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Comparisons of Myofibrillar Protein using Gel Electrophoresis in Mouse Skeletal Muscle

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Abstract

Mice were conditioned to an exercise regimen of either fast rpm's at 15 minutes or slow rpm's at 30 minutes. There were two groups of mice containing 15 in the fast exercise group and 13 in the slow group. Each day the mice were exercised in their prospective groups for the alloted amount of time. On day 35, the triceps brachii and soleus muscles were removed from each mouse and prepared for gel electrophoresis to identify any protein differences within each group. The mice that were conditioned showed no significant changes in myofibrillar proteins. The exercise regimen did not seem to have any effect on the proteins contained in the triceps or soleus muscle fibers. Therefore, it is concluded that procedures used were not sufficient to detect any changes in proteins within the muscle fibers due to exercise regimens.

Introduction

For experimental purposes, muscle fiber types of humans and animals can be compared. Several studies on the muscular fibers of animals have been conducted to help us better understand what occurs within the human musculature. For instance, there are particular muscle fibers which contract more slowly and have lower myosin ATPase activity than others in a mouse. This is also the case for human muscle fiber types. The myosin ATPase activity and the maximum velocity of muscle shortening determines the fiber types (Brown et al., 1983).

This is how fast and slow-twitch muscle fibers are named. These fiber types not only differ in ATPase activity, but also in most of the contractile and regulatory proteins as well. These proteins exist in different isoforms which have similar biological function, but a slightly similar amino acid sequence. The diffences are because of the various physiological properties between different fiber types (Barnard et al., 1970). Consequently, various levels of proteins are developed within muscle fibers. This can be done by muscle innervation, but also independently through varying levels of exertion placed on the muscle fiber. Therefore, different regimens of exercise will develop a variation in protein content of each muscle fiber group. The two major muscle groups are fast-twitch and slow-twitch. Fast-twitch muscle is found predominately in sprinters and slow-twitch predominately in long distance runners (Eisen et al., 1975).

To differentiate between these fiber groups, a protein analysis may be done. In a controlled environment, protein content is measured using an electrophoresis gel (Hames and Rickwood, 1981). Therefore, upon comparison of proteins, the fast and slow-twitch fiber types should have different levels of protein or isoform content (Hames and Rickwood, 1990).

There is another major muscle fiber group called the intermediate fibers. These fibers may be either a different variation, naturally, from fast- or slow-twitch fibers, or they may result from a protein content change in either one of the groups. Therefore, when each muscle group is exerted, whether it be through sprinting or long distance running or both, they will develop accordingly (Brooke and Kaiser, 1970).

However, when a fast-twitch muscle fiber is trained to do continuous long distance exertion, it may develop proteins to compensate for that particular activity. A fast-twitch muscle fiber may develop some properties of a slow-twitch, which is where the intermediate muscle fiber group emerges. The intermediate muscle fiber group can emerge as a larger mass because of varying exercise, therefore, the myofibrillar protein content within that particular muscle mass has been altered. Through extensive training, the untrained muscle fibers will develop different amounts of proteins (Barnard et al., 1971).

The objectives of this research are to find out whether extensive training of untrained muscles can induce changes in myofibrillar protein content of unidentified proteins. Proteins will be allowed to migrate, according to their molecular weight, through a polyacrylamide electrophoresis gel to determine any possible changes within specific muscle groups due to exercise regimine.

Materials and Methods

The mice were divided randomly into 3 groups (with at least half male and half female): Group I--fast exercise, Group II--slow exercise, and Group III--cage activity. There were 15, 13, and 14 mice per group, respectively. The fast group exercised on an electrically driven wheel for 15 minutes at 40 rpm. The slow group exercised on the same wheels, but for 30 minutes at 23 rpm. The cage activity mice were left to spontaneously exercise in their cages. The wheels were constructed by using a large wire rat cage as a

base and attaching a standard, plastic round mouse wheel through a hole in the side of the cage. A Variac power control was connected to each wheel to insure constant velocity.

The mice were exercised every evening for a peiod of 3 weeks. Upon completion of the period, all mice were killed by using the cervical dislocation method. Biopsies were taken from the left triceps brachii and left soleus muscles. The muscle samples were place into labeled microfuge tubes and stored at -20° C. The samples were prepared for insertion into a one dimensional electrophoresis gel according to procedures described by Gasque (1989). Stock solutions of acrylamide-bisacrylamide, TEMED, ammonium persulfate, and SDS were made according the procedures outlined in Hames and Rickwood (1990,Table 1). An SDS continuious polyacrylamide gel was prepared according to Hames and Rickwood (1990). The final concentrations for buffers were as follows: stacking gel - 0.125 M Tris-HCL, pH 6.8; resolving gel - 0.375 M Tris-HCL, pH 8.8; reservoir buffer - 0.025 M Tris, 0.192 M glycine, pH 8.3. Samples were loaded into the wells of the gel with a microsyringe and electrophoresed for 8 to 9 hours at 55 mAmps. Gels were stained with Coomassie blue and a silver nitrate staining kit (Sigma Chemical Co.; Gasque, 1989). Gels were stored in deionized water, in large ziplock bags, at 4° C.

Bands were scored on a scale of dark (3), medium(2), light(1), and nonexistant(0) as compared to the other proteins of a similar molecular weight (in the same row). Band were assigned numbers relative to their mobilities; faster migrating bands were assigned larger numbers. A high molecular weight marker was run in a lane beside the other samples in each gel. A Kruskal-Wallace one-way analysis of variance was done to compare band-staining intensities among treatments using SYSTAT.

Table 1. Recipe for gel preparation using the SDS-discontinuous buffer system from Hames and Rickwood (1990). Values are mL of reagents required to make solutions.

Stock Solution	Stacking Gel	Resolving Gel		Reservoir Buffer
		11%	5.5%	
Acrylamide-b bisacrylamide (30:0.8)	2.5	20.0	10.0	
Stacking gel buffer stock	5.0			
Resolving gel buffer stock		3.75	3.75	
Reservoir buffer stock				100
10% SDS	0.2	0.3	0.3	
1.5% ammonium persulfate	1.0	1.5	1.5	
Water	11.3	4.45	14.45	900
TEMED	0.015	0.015	0.015	

Table 2. Staining intensities of mouse triceps muscle from SDS gels. Groups represent different treatments: Group I = fast exercise, Group II = slow exercise, and Group III = cage activity. The staining intensities ofeach band are identified as follows: nonexistant = 0, faint = 1, medium = 2, and dark = 3.

	Group I														Group II													Group 3													
Band Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	0	0	1	1	1	1	1	0	2	1	1	1	1	0	0	1	1	1	2	0	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	2	1	1	1	1	1
2	0	0	0	1	1	0	0	1	0	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	1	1	1	0	0	1	1	1	0	1	0	0	1	0	1	1	1	1
3	2	2	2	2	2	2	1	2	1	2	1	0	1	1	2	2	2	2	2	3	1	2	2	1	2	2	2	2	2	2	2	2	1	2	2	2	2	2	1	2	2	2
4	3	3	3	3	3	2	3	3	0	2	1	1	1	1	3	3	3	3	3	3	3	3	3	3	1	1	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	2	2	2	2	2	2	3	3	1	2	2	2	2	2	2	2	2	2	3	3	3	3	1	1	1	1	1	2	2	2	2	3	3	3	3	1	1	1	1	1	1	1
6	2	2	2	2	2	2	3	3	1	2	2	2	2	2	2	2	2	2	3	3	3	2	1	1	2	2	2	2	2	2	2	2	3	3	2	1	2	1	2	2	2	2
7	1	2	2	1	1	1	1	2	0	0	1	1	1	1	1	2	2	1	1	1	1	2	1	1	2	2	2	1	2	1	1	1	2	2	2	1	1	0	1	2	2	1
8	2	2	2	2	0	2	2	3	0	0	1	1	1	1	2	2	2	2	3	3	2	2	0	1	1	1	1	2	2	2	2	2	3	3	2	0	0	0	0	0	0	0
9	2	2	2	2	2	2	3	3	0	1	1	1	1	1	2	2	2	2	3	3	3	3	0	1	1	1	1	2	2	2	2	3	2	3	2	0	0	0	0	0	0	0
10	3	2	3	3	1	1	1	1	2	3	1	3	3	3	3	3	3	2	1	1	1	1	3	1	3	3	3	2	3	3	3	1	1	1	1	3	3	1	1	3	3	3
11	3	1	1	1	0	1	1	1	0	1	2	2	2	2	1	3	1	1	1	1	1	1	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	1	2	2	2	2
12	1	1	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	0	2	2	2	2	1	1	1	1	1	1	1	1	0	0	0	2	2	2	2
13	1	1	1	1	1	1	1	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2

Table 3. Staining intensities of mouse soleus muscle from SDS gels. Groups and staining intensities as in Table 2.

	Group I														Group II													Group III												
Band Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	2	3	4	5	6	7	8	9	10	11	12	13	14
1	3	0	2	2	2	2	2	2	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	2	2	2	2	2	2	3	3
2	0	2	2	2	2	2	2	2	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	2	2	2	2	2	2	2	2
3	2	0	3	3	3	3	3	3	0	2	2	2	0	2	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	3	3	3	3	3	3	3	2
4	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	2	2	3	3	3	3	3	3	0	1	1	1	0	1	1	2	1	1	1	1	2	1	2	1	1	1	2	1	1	1	0	0	3	3	3	3	3	3	3	2
6	1	1	3	3	3	3	3	3	0	0	1	1	0	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	0	0	3	3	3	3	3	3	3	1
7	1	1	2	2	2	2	2	2	0	0	0	1	0	1	1	2	1	1	1	1	2	1	2	1	1	1	1	0	0	0	0	0	2	2	2	2	2	2	1	1
8	1	1	1	1	1	1	1	1	0	0	0	2	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	1	1	1	1	1	1	1	1
9	2	1	3	3	3	3	3	3	1	2	2	2	0	2	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	3	3	3	3	3	3	2	1
10	3	3	3	3	3	3	3	3	1	1	2	2	1	2	2	3	1	2	3	1	2	2	3	0	3	0	3	2	2	1	1	1	3	3	3	3	3	3	3	3
11	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	1
12	1	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
13	3	3	3	3	3	3	3	3	3	3	1	1	1	1	1	2	1	2	2	1	2	2	2	2	1	1	2	1	1	1	1	1	3	3	3	3	3	3	3	3
14	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Results

Staining intensities for each band for each individual are presented in Tables 2 and 3. Band staining intensities were highly variable among individuals for some groups and not variable for others. For example, staining intensities for band 9 of Group I triceps muscle varied from 1 to 3, while the intensity of band 14 of group I, II, and III of the soleus muscle remained constant (3). This indicates that there was more variance within the groups than between groups. Several bands had an inconsistant band intensity pattern within the same muscle group. Therefore, a consistent pattern was not detected and results from a nonparametric were inconclusive.

The identity of several bands was hypothesized from comparisons with mobilities of known molecular weight standards (in daltons). Possible identities are as follows:

Band 1-29,000d	(carbonic anhydrase)
Band 2-45,000d	(Albumin, Egg)
Band 3-66,000d	(Albumin, Bovine--BSA)
Band 4-97,400d	(Phosphorylase b)
Band 9-116,000d	(Beta-Galactosidase)
Band 11-205,000d	(myosin)

Discussion

There are several possible explanations why the results did not reveal a significant difference between groups of mice according to their protein content. The exercise regimen may not have been adequate to successfully display different protein contents. Perhaps the mice were not exercised for a long enough period; two months may have been a better time period for conditioning. Also, the fast exercised mice probably should have had a regimin of twice a day with running for 1 minute at a fast pace and resting for 30 seconds. Three sets of five with a five minute rest in between sets might show better results than running continuously for 15 minutes.

The staining procedure at first appeared to work, however, some bands which looked like they should have been more intense were faded because the stain did not take well at that point. A better staining technique could show the bands' intensities at a consistant rate. Histochemical staining procedures seem to be the choice of most of the detailed experiments such as this one.

The variation within the muscle groups instead of between muscle groups was the major problem in determining an accurate analysis in this instance. However, the reason for this occurrance can not be explained in this case. The molecular weight marker shows that band 4 (presumably phosphorylase b) was definitely present in all groups (with a rating of 2-3). However, the presumed myosin protein would be of most interest to this study. This was band 11 for all groups. In group I, II, and III of the triceps muscle, there is some variance in intensities. Predominately, 1 and 2 are assigned, but they are not in any particular pattern or group. Band 11 for the groups in soleus muscle has only one intensity rating of 3. This protein did not change in these groups because the intensity remained the same. However, it is a possibility that this specific protein content might have differed in the groups for the triceps muscle because of the variation in intensity.

There were only a few more, unidentifiable, bands left on the gel to analyze. Either the gel needed to be run longer or the pores in the gel were too small for high molecular weight proteins to migrate. Therefore, the protein stays at the top of the gel. A 10% polyacrylamide gel (total acrylamide/bisacrylamide is 10g/100ml) should be

used so that the molecular mass of proteins with a higher molecular weight, such as myosin, can migrate through the gel. These discoveries, after the project was completed, would have helped intensely in receiving the results which were anticipated. More analysis of the molecular weights and trying to identify particular proteins found in muscle would improve the analysis of the results.

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