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Changes in muscle morphology in chronic trapezius myalgia

by Rolf Lindman, DDS, Mats Hagberg, MD, Karl-Axel Ångqvist, MD, Karin Söderlund, Eric Hultman, MD, Lars-Eric Thornell, MD

LINDMAN R, HAGBERG M, ÅNGQVIST K-A, SÖDERLUND K, HULTMAN E, THORNELL L-E. Changes in muscle morphology in chronic trapezius myalgia. Scand J Work Environ Health 1991;17:347—55. Muscle biopsies of the descending portion of the trapezius muscle from female patients with chronic trapezius myalgia and from healthy women were analyzed with enzyme histochemical and immunohistochemical methods. Frequency, area, and capillarization of the muscle fiber types were determined. A biochemical analysis determined the lactate concentration of mixed muscle samples and the adenosine triphosphate (ATP) and phosphocreatine levels in single muscle fibers. The patients had larger type I fibers and a lower capillary:fiber area ratio for type I and type IIA fibers. The patients also exhibited lower levels of ATP and phosphocreatine in both type I and type II fibers. It is suggested that there might have been an imbalance between the capillary supply and the cross-sectional fiber area of type I and type IIA fibers in the patients. This imbalance might be of significance in the development of muscular fatigue and pain.

Key terms: adenosine triphosphate, capillary supply, chronic pain, fiber types, trapezius muscle.

The descending portion of the trapezius muscle is normally subjected to frequent loading to support and stabilize the shoulders during movements of the upper extremities (1). Work conditions with static and repetitive tasks cause an additional load on the shoulder muscles, and it appears that low-intensity loads over a long period of time and static loads cause a particular problem (2—4). The major complaint reported among patients with work-related disorders is chronic tenderness and pain in the muscles of the neck and shoulders (5—10). A myogenic cause of chronic work-related neck-shoulder pain is suggested by the increased rate of fatigue indicated by electromyography (8), the low elevation strength (11), and the inability to generate high amplitude motor units (12). Ischemia is thought to be a significant factor in occupational muscle disorders (11—14). Recent findings of morphological and biochemical changes in muscle biopsies from patients with work-related trapezius myalgia have been suggested to be related to local temporary hypoxia and reduced muscle oxygenation (15), possibly due to reduced blood flow (16). Furthermore, an energy crisis within the muscle fibers has been suggested as a possible cause of chronic muscular pain (15, 17).

The use of enzyme histochemistry and the concept of muscle fiber types has been of great importance in the understanding of muscle physiology and pathology. Muscle fibers can be classified into different fiber types according to the reaction of myofibrillar adenosine triphosphatase (mATPase) at different levels of alkaline and acidic preincubations (18, 19). The histochemical fiber type composition can be correlated with the physiological properties (20—24) and can thereby give an indication of the contractile and metabolic properties of the muscle. Thus type I fibers are slow contracting and fatigue resistant, type IIA fibers are fast contracting and fatigue resistant, and type IIB fibers are fast contracting but fatigue sensitive.

Although previous results have shown morphological changes thought to be related to muscle oxygenation (15, 16) and also changes in the fiber type population in patients with chronic trapezius myalgia (15), there are no studies on fiber type composition which include the type II fiber subtypes and muscle fiber capillarization in such patients. Therefore, we have carried out such a study on female patients with longstanding static and repetitive loading of the neck and shoulder muscles and a long history of pain. We have then compared these results with that of healthy female subjects.

Subjects and methods

Muscle biopsies were obtained from patients who had been referred to the Department of Occupational Medicine due to suspected chronic work-related myalgia in the muscles of the neck and shoulders. In the present study, male subjects were excluded, and also one female subject who was not subjected to static or repetitive work was omitted. In addition, two female subjects were later excluded due to artifacts in the biopsies. Thus 10 female patients with a mean age...
of 45.5 (range 27—58) years were included. According to detailed work history recordings, these patients had been exposed to static and highly repetitive work tasks for an average of 15 (range 4—22) years. Their job titles were cashier (N = 2), charwoman (N = 1), assembly worker (N = 5), microscopist (N = 1), and spray lacquerer (N = 1). No exposure measurements were performed. Their mean duration of symptoms was 7 (range 4—13) years. At the time of the biopsy, all the patients were on sick leave and had been so for an average of 2.4 (range 14 days—7 years) years. Two patients were smokers and three were ex-smokers. For eight patients, the diagnosis did not conform to fibrositis or primary fibromyalgia since the pain was localized in the shoulder and neck region only. Two patients had signs of more generalized pain, but both had been exposed to static and repetitive load and, therefore, occupational trauma could not be excluded. An extensive clinical, radiographic, and laboratory examination of the patients showed no signs of inflammatory rheumatic disease, neuromuscular disease, or cervical root engagement. Two patients had degenerative changes of the osteophytic type, however, not on the biopsy side. None had a radiograph indicative of severe spondyloarthrosis or impingement of cervical nodes or the spinal cord. During time on sick leave, all the patients went through a program of physiotherapy, however, at very low intensities and loads and for a short duration because of the pain. Attempts at rehabilitation were unsuccessful.

Five healthy females with a mean age of 35 (range 32—39) years with no history of chronic shoulder or neck muscle pain served as the referents. Three of the referents were medical doctors, one was a physiotherapist, and one was a nurse. None of the referents performed static or repetitive work tasks, and they were all only moderately physically active.

**Biopsy, enzyme histochemical and immunohistochemical procedures**

Biopsies were taken with an open surgical technique. For the patients, a biopsy was taken from a tender point in the upper region of the descending portion of the trapezius muscle. In the reference group, the biopsy was taken from the same area. A portion of the muscle sample was separated and immediately frozen in liquid nitrogen for the biochemical analyses. Another part was oriented in OCT® embedding medium (Miles Laboratories, Naperville, IL, United States) on cardboard and rapidly frozen in propane chilled to −160°C and stored at −80°C until use. Serial transverse sections, 10 mm thick, were cut in a cryostat ultramicrotome at −20°C and mounted on glass slides. The sections were then stained for the reaction of mATPase (EC 3.6.1.3) at pH 9.4 (18, 25) and at different levels of preincubation, pH 4.6 and 4.3 (19). In addition we used preincubation at pH 4.7.

Nicotinamide dinucleotide dehydrogenase tetrazolium oxido-reductase (NADH-TR) (EC 1.6.99.3), glycerol-3-phosphate oxidoreductase (EC 1.1.99.6), and phosphorylase were used to show oxidative and glycolytic activity. Modified Gomori trichrome staining (26) was used to identify nuclei and cell borders. Oil red O was used to identify the lipids.

Rabbit polyclonal antibodies against laminin isolated by affinity chromatography from sarcomas and produced by E-Y lab, INE, San Mateo, California, United States, were used to stain the basement membranes. The peroxidase-antiperoxidase technique (27) was used to visualize the presence of the laminin, displaying the basement membrane of the capillaries in sections serial to those used for the fiber typing.

**Sampling of fibers, fiber area measurements and capillary count**

The muscle fibers were typed, according to their mATPase lability at different levels of preincubation acidity, into type I, IIA, IIB, IIC (19), IIAB (28), and IM (29) fibers. The analyses were based on three randomly selected areas from each sample. Photographs were taken with a Leitz (Dialux 20) light photomicroscope, magnified to 620X before the measuring on a Hipad digitizing table (Huston instruments) connected to an ABS 800 personal computer programmed for morphometric analyses. Capillaries in contact with each fiber type (CF) and the CF relative to the cross-sectional fiber area of the fiber types (CA) was determined.

**Biochemistry**

One part of the samples was dissected free from blood and connective tissue and extracted with 0.5 M perchloric acid containing 1.0 mM ethylenediaminetetraacetic acid (EDTA). These pieces were neutralized with 2.2 M potassium hydrogen carbonate (KHCO₃). The neutralized extracts were analyzed enzyme histochemically for lactate as described by Harris et al (30).

One part of the freeze-dried muscle sample was used for fiber separation and the analysis of adenosine triphosphate (ATP) and phosphocreatine in single muscle fibers. To exclude the admission of extramuscular tissue in the analyzed material, we separated the fibers and analyzed individual characterized fibers. Two small pieces were cut from each fiber and used for classifying the fibers into type I and type II according to their staining characteristics for mATPase. The remaining part of the fibers was weighed on a quartz-fiber fishpole balance as described by Lowry & Passonneau (31). Each fiber was then extracted in 200 ml of 2.5 % trichloroacetic acid for approximately 2—3 min and neutralized with 20 ml of 2.2 M KHCO₃ and stored at −70°C until analyzed. By using a bioluminescence technique, we were able to determine the ATP and phosphocreatine content in single fibers (weighing 1—4 mg) using a creatine kinase (EC 2.7.3.2.) total kit 1243-100 LKB Wallac (32) modified for this purpose (33). The analyses were performed with a Pharmacia LKB-Wallac 1251 luminometer (Turku, Finland).
**Statistical methods**

Mean values and standard deviations were calculated. Since the sample size was small, differences in the frequency and area of the muscle fibers, as well as the fiber capillarization between the groups, were tested for significance with the Mann-Whitney analysis of variance. Significance was set at the 0.05 level.

The mean value for ATP and phosphocreatine in single fibers from five patients was compared with the mean value from five healthy subjects (reference value). The test used was "simple significance tests based on normal distribution comparison of means of two large sample sizes" (34).

**Results**

**Muscle fiber types, frequency and size**

Generally, type I, type IIA, type IIAB, and type IIB fibers were identified (figure 1). Type I and type IIA fibers constituted the majority of the fiber population. Type I fibers predominated in both the patients and the referents and were the most marked in the patients. Type IIB fibers were absent in one patient, and both type IIAB and type IIB fibers were absent in two patients and one referent. Type IIC and IM fibers rarely occurred. However, in one patient type IIC fibers constituted 2% of the total fiber population.

![Figure 1. Serial cross-sections from the trapezius muscle showing the different muscle fiber types when stained for myofibrillar adenosine triphosphatase at pH 9.4 (A), 4.6 (B), and 4.3 (C). Type I, type IIA, IIAB, and type IIB fibers are marked. (bar = 40 μm)](image)

**Table 1.** Mean frequency, cross-sectional area, capillaries per fiber type ratio (CF ratio), and CF ratio relative to the fiber type area (CA) of the patients and referents.

| Fiber type | Fiber type frequency (%) | Fiber type area (μm²) | CF ratio | CA |
|------------|--------------------------|----------------------|----------|----|
|            | Mean | SD     | Mean | SD | Mean | SD | Mean | SD |
| I          |       |        |      |     |       |     |       |     |
| Patients   | 64.3  | 12.6   | 4720 | 1262 | 3.54  | 0.69 | 0.80  | 0.24 |
| Referents  | 55.8  | 26.0   | 2631 | 721  | 3.15  | 0.94 | 1.12  | 0.26 |
| IIA        |       |        |      |     |       |     |       |     |
| Patients   | 25.9  | 10.6   | 4159 | 1728 | 2.58  | 0.85 | 0.64  | 0.30 |
| Referents  | 29.2  | 21.0   | 2752 | 747  | 2.69  | 0.33 | 1.04  | 0.33 |
| IIAB       |       |        |      |     |       |     |       |     |
| Patients   | 5.1   | 4.2    | 4003 | 1767 | 2.36  | 0.39 | 0.66  | 0.18 |
| Referents  | 4.2   | 2.4    | 2674 | 875  | 1.81  | 0.24 | 0.75  | 0.31 |
| IIB        |       |        |      |     |       |     |       |     |
| Patients   | 4.7   | 8.4    | 4144 | 2088 | 2.62  | 0.47 | 0.60  | 0.17 |
| Referents  | 10.8  | 10.9   | 2408 | 760  | 1.84  | 0.52 | 0.81  | 0.28 |

* P < 0.05, ** P < 0.01.
The relative frequency and the mean cross-sectional fiber area of the fiber types are presented in Table 1. When stained by nicotinamide dinucleotide dehydrogenase tetr唑olium oxido-reductase, the type I fiber population showed differences in the staining pattern. In general, the type I fibers showed an even distribution of formazan deposits, and the intermyofibrillar network had a regular honeycomb pattern. Fibers with an irregular intermyofibrillar network and clusters of formazan deposits were also observed. We refer to them as "irregular fibers" (I_{IR} fibers) (Figure 2).

Fibers with focal loss of enzyme activity and whorling of the intermyofibrillar network are referred to as moth-eaten fibers (I_{ME} fibers) (Figures 2 and 3). Within the I_{ME} fiber population there were fibers with large patches of activity loss. We refer to them as "very" moth-eaten fibers (I_{vME} fibers) (Figures 2 and 3).

Generally speaking, the moth-eaten fibers were evenly distributed over the cross-section and they were always of type I. Irregular fibers and moth-eaten fibers were observed in six patients and four referents. "Very" moth-eaten fibers were seen in four patients and three referents. The frequency of these fibers with-
in the type I fiber population and their mean cross-section fiber area and standard deviation are presented in table 2.

Fibers with a subsarcolemmal accumulation of formazan particles in NADH-TR in the same zones as reddish blue material in the Gomori trichrome staining were referred to as ragged-red fibers (figure 2). The ragged-red fibers showed higher levels of intracellular lipids than the ordinary type I fibers when stained by oil red O (figure 4). Ragged-red fibers were observed in one patient and one referent, one in each biopsy.

The average number of capillaries in contact with each fiber type (CF) was higher for the type I fibers than for the type II fibers in both the patients and the referents. When the CF was expressed relative to the mean cross-sectional fiber area, this value (CA) was also higher for the type I than the type II fibers in both groups. Both the CF and CA varied between individuals in both the patient group and the reference group. The mean values and standard deviations for the CF and CA ratios are given in tables 1 and 2.

Significant differences in the fiber type composition and capillary supply
The type I fibers and the irregular fibers were significantly larger in the patients than in the referents. In the patients the \( I_{ME} \) fibers were larger than the type IIA fibers. In the referents the \( I_{ME} \) fibers were larger than the type IIB fibers. There was a significantly higher CF ratio for the type IIA fibers in the patients than in the referents. Within the patient group, the CF value was higher for the type I and the \( I_{IR} \) fibers than for the \( I_{ME} \) fibers. However, in the reference group, the CF value was higher for all type I fibers than for the type IIA and type IIB fibers. The CA ratio was significantly lower for the patients than for the referents regarding type I, \( I_{IR} \), and IIA fibers.

Biochemistry
The ATP and phosphocreatine concentrations determined for fibers isolated from the five freeze-dried muscle samples from the patient group were significantly lower than corresponding concentrations ob-
Figure 4. Serial cross-section of a ragged-red fiber (center) stained for nicotinamide dinucleotide dehydrogenase tetrazolium oxido-reductase activity (A) and with oil red O (B). Note the accumulation of lipid droplets within the ragged-red fiber. (bar = 26 µm)

Table 3. Concentration of adenosine triphosphate (ATP) and phosphocreatine in single fibers and the lactate concentration in whole muscle samples from the trapezius muscle of the patients with chronic trapezius myalgia. Reference values represent the corresponding values of five healthy women.

|          | ATP            | Phosphocreatine (mmol/kg of dry muscle tissue) | Lactate |
|----------|----------------|-----------------------------------------------|---------|
|          | Number of samples | Mean ± SD | Number of samples | Mean ± SD | Number of samples | Mean ± SD | Number of samples | Mean ± SD |
| Patient values |                  |          |                  |          |                  |          |                  |          |
| Type I   | 49              | 14.8 ± 2.47** | 28              | 18.8 ± 1.79*** | 49              | 56.7 ± 9.59** | 28              | 86.5 ± 13.8** |
| Type II  | 113             | 25.2 ± 3.09 |                  |          |                  |          |                  |          |
| Reference values |              |          |                  |          |                  |          |                  |          |
| Type I   | 60              | 20.2 ± 4.70 | 95              | 97.1 ± 16.2 | 5               | 5.01 ± 1.7  |          |          |
| Type II  | 113             | 25.2 ± 3.09 |                  |          |                  |          |                  |          |

** P<0.01, *** P<0.001.

Determined from the five healthy female volunteers (table 3). Lactate concentration determined in whole muscle samples had similar values in both groups.

Discussion

All the fibers, irrespective of type, tended to have a larger mean cross-sectional fiber area in the patients than in the referents, the type I and type IIb fibers being significantly larger. The type I fibers belong to motor units which are the first to be recruited during repetitive movements of relatively low tension (21). The larger area of the fibers in the patient biopsies, obtained from tender points, may reflect an adaptive response to frequent occupational use of this region of the muscle since muscular usage might cause an increase in fiber area (35, 36). However, one might then expect the fiber size to be normalized in the patients since they had been on sick leave for a long period of time. One possible explanation is that this assumed increase in fiber area may persist, despite absence from work, due to altered central motor control mechanisms. Edwards (37) has advocated that work-related muscle pain might be a consequence of an imbalance between motor control and postural muscle activity. Thus the muscular changes in the patients in this study might be secondary to altered central motor control mechanisms.

The difference in fiber size between the patients and the referents in the present study is in contrast to the findings of Larsson et al (15). There are, however, difficulties associated with studying the trapezius muscle which may to some extent explain the discrepancies between the two observations, and perhaps also between the two groups in the present study. As we have previously shown, the muscle fiber composition of the trapezius muscle is inconsistent throughout different portions of the muscle, the inconsistency being most apparent in the descending portion of the muscle (38, 39). In the lower regions of the descending portion type I fibers predominate, and the fibers have a larger cross-sectional area. In the upper regions the fibers are smaller and the type II fibers increase in number, especially the type IIB fibers. Consequently biopsies obtained at slightly different levels in this region may show differences with respect to the frequency and size of the fiber types. However, the
marked difference in the fiber cross-sectional area between the patients and the referents cannot be explained by differences in biopsy sites within this region since these large differences were not found in our previous study on the female trapezius muscle. Interindividual differences and the differences related to gender might also be large, and other factors, such as present and previous physical activity, posture and biomechanical relationships, might also influence muscle morphology. In order to overcome some of the factors, we only included women, all subjects had comparable leisure-time physical activities, and the subjects of the reference group were from the same group as that of Larsson et al (15).

The capillary fiber contact (CF) ratio of the reference group, and also that of the patient group, was higher for the type I fibers than for the type II fibers. These results are similar to findings for human female quadriceps muscle (40). However, our results indicate that the trapezius muscle fibers are less supplied by capillaries than limb muscle fibers. The CF ratio for the trapezius muscle was lower than that reported for the normal female quadriceps (40), the male quadriceps (40—43), and the triceps brachii (43) muscles. The significantly higher CF ratio for the type IIA fibers in the patients than the corresponding ratio of the referents might be of some interest since an increase in capillaryization seems to precede changes in myofibrillar proteins in fiber type transformation (44). Further information regarding this matter may be obtained from the immunohistochemical analyses of trapezius muscle fiber types in progress in our laboratory.

Another significant observation of this study was that, in the patient group, when the CF value was expressed relative to the mean fiber type area, this ratio (CA) was clearly lower for the type I, the I_{RR_1}, and the type IIA fibers. This finding might be a result of an increase in muscle fiber area not matched by an increased capillary supply, the result being a larger diffusion distance. Another possibility is a genetic predisposition of the muscle morphology resulting in a low CA ratio. A low CA ratio, regardless of cause, could mean that a smaller blood volume is available for these fibers, and this lower volume could be critical for the uptake of substrates and the removal of metabolites.

Generally, the biochemical analysis of samples from resting muscle of the patients showed a normal content of lactate but significantly lower levels of ATP and phosphocreatine in both fiber types in comparison with that of the healthy referents. There is no easy explanation for this finding. A low resting level of ATP in muscle tissue has been described in patients with rheumatoid arthritis (45), and a minor decrease was observed in patients with chronic trapezius myalgia (15). Interestingly, biopsies obtained from male subjects with the same symptoms all showed normal ATP levels (our unpublished results). This phenomenon might indicate differences between men and women with respect to muscle function and work tolerance, as has previously been suggested (39).

Moth-eaten fibers are of special interest since they have not only been found in trapezius muscle biopsies from patients with chronic muscle pain, but also in the trapezius muscle from perfectly healthy individuals (15, 46). They are found only in the type I fiber population and only in the descending portion of the muscle (38, 39). It is suggested that the specific anatomic location of these fibers may indicate that this region of the muscle is subjected to strain even during ordinary conditions with no excessive loading (38, 46). The different staining patterns of the moth-eaten fibers in the trapezius muscle presented in this study may reflect different mitochondrial reactions. Noteworthy, moth-eaten fibers have also been found in ischemic rat muscle (47).

Ragged-red fibers, which are typical for mitochondrial myopathies (19), have also been found in ischemic rat muscle (47). Larsson and his co-workers (15, 16) found ragged-red fibers to be frequent in the trapezius muscle of a group of patients with work-related trapezius myalgia. However, we found ragged-red fibers only in two subjects, one patient and one referent. Serial sections have, however, revealed that the ragged-red fibers may only be present segmentally (48); if so, the segmentation might account for the discrepancies in the number of ragged-red fibers between our studies. The accumulation of lipid droplets in the ragged-red fibers (figure 4) suggests that the fiber metabolism is disturbed. Nevertheless, ragged-red fibers cannot give rise to the low values of energy phosphates found in the trapezius muscle biopsies from our patients. Interestingly, as in mitochondrial myopathies (49), the ragged-red fibers found in the present study were extremely well supplied with capillaries, a finding indicating that the fibers themselves could not be ischemic.

In conclusion, although the results of the present study are based on a small number of subjects and selected cases, they indicate that changes in muscle morphology in chronic trapezius myalgia does exist. However, further studies are needed to confirm whether such changes are related to the development of chronic muscular fatigue and pain caused by static and repetitive worktasks.

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