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Research Article

Morphological Adaptation of Rat Skeletal Muscle to A Cold Environment

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Abstract: Male Sprague-Dawley rats (SD) were kept in medium between the postnatal 80th and 130th day while environmental temperature (from 20 to 5 ºC) and photoperiod were decreased gradually. The weight of the animals adapted to the cold and the weight and length of the external digitorum longus (EDL) muscle were measured. When compared with controls, in a cold environment the weight and fiber length of the muscle was reduced significantly. In addition, EDL muscle was separated into two (red and white) by using the succinic dehydrogenase (SDH) method and the percentage of fiber types and fiber diameters according to mitochondria contents were measured. Transformations in muscle fibers (from Type IIb to Type IIa), decreases in fiber diameters (particularly Type IIb) and atrophic fibers (in Type IIa and Type IIb) were observed. However, normal values were obtained when the temperature returned to normal.

Key Words: Cold environment, Muscle, Mitochondria, SDH, Histochemistry, Rat

Sıçan İskelet Kasının Soğuk Çevreye Morfolojik Adaptasyonu

Özet: Erkek Spraque-Dawley sıçanlar (SD) doğumdan sonraki 80.günden 130.güne kadar çevre ısısı (20'den 5ºC) ve fotoperiyodun dereceli olarak düşürüldüğü ortamda tutuldular. Soğuğa adapte olmuş hayvanların ağırlığı, EDL (Extensor digitorium longus) kasının ağırlığı ve uzunluğu ölçüldü. Kontrollerle karşılaştırıldığında soğuk çevrede, kasın ağırlığı ve lif uzunlukları belirgin bir şekilde azaldı. Aynı zamanda Succinic Dehydrogenase metodu (SDH) ile EDL kası iki bölgeye ayrıldı (kırmızı ve beyaz) ve mitokondriya içeriklerine göre yüzde lif tipleri ile lif çapları ölçüldü. Kas liflerinde transformasyonlar (Tip IIa'dan IIb'ye), lif çaplarında azalmalar (bilhassa Tip IIb) ve atrofik liflerin (Tip IIa ve Tip IIb'de) ortaya çıktığı görüldü. Ancak normal sıcaklığa dönüldüğünde kontrol değerler tekrar kazanıldı.

Anahtar Sözcükler: Soğuk çevre, Kas, Mitokondriya, SDH, Histokimya, Sıçan

Introduction

Favorable developments have been reported in muscle fibers with regards to growth (1) and decrease in the muscle mass (2-4), fiber size (3), length and number of sarcomers (4,5), fiber transformations, and atrophic and angular type fibers (2,6).

The variations in the activities of oxidative enzymes observed by histochemical methods were found to be of value in explaining physiologic variations (7). Heat stress does not affect dehydrogenase activity in the mitochondria, although it reduces the activity of cytochromic enzymes (8). Cold environment prevented mitochondrial dysfunction efficiently (9), and caused an increase in mitochondria volume (soleus (SOL) and diaphragm muscle (10) and mitochondrial enzyme activity (11). By cold application, succinate dehydrogenase activity in rat mitochondria increased (12), and cytochrome oxidase activity was affected in different ways (13).

Three types of fibers were distinguished in external digitorum longus (EDL) muscle of Sprague-Dawley (SD) rats by mATPase (SO: slow-twitch oxidative, FOG: fast-twitch oxidative glycolytic, FG: fast-twitch glycolytic) (14). These fibers were determined by

Eddinger et al. (15) and Brook and Kaiser's (16) fiber type terminology to be Type I, Type IIa and Type IIb. Succinic dehydrogenase (SDH) revealed significant activity in subgroup Type IIa, a very small activity in subgroup Type IIb (17), and an intense activity in group Type I (18).

Experimental cold-adaptation made possible the use of different thermoregulation mechanisms (19). Thermal sensitivity decreased with age (20), and the most prominent change was observed in the extremities (21). In this study, SD rat hindlimb EDL muscle was selected and morphologic and histochemical studies were performed to see the effects of cold exposure.

Materials and Methods

Thirty-six male SD rats (Rattus norvegicus) were kept at 20 ºC until the postnatal 80th day. They were separated into two groups as a control and experiment group. The control and experiment group were also subdivided into subgroups of six rats. The experimental group was held in a medium where the environmental temperature was decreased from 20 ºC to 5 ºC and the photoperiod was reduced gradually (22) over 50 days. On the 130th day, the experimental group was compared with the control group of the same weight kept in a normal medium. The animals were sacrificed by an overdose of anesthesic. EDL was dissected quickly, the required measurements were obtained (Table 1), and it was frozen (22). Cross sections (12 µm) were taken along the muscle under –20 ºC cryostat (15). The cross sections were stained with SDH to determine the mitochondrial content in the muscle fibers (23,24). Succinate, which is used to measure the maximal capacity of the respiration chain in the liver mitochondria of cold-exposed rats (25), was used in our study to measure specific SDH activity (26). For the histochemical demonstration of unfixed sections were incubated in an appropriate solution (stock solution) for 30-60 minutes. Stock solution was prepared with tetrazolium and substrate as described by Bancroft and Stevens (23). The sections were transferred to formal saline solution (15%) for 15 min and washed with distilled water and then mounted in glycerine jelly. Photographs were taken within 30 min of mounting. The areas of each section were the same. These areas were divided into two groups in EDL (oxidative and glycolytic). The rats in the latter experimental group were kept at normal temperature until they reached the body weights of the control group and the same studies were carried out in both groups. The mitochondria content of fiber types (Type I, Type IIa and Type IIb) (16) were examined in the control and experimental groups, and the fiber type percentages and diameters were compared (4,27). The results were evaluated using Student's t-test.

Results

Chronic exposure to cold caused a significant decrease in rat and EDL weight and fiber length ($P \leq 0.0001$, $P < 0.005$, respectively), but control levels were restored after transfer to normal environmental temperature (Table 1).

In addition, the EDL was divided into two regions qualitatively (red and white) (Figure 1) and a quantitative study was performed in these regions. The variations in fiber diameters and the percentages of Type I and Type II fibers were evaluated by comparing control and experimental animals (Table 2).

Table 1. The effects of cold environment on body weight, muscle weight and muscle lengths in male Sprague-Dawley rats.

Treatment	Age (days)	Body weight (g)	Muscle weight (mg) EDL	Muscle length (mm)
Control	80	458.7 ± 1.2	225.4 ± 1.2	28.0 ± 0.3
Cold environment	130	449.6 ± 1.0****	215.0 ± 1.7****	26.9 ± 02**
Control	130	468.3 ± 1.2	229.4 ± 0.9	28.7 ± 0.2
Cold, normal environment	178	464.2 ± 0.8	235.5 ± 0.8	28.9 ± 0.2
Control	178	464.6 ± 0.7	235.3 ± 1.2	28.9 ± 0.2

1 Values are means ± standard error.
Mean values were significantly lower than the corresponding control values : ** $P < 0.005$, **** $P \leq 0.0001$

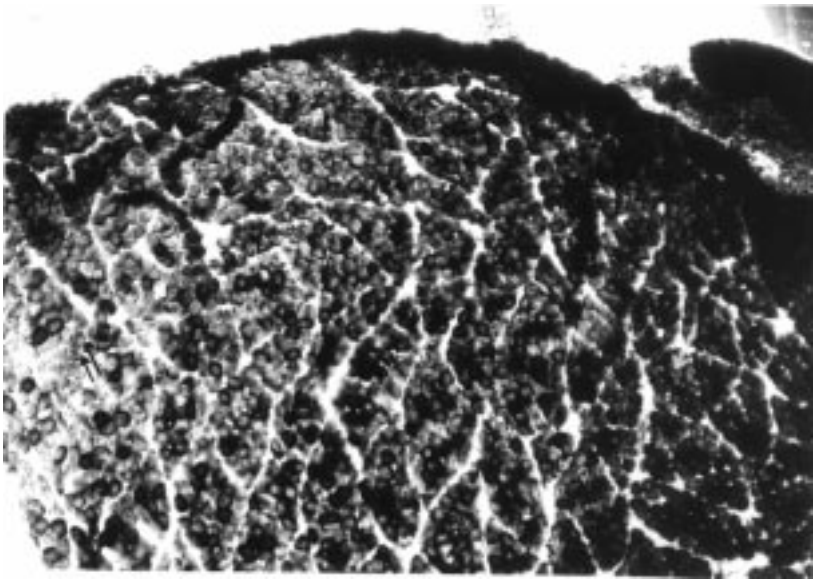

Figure 1. EDL muscle of Sprague-Dawley rat. Predominant fibers are Type IIb in white region and Type IIa in red region. Double arrow: IIb, Arrow: IIa, SDH (original magnifigation x 31).

Table 2. The effects of cold environment on diameter and percentage of fiber types in male SD rats.

Treatment	Muscle (EDL)	%Fiber type			Fiber Diameter (µm)		
		I	II a	II b	I	II a	II b
Control	r	9 ± 1.3	62 ± 2.7	29 ± 2.1	26.9 ± 1.2 (20.4-33.5)	30.4 ± 2.3 (25.2-35.7)	36 ± 3.1 (32.1-40.8)
	w	3 ± 2.0	29 ± 30	68 ± 3.3	19.0 ± 1.0 (14.9-23.4)	27.0 ± 3.2 (20.3-33.7)	43 ± 2.2 (38.2-48.5)
Cold environment	r	10 ± 2.7	64 ± 2.1**	26 ± 1.5***	25.0 ± 2.3** (22.3-28.2)	28.5 ± 0.8 (23.6-33.0)	36.4 ± 2.5 (28.5-44.7)
	w	2 ± 3.2	39 ± 2.9****	59 ± 2.4****	20.1 ± 1.0 (15.2-25.4)	29.4 ± 1.5 (25.1-34.0)	31 ± 3.0**** (23.4-39.2)
Control	r	9 ± 1.5	61 ± 1.1	30 ± 1.3	28.0 ± 2.0 (25.6-31.2)	29.9 ± 1.6 (24.7-35.1)	37.0 ± 3.1 (32.2-42.7)
	w	3 ± 3.0	31 ± 1.4	66 ± 0.7	20.3 ± 2.8 (18.3-22.2)	29.7 ± 0.9 (24.5-35.0)	46.2 ± 1.4 (39.4-53.6)
Cold, normal environment	r	10 ± 2.4	58 ± 1.8	32 ± 2.3	28.3 ± 1.2 (25.3-31.4)	32.3 ± 2.9 (27.1-37.7)	38.9 ± 1.0 (32.5-44.0)
	w	2 ± 1.7	33 ± 4.0	65 ± 2.4	20.1 ± 3.0 (19.2-21.5)	30.5 ± 1.4 (27.7-33.2)	50.1 ± 4.2 (45.6-55.2)
Control	r	10 ± 3.1	58 ± 3.5	32 ± 1.0 (24.6-32.3)	28.5 ± 2.8 (28.1-36.3)	32.9 ± 3.4 (34.4-42.7)	38.8 ± 1.5
	w	2 ± 4.0	33 ± 1.8	65 ± 1.1	20.3 ± 3.3 (19.0-21.4)	30.7 ± 2.0 (29.2-31.5)	50.3 ± 1.1 (47.5-53.2)

Values are means ± standard error. Numbers in parentheses indicate the range (minimum-maximum) of values obtained. **P < 0.005, ***P < 0.001, ****P ≤ 0.0001. Mean values (IIb) were significantly lower than the corresponding control values. %Fiber type (P ≤ 0.0001) and fiber diameter of Type IIb (P ≤ 0.0001) in the white region were significantly different from Type IIb fibers in the red region. In addition, mean values (IIa) were significantly higher than the corresponding control values: %Fiber type of Type IIa in the white region were significantly different from Type IIa fibers in the red region (P ≤ 0.0001).

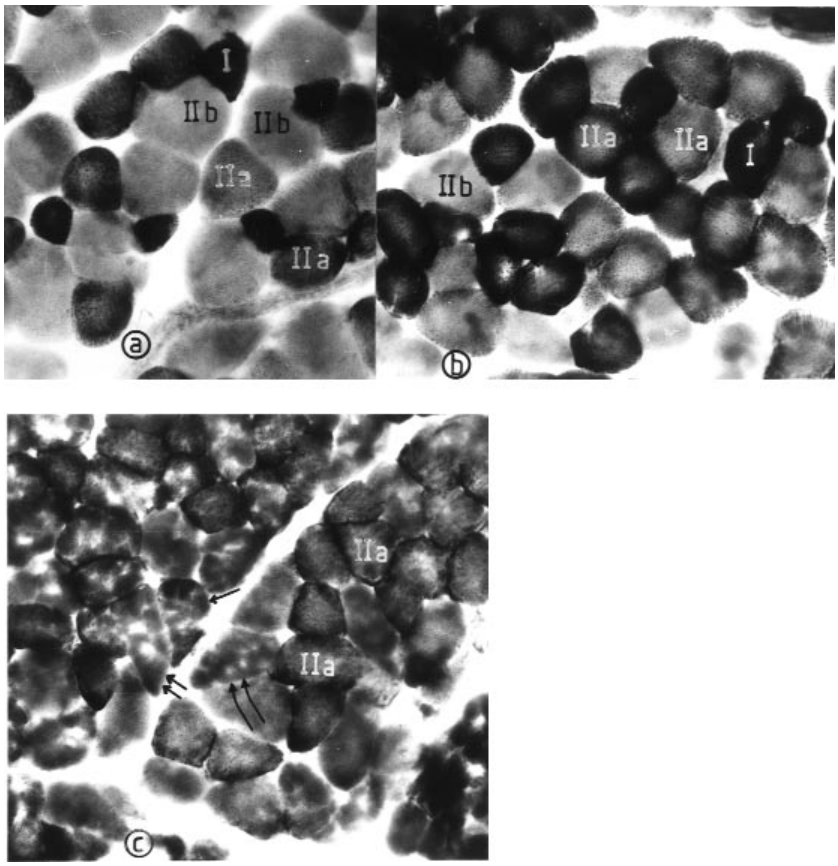

Figure 2. EDL muscle. A) White region. I: Type I, IIa: Type IIa and IIb: Type IIb, B) Fiber types in red region, I: Type I, Iia: Type IIa and IIb: Type IIb, C) Significantly increased Type IIa fibers and atrophy in the white region of EDL muscle in cold-exposed animal. Double arrow: atrophic Type IIb fibers, Arrow: atrophic Type IIa fibers, SDH (original magnification x 400).

It was observed that Type IIb fibers (with very little mitochondria content) were predominant in the white region of EDL muscle (68%) whereas Type IIa fibers (with a moderate level of mitochondria content) were predominate in the red region in control group (62%) (Figures 2a and b). In a cold environment, the mitochondria content of Type IIb fibers increased particularly in the white region and these fibers reacted similarly to Type IIa fibers. Thus, Type IIb fibers decreased from 68% to 59% and Type IIa increased from 29% to 39% ($P \leq 0.0001$) (Table 2). In addition, atrophy was observed both in Type IIa and Type IIb fibers (Figure 2c).

Discussion

Morphological changes in SD rats observed in this study (Table 1) might have occurred due to the use of body fat reserves, as reported in cold-stressed mice (28). In the same manner, cold increased the basal metabolic rate in the EDL muscle of rats (29). Thus, the use of triacylglycerol was increased in the muscles to create a response for altered mitochondrial respiration in the muscle (30,31). This shows that the muscle has a huge energy power (32) that plays a role in preserving thermal homeostasis (33).

Differences in the fiber type composition in EDL muscle among different rat species have been noted (27,34,35). However, generally EDL is considered to be as a fast muscle (22), rich in FG (Type IIb) fibers (14,27). This muscle in SD rats is separated into two regions, red and white (27). Histochemical fiber transformations observed in the red and white regions of EDL muscle as a result of the cold acclimation in our research (Tables 1 and 2) were also determined morphologically and metabolically as a result of the cold acclimation in different rat muscles (6). Thus, the significant mitochondriogenesis in EDL muscle fibers can be interpreted as a marker of a high metabolism occurring as a result of an unexpected depression in energy capacity (36). In addition, these fiber transformations might be associated with decreased twitch contraction power and

maximum contraction time in the cold as reported for the gastrocnemius muscle of the rat (37). Under similar conditions it is also determined that neuro-muscular junctions of rat EDL and diaphragm muscles are functional with slow rhythmic stimulation (38).

According to a histochemical method used for measuring Mg^{+2} –ATPase activity of mitochondria, cold caused an increase in the staining intensity of all fibers except for FG fibers (Type IIb) and also increased numbers of SO fibers (Type I) (39). Nevertheless, cold did not always affect the composition of the muscle fibers (40) but could create differences in the mitochondria functions (cold-induced changes in calcium transport) (41). These observations demonstrated that the muscles respond in a different way to changes under physiological conditions (42). In our study with cold acclimation, we also observed a decrease in fiber diameters, especially Type IIb (Table 2). This condition was thought to be a result of the high oxidative metabolism that is seen in fibers with small diameters, similar to that reported by Rosser et al. (35). But the atrophic fibers (Type IIa and IIb), fiber type percentages and fiber diameters were obtained again when the normal conditions returned, as reported for diet limitation (4) and denervation (2) (Table 2).

In conclusion, cold exposure resulted in the adaptation of EDL muscle affecting its weight, length, fiber percentage, fiber diameter and shape. Our study supplied additional information about morphologic and histochemical aspects to previous literature considering the metabolic and physiologic aspects.

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