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REDUCED HIGH-ENERGY PHOSPHATE LEVELS IN THE PAINFUL MUSCLES OF PATIENTS WITH PRIMARY FIBROMYALGIA

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Muscle energy metabolism was studied by chemical analysis of biopsy samples from: 1) trigger points in the trapezius muscle from 15 patients with primary fibromyalgia (PF), 2) nonpainful, anterior tibial muscle from 6 patients with PF, and 3) the trapezius muscle from 8 healthy controls. We found a decrease in the levels of adenosine triphosphate, adenosine diphosphate, and phosphoryl creatine, and an increase in the levels of adenosine monophosphate and creatine, in the trapezius muscles from the patients. These findings support the notion that the pain in patients with PF is of muscular origin.

Primary fibromyalgia (PF) is a nonarticular rheumatic disease that is characterized by chronic pain and stiffness in the skeletal muscles and in the joints, but without arthritic manifestations. The painful "trigger points" in muscles and tendon insertions are a typical feature (1). The cause of muscle pain and fatigue in these patients has remained unknown. Several factors are thought to interact to produce the PF syndrome, e.g., muscle overload, disturbed sleep, and psychogenic factors, all of which cause muscle tension or spasm (2).

In 1973, Fassbender and Wegner (3) published a morphologic study of PF. Electron microscopic examination of the trapezius muscle had revealed swollen capillary endothelial cells, and the investigators hypothesized that local hypoxia caused degenerative changes in the muscles and was a predominant factor in the development of symptoms of the disease. Measurement of oxygenation in the muscles of PF patients, with a multipoint oxygen electrode placed on the muscle surface, has given further evidence for the existence of abnormal oxygenation in these patients (4).

Earlier studies of muscle energy metabolism have shown that there are significant metabolic changes in human muscles during ischemia (5–7): these include a decrease in adenosine triphosphate (ATP) and phosphoryl creatine (PC) and an increase in adenosine monophosphate (AMP) and creatine. If muscle hypoxia is an important factor in PF, similar changes in intermediary energy metabolism could occur.

The aim of this study was to investigate energy metabolism by chemical analysis of muscle biopsy specimens taken from trigger points within the trapezius muscle of PF patients and to compare these findings with those of the same tests done on samples of PF patients' muscles having no trigger points,

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anterior tibial muscles, and trapezius muscles from healthy volunteers.

PATIENTS AND METHODS

The study subjects were assigned into 3 groups. In group 1, there were 15 patients (14 women, 1 man). Their mean age was 46 years (range 25–63). Their mean duration of PF symptoms was 6 years (range 1–12 years). These were the patients whose trapezius muscles were analyzed. Group 2 included 6 women with PF. Their mean age was 45 years (range 31–53), and their mean duration of symptoms was 6.5 years (range 3–12 years). We analyzed the anterior tibial muscles from this group. All patients had symptoms at the time of the study. All fulfilled the diagnostic criteria for PF as proposed by Yunus et al (1).

Muscle biopsies were also taken from 8 healthy volunteers, who served as controls. There were 7 women and 1 man. Their median age was 40 years (range 26–43).

Blood samples were taken from all patients and were analyzed for erythrocyte sedimentation rate, blood cell counts, electrolyte levels, creatinine level, alanine aminotransferase value, aspartate aminotransferase value, creatine kinase level, thyroid function (thyroxine, triiodothyronine, T_3 uptake, and thyroid stimulating hormone), and for the presence of rheumatoid factor (by latex fixation and Rose-Waaler tests).

Among the 15 patients in group 1, 5 were taking no medications and 10 patients were taking a nonsteroidal antiinflammatory drug (NSAID) (in most cases aspirin) or mild tranquilizers. Of the 6 patients in group 2, 5 were taking an NSAID.

The biopsy samples from group 1 patients were taken from a trigger point area in the upper part of the trapezius muscle. Two of the patients were biopsied twice; 1 sample was from the trigger point area, and 1 was from an area outside that of the trigger point. For ethical reasons, only 1 specimen was taken from each of the other patients.

The muscle biopsy samples from group 2 patients were taken from the anterior tibial muscle. These patients had no trigger points in the anterior tibial muscle, but had trigger points in other muscles. Biopsy specimens from the controls were taken from the same portion of the trapezius muscle as those obtained from the patients in group 1.

Biopsies were performed using an open surgical technique and were carried out under the same conditions both in patients and in controls. The samples were taken with the subjects resting on their side. Mepivacaine hydrochloride (Carbocaine; 1%) was used for local anesthesia. No sedatives were given prior to the biopsy procedure.

The muscle samples were immediately frozen in liquid nitrogen and were kept frozen until freeze-drying. The samples were powdered, the connective tissue, fat, and blood were removed, and the remaining tissue was extracted as described elsewhere (8). The samples were analyzed for levels of ATP, adenosine diphosphate (ADP), AMP, PC, creatine, lactate, pyruvate, and glycogen. The energy charge potential (ECP) was calculated from the formula (9):

$$ECP = \frac{1(ADP + 2ATP)}{2(ATP + ADP + AMP)}$$

The total adenine nucleotide pool (TA) was calculated as ATP + ADP + AMP. The total creatine value (PC + creatine) has been shown to be resistant to change and was used as a reference for the intracellular substances ATP, PC, and glycogen (10). All trapezius specimens were analyzed in 1 session, and all anterior tibial muscle specimens were analyzed in 1 session. Chemical analyses of all samples were performed within a timespan of 10 days.

The muscle samples were examined according to routine histopathologic and histochemical methods. For fibertyping, the trapezius samples were stained for myofibrillar ATPase by preincubation at pH 9.4 and at pH 4.6, and the proportions of type I and type II fibers were determined. Type I fibers were those which stained light for ATPase at pH 9.4; type II fibers stained dark for ATPase at pH 9.4. Staining for myoadenylate deaminase was performed on the trapezius biopsy specimens according to a modification of the method described by Fishbein et al (11).

For the statistical analyses, the differences between means were tested for significance by Student's unpaired 2-tailed t-test. A value of $P < 0.05$ was considered significant.

RESULTS

The results of laboratory tests on the blood samples were normal in all subjects. Results of the chemical analyses of the muscle samples are presented in Table 1. The values obtained on samples from the healthy controls were similar to those previously reported for human quadriceps muscle at rest (8), except for the levels of glycogen, which tended to be lower in the trapezius muscle.

Levels of ATP and ADP were significantly lower in the trapezius muscles of the PF patients compared with the controls ($P < 0.001$ for ATP and $P < 0.05$ for ADP), whereas the AMP values were increased in the PF patients ($P < 0.005$). The TA value was lower ($P < 0.001$) in the trapezius muscles of the patients. There was also a significant decrease in the ECP values. A significantly lower ($P < 0.001$) level of PC and a higher ($P < 0.001$) level of creatine was found in the trapezius muscles of the patients with PF as compared with the normal subjects. There was a significantly higher ($P < 0.005$) total creatine value in the PF patients' trapezius muscles. The changes in ratios of ATP:total creatine and PC:total creatine were even more pronounced (Figure 1).

There were no differences in the lactate and pyruvate contents of the PF trapezius muscles compared with those of the controls. There were no significant differences in chemical content between the PF patients' anterior tibial muscles and the muscle samples from the healthy controls, except for the levels of ADP and AMP. In the PF patients' trapezius muscles, there was a mean (±SD) of 64.5 ± 8.3% type

Table 1. Levels of selected chemical components of muscle in patients with primary fibromyalgia and in control subjects*

Variable†	Controls (n = 8)	Group 1 (n = 15)	P‡	Group 2 (n = 6)	P‡
ATP	23.9 ± 1.0	19.9 ± 2.2	<0.001	22.3 ± 2.0	NS
ADP	3.3 ± 0.30	3.0 ± 0.30	<0.05	2.9 ± 0.28	<0.05
AMP	0.12 ± 0.03	0.22 ± 0.08	<0.005	0.17 ± 0.02	<0.01
TA	27.3 ± 1.16	23.4 ± 2.49	<0.001	25.4 ± 2.08	NS
ECP	0.935 ± 0.0045	0.925 ± 0.0113	<0.02	0.936 ± 0.0062	NS
PC	69.5 ± 3.9	54.6 ± 7.5	<0.001	68.4 ± 9.8	NS
Creatine	50.4 ± 4.2	78.4 ± 12.5	<0.001	47.2 ± 4.7	NS
Total creatine	119.9 ± 5.3	133.0 ± 10.8	<0.005	115.7 ± 13.6	NS
Lactate	5.0 ± 1.7	4.2 ± 1.7	NS	3.5 ± 2.1	NS
Pyruvate	0.46 ± 0.14	0.54 ± 0.17	NS	0.42 ± 0.16	NS
Glycogen	277 ± 39	267 ± 46	NS	247 ± 58	NS

* The trapezius muscle was sampled in the control subjects. In group 1 patients, the samples were taken from trigger point areas of the trapezius muscle. In group 2 patients, samples were taken of the anterior tibial muscle. See Patients and Methods for details.
†ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; TA = total adenine nucleotide pool; ECP = energy charge potential; PC = phosphoryl creatine. All values are given in mmoles/kg of dry muscle, except for glycogen for which the value is in mmoles of glycosyl units/kg of dry muscle.
‡Versus controls, by Student's unpaired 2-tailed *t*-test. NS = not significant.

I fibers. In the healthy controls the value was 57.0 ± 8.2%. The difference was not significant. No increase in endomysial or perimysial tissue was seen in the patients compared with the controls. Staining for myoadenylate deaminase was normal in all PF patients' trapezius muscle samples and in all but 1 of the controls.

Table 2 shows the results of the analyses of the trapezius muscle specimens taken from the 2 patients who had 2 biopsies, 1 from the trigger point and 1 from outside the trigger point area.

DISCUSSION

PF is a disease which predominantly affects females and is a disorder that is commonly encountered in rheumatology practice (12). It is a disease that has long been recognized (13), but there has been some confusion because of a lack of uniform diagnostic criteria and a lack of knowledge of the pathogenic mechanisms involved. In 1981, Yunus et al suggested criteria for the diagnosis of PF (1). All our patients fulfilled these criteria, and all had at least 10 trigger points (1).

The pathogenic mechanisms of PF are not clearly known, but there are many hypotheses. Many authors have postulated that the muscle pain is caused by inflammation and swelling of the muscle, but because this theory could not be confirmed by light microscope examinations, a psychosomatic cause for PF was proposed (for review, see refs. 14 and 15). Moldofsky et al (16) found a disturbed non-REM (rapid eye movement) sleep pattern in PF patients and thought this to be an important factor. Another hypothesis is that muscle tension, due to poor posture, anxiety, muscle overload, etc., gives rise to hypoxia in the muscle and leads to degenerative changes in the muscle cell (3). It should be noted, however, that at present there is no real evidence of a more or less continuous muscle contraction of the painful muscle.

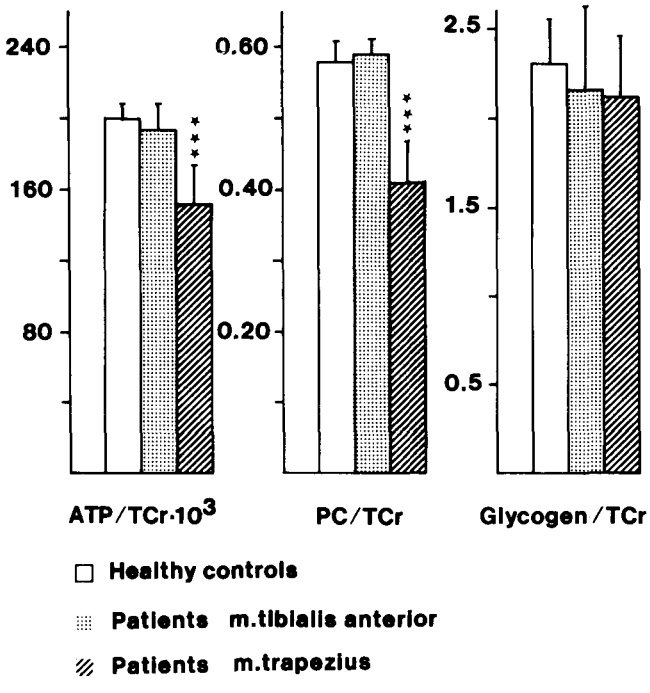

Figure 1. Ratios of adenosine triphosphate (ATP)/total creatine (TCr), phosphoryl creatine (PC)/TCr, and glycogen/TCr in the trapezius muscles of 8 controls, the anterior tibial muscles of 6 patients with primary fibromyalgia (PF), and the trigger point areas of the trapezius muscles of 15 PF patients. Values are mean ± SD. ★★★ = $P < 0.001$ versus controls, by Student's unpaired 2-tailed *t*-test.

Table 2. Levels of selected chemical components of trapezius muscle in 2 patients with primary fibromyalgia, from whom 2 biopsy samples were obtained*

	ATP	ADP	AMP	PC	Creatine
Patient 1					
Trigger point	17.7	3.0	0.22	58.8	93.4
Outside trigger point	20.4	3.2	0.33	61.3	75.0
Patient 2					
Trigger point	17.0	3.0	0.38	42.7	97.1
Outside trigger point	19.2	3.3	0.10	51.5	99.4

* Values are given in mmoles/kg of dry muscle. See footnotes to Table 1 for abbreviations.

As mentioned above, there is evidence for pathologic distribution of muscle surface oxygenation in muscle from PF patients (4). Measurements were made in vivo with an oxygen electrode placed on the muscle surface over trigger points of the trapezius and the brachioradial muscles. Abnormal muscle oxygen tension, which indicates an uneven capillary perfusion, was found in all PF patients, compared with results of the same tests on healthy controls. The cause of this relative hypoxia, however, is unknown.

In this study, a decrease in levels of ATP, ADP, and PC, and an increase in levels of AMP and creatine, were found in the painful muscles of the PF patients. All trapezius biopsy materials (Table 1) were taken from trigger points. In 2 patients, samples were also taken from nontender parts of the same muscle for comparison. Values for ATP and PC in this nontender area were also low compared with those of the controls, which might indicate that the changes found are not restricted to the trigger point area, although no definite conclusion can be made from only 2 observations.

There were no significant differences in the chemical values found in patients' nontender muscles compared with those in the specimens from healthy controls, except for the levels of ADP and AMP. However, the slight differences are probably related to the total creatine value. In this study, the biopsy specimens of nontender and tender muscles were taken from different muscles. This should be of only minor importance since variations in the high-energy phosphate contents between muscles are small (17). It can thus be concluded that pronounced changes of high-energy phosphate levels are found only in muscles with pain and trigger points.

There are 2 possible explanations for the present findings. One explanation is that the chemical changes are secondary to hypoxia. The second is that there is a metabolic change which leads either to a defective synthesis of energy-rich phosphates or to an increased degradation of these substances. Our results do not allow a definite conclusion; however, the results do confirm that there are real metabolic changes in painful muscles in PF patients.

Earlier studies have shown that there are significant metabolic changes in human muscle energy metabolism during ischemia. Harris et al (5) found a significant decrease in PC within 4 minutes of total circulatory occlusion of the quadriceps muscle. The changes in the levels of ATP were minor, and other studies (18) have shown that there is no significant decrease in the ATP content until 2 hours after induction of total ischemia. Tissue ADP and AMP levels increase during the first hour of ischemia (5), whereas prolonged ischemia results in a decrease in these levels because of a loss of adenine nucleotides from the cell (18).

Bergström et al (7) studied energy-rich phosphates in muscle biopsy material from severely ill patients. In the acutely ill patients with circulatory or respiratory insufficiency, those investigators found a decrease in the levels of ATP, PC, and TA. The changes in the adenylate pool were even more pronounced in patients with prolonged disease. In those patients, the ATP content was only 50% of normal. The reason for the changes was thought to be hypoxia in combination with a decreased rate of purine synthesis in the liver and/or a decreased capacity for "purine salvage" in the muscle.

One possible mechanism for the occurrence of hypoxia in PF patients is an uneven capillary perfusion. Fassbender and Wegner (3) examined muscle biopsy specimens from PF patients and found swollen endothelial cells. Such a condition could cause an intravascular obstruction and could sustain hypoxia. It is not known whether the swollen endothelial cells are the cause of, or whether they are caused by, the hypoxia. Gidlöf et al (19) also found swollen capillary endothelial cells in muscle samples taken from the quadriceps muscle after 150 minutes of induced ischemia.

Muscle pain and fatigue are common symptoms in patients with rheumatoid arthritis (RA); therefore it is of interest that similar changes in energy metabolism have been found in RA patients. Muscle blood flow has been shown to be reduced in RA patients (20). Muscle energy metabolism in RA patients was studied by Nordemar et al (21), who found a decrease in levels of ATP, ADP, and PC and an increase in creatine in the RA patients they evaluated. We found that the change in the content of these high-energy phosphates in our PF patients was of the same magnitude.

In other studies, lactate and pyruvate levels in muscle were found to be elevated in patients with ischemia (6,7). There was no increase in the lactate

content of muscle in our patients. This probably indicates that, despite a probable hypoxic situation, the circulation is good enough to clear the lactate that is produced. It has also been found that there is no increase in the lactate content of muscles from RA patients (21).

The decrease in ECP found in our patients is noteworthy; it indicates a decreased capacity for biosynthetic reactions. The ECP is a constant factor, with a normal value of 0.94–0.95 in humans. The ECP level seems to be independent of pH and of variations in the size of the adenine nucleotide pool. Low energy charge has previously been found in severely ill patients and in patients with untreated malnutrition (7).

Because histochemical staining for myoadenylate deaminase gave normal results in all patients, it is unlikely that a deficiency of this enzyme is the cause of any of the changes we observed.

There was no significant difference between patients and controls regarding the proportions of type I and type II fibers. Thus, differences in the adenine nucleotide content between the different fiber types could not explain the present findings.

In conclusion, this study has shown that there is a marked change in muscle energy metabolism in painful muscles that have trigger points in PF patients, compared with normal controls. This change was shown by a decrease in levels of ATP, ADP, and PC and an increase in levels of AMP and creatine. The reason for this change is unknown. Hypoxia is probably present in the painful muscle, and might at least contribute to the deficiency of energy-rich compounds.

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