–F5) in the EDL muscle of young male Wistar rats at 35 postnatal days.

2. Materials and methods

2.1. Animals

All experiments were performed in accordance with the guidelines contained in the Guide for the Care and Use of Laboratory (National Research Council, 2010; National Institutes of Health, Bethesda, MD, USA; Animal Welfare Assurance #A5036-01). The animal protocols were approved by the Institutional Bioethical Committee for the Care and Handling of Laboratory Animals (UPEAL-Protocol 013-02, CINVESTAV). Two groups of female Wistar rats (mean body weight: 257.4 ± 16.3 g) were subjected to the following feeding conditions: (i) Control group (C): adult female rats (n = 16) and their offspring, which had free access to commercial food (Formulab 5008, Lab diet, Framingham, MA, USA); and (ii) Chronic undernourished rats (U): adult female rats (n = 18) which were fed with approximately half of the mean food intake given to control animals, starting 2 weeks before mating and continuing during pregnancy and lactation (Quiróz-González et al., 2013). The feeding protocols were maintained during the gestation, birth and lactation (postnatal day 21). Litters were adjusted to 9 pups (5 males and 4 females). After weaning, pups continued with the same feeding protocol as their mothers (C and U) until postnatal day 35. Each mother and offspring were housed in large acrylic cages (43 × 53 × 20 cm) and subjected to the same feeding protocols until pups weaning (post-natal day 21). From weaning to the day of the experimental session (35 postnatal day), the male rats were housed in individual acrylic cages (32 × 47 × 20 cm) and on the day of the experiment C and U male rats were randomly selected and weighted (Body Weight). All animals had free access to water and were maintained under an identical light and dark cycle (12 h–12 h) and temperature (22–24 °C). No supplementary mineral, trace elements or vitamins were added to the food supply of undernourished animals.

2.2. Histoenzymatic analysis

At postnatal day 35, the animals were anesthetized with urethane (1.6 g/kg of body weight). The EDL muscles were quickly removed and weighed. Subsequently, the four fascicles of each muscle (F2–F5) were carefully separated and their length was measured. After that, fascicles were immersed in 2-methylbutane, cooled to near freezing point with liquid nitrogen and stored at −80 °C until their processing. At the end of tissue extraction, the animals were euthanized using an overdose of anesthetic (urethane). Subsequently, the middle segment of each fascicle was sectioned and mounted on specimen holder in a cryoprotectant solution (Tissue-Tek® O.C.T Compound, Sakura® Finetek, Torrance, Ca). Serial transverse sections (10 μm thick) of each specimen tissue were obtained by means of cryostat at −25 °C (CM-1520; Leica Biosystems, Nussloch, Germany). The sections were subsequently mounted on glass coverslips for staining.

The metabolism of fibers was inferred using the NADH-TR (nicotinamide adenine dinucleotide tetrazolium reductase) histochemical technique (after Nachlas et al., 1958). Briefly, the fascicle's sections were incubated for 1 h at 37 °C in 1:1 (v/v) NBT-NADH solution (nitro-blue tetrazolium diluted in 50 mM tris buffer, pH 7.6; 2.25 mM nicotinamide adenine dinucleotide diluted in 50 mM tris buffer). Later, samples were washed three times with distilled water. The unbound NBT from the section were removed with three exchanges each of the 30, 60 and 90% acetone solutions and coverslips were mounted as described above.

In subsequent serial tissue sections, myofibrillar ATPase activity of muscle fibers was visualized using an alkaline ATPase (pH 9.4) technique (after Guth and Samaha, 1970). In brief, non-fixed sections were pre-incubated at 37 °C in a solution of 0.01 M Tris base and 0.018 M $CaCl_2$ (pH 9.4) for 20 min. After pre-incubation, samples were washed 15 times with distilled water and subsequently incubated at 37 °C for 1 h in 1.5% w/v of adenosine-5′-triphosphate in a pre-incubation solution at pH 9.4. Later, samples were washed 15 times with distilled water and incubated consecutively in a 2% w/v $CaCl_2$ solution for 3 min and 2% w/v $CoCl_2$ solution for another 3 min. After this, the samples were washed with distilled water 20 times and transferred to 10% v/v $(NH_4)_2SO_4$ solution for 1–3 min. Finally, the sections were washed, dehydrated with increasing and decreasing ethanol solution, and coverslips then were mounted with glycerogel (2% gelatin, 50% glycerol, 0.5% phenol).

2.3. Fiber type characterization

Photomicrographs of each fascicle section were taken using a digital camera mounted on a light microscope with a 10X objective. Individual photographs were merged using Adobe Photoshop (v 2014.2.2, Adobe