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Three Clusters of Different Properties Characterize Women with Chronic Trapezius Myalgia

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ABSTRACT

Objectives: A correlative study on data from 14 women with unilateral chronic shoulder pain was undertaken.

Methods: Data were obtained on evoked pain and pressure-pain thresholds [PPTs] changes upon muscle exertion and biopsy findings on capillary density and muscular pathology. The PPTs were measured in the trapezius muscle, before and after a static abduction endurance test of the shoulder [electronic algometer]. Holding time and pain intensity was registered. Capillarization and ragged red fibers, cytochrome-c-oxidase negative fibers, and moth-eaten muscle fibers were analyzed in the same trapezius muscles.

Results: Principal component analysis was used for multivariate analysis, showing a model with three statistically significant components. The first component explained 33 percent of the variation. Pressure-pain threshold changes were positively correlated with capillarization, and negatively correlated with prevalence of moth-eaten fibers and cytochrome-c-oxidase negative fibers. The second component explained 23 percent of the variation, and reflected the correlations between holding time, differences in pain and PPTs, i.e. between various aspects of perceived pain after exertion. The third component explained 19 percent of the variation. The pain difference correlated positively with the prevalence of cytochrome-c-oxidase negative fibers and ragged-red fibers; subjects with high prevalence of these two fiber types presented increased pain. In summary, our results suggest that not only capillarization and histopathological findings of the trapezius muscle, but also centrally modulated pain intensity and PPT changes after muscle exertion are associated.

Conclusions: Three clusters of different properties were revealed in women with trapezius myalgia, highlighting the multifactorial mechanisms responsible. These components may have prognostic value.

KEY WORDS: Pressure-pain threshold, shoulder pain, trapezius muscle, muscle pathology, muscular fatigue, endurance test, central processing

INTRODUCTION

The trapezius muscle is frequently affected in neck/shoulder pain (1,2). The pathophysiological mechanisms of trapezius myalgia are only partially understood (3). Injury, inflammation and changes in muscle blood flow causing peripheral sensitization of nociceptors and hyperreflexia are possible precipitating factors in the acute and in the chronic phase (4-7). Biopsy studies have shown immunohistochemically confirmed alterations of oxidative metabolism and capillarisation in type I muscle fibers (4,6,8). The presence of tender points, i.e. a rough manual assessment of pressure-pain thresholds [PPTs] was associated with a high prevalence of so-called ragged-red fibers, which are indicative of a disturbed oxidative metabolism (8).

The prevalence of neck/shoulder pain is high in the general population (1). In cross-sectional studies, repetitive muscular work, prolonged static work, or extreme working postures (9) have been associated with such pain, and have therefore been considered major contributory factors. This has been partly confirmed in recent prospective studies by Macfarlane and co-workers (10,11), especially for shoulder pain. Moreover, adverse work related psychosocial factors, particularly aspects of job demand and lack of job control, influence the reporting of regional musculoskeletal pain even after only short term exposure (12). In another study, three occupational factors identified a high risk of developing shoulder pain: duration of lifting with one hand, duration of working above shoulder level, and whether employees found their work stressful (13).

In addition, global or bilateral increases of PPTs after strenuous muscle activity indicate that the involvement of a central pain regulating mechanism is important for sensory function both in healthy humans (14,15) and in persons with musculoskeletal pain (16,17). Ongoing pain seems to be another modulator for this mechanism (18).

Here we present a within group post hoc analysis on data from 14 individuals with chronic shoulder pain in whom it was possible to obtain unique data sets both on evoked pain and on PPT changes upon muscle exertion (16), as well as on capillary density and on muscular pathology (8,19). The histopathological data (8) were obtained one year prior to the muscle exertion study (16). However, the subjects had reported chronic pain prior to the first study (8,19), and continued to do so while keeping on with their work as cleaners at the time of the second study (16). Therefore we considered their pain stable enough to perform this correlative analysis on the compiled data set.

MATERIALS AND METHODS

Subjects

Fourteen female hospital cleaners with predominantly unilateral shoulder pain had previously participated in two studies [Study I (8,19)], which investigated muscle pathology and capillary density in the development of myalgia in the shoulder region. An open surgical muscle biopsy of the descending part of the painful trapezius muscle was performed on them in order to classify muscle fibers, count capillaries, and perform biochemical characterizations [for details see Methods]. One year thereafter, the same study group participated in another study [Study II (16)] where PPTs were measured in the shoulder region before and after a standardized static endurance test [for details see Methods]. The main work task for the hospital cleaners was manual mopping of floors. They also cleaned toilets, carried and emptied garbage bins, and equipped their cleaning cart with materials. Moving and stabilizing the mop implies long lasting static muscle activation bilaterally for the trapezius muscle. Thus, the subjects were occupationally active in moderately physically demanding manual cleaning work using both arms so we did not consider them as being untrained. However, only a few of the subjects performed regular physical training on leisure time.

To diagnose trapezius myalgia, a validated structured clinical examination (20), including a pain drawing was used. Only subjects with regional pain in the neck and shoulder area were included in the studies. Obvious tightness and tenderness on general manual palpation of the trapezius muscle and a normal range of motion in the shoulder joints and in the cervical spine were found in the included patients, strongly indicating myalgia as the cause of pain. Women diagnosed as having fibromyalgia syndrome were excluded by using the American College of Rheumatology Criteria for the Classification of Fibromyalgia (21), e.g. widespread pain in combination with tenderness at 11 or more of the 18 specific tender point sites.

Thus the 14 cases included in the current study are the subjects where the most painful side [right side N = 10, left side N = 4] was the same at the time for both Study I and Study II and, consequently, also the side that had been exposed to the endurance test. Individual work technique might explain why some cleaners are affected on the right hand-side and some on the left hand-side. All the participants were right-handed. For the 14 subjects the average age was 47 ± 14.8 years, the average height was 162 ± 7.2 cm, and the average weight was 61 ± 12.0 kg. They had been working as cleaners on average 15 [range: four to 24] years. One inclusion criterion common to both studies was pain for at least one year. The duration of the shoulder pain was in mean 2.9 ± 1.6 years and in median 2.5 [range: one to seven] years as far back as in Study I, and the onset of pain was reported to be related to the cleaning work. Study II was performed one year later. Prior to both studies, the subjects had received common treatment for chronic pain such as physical therapy, pain-relieving pharmacological therapy, or acupuncture. Data on treatment and treatment effects were not systematically registered.

Verbal and written information about the procedures were given in the two studies both before and at the time of the test. Both studies were approved by the Ethics Committee of Lund University.

Sampling of Muscle Biopsies

Open surgical muscle biopsies were obtained from the descending part of the trapezius muscle from the myalgic side of the subjects. The incision was placed two centimeters lateral to the midpoint between the seventh process of the cervical spine and the lateral part of the acromion process, a localization which has been in frequent use by others (4,6). The skin and the subcutaneous area were infiltrated with 3 to 5 ml five percent Xylocaine. Care was taken not to infiltrate the fascia or the muscle. A piece of muscle tissue, approximately 0.5 x 0.5 x 0.5 cm large, was carefully removed and placed in a humid chamber for 15 minutes. The samples were then oriented and mounted for transverse sectioning in Tissue Tek[®] Optimal Cutting Temperature Compound, (Miles laboratories, Naperville, Illinois, USA) and frozen in chilled liquid propane and stored at -60°C until use.

Enzyme histochemistry and Immunohistochemistry

Serial cross-sections, 10 µm thick, were cut at -20°C in a cryostat microtome and mounted on glass slides. Identification of capillaries was performed with a monoclonal antibody against a basal lamina specific protein, laminin A chain [Laminin4C7, DAKO]. Visualization of bound antibody was performed using the indirect conjugated immunoperoxidase technique (22).

Identification of so called ragged-red fibers was based on the fiber appearance in Gomori trichrome [GT] staining (23). Ragged-red fibers, the hallmark of mitochondrial myopathies, were identified by their characteristic subsarcolemmal and intermyofibrillar accumulation of reddish material [mitochondria] when stained with the GT staining

compared to ordinary fibers. Such fibers have also been found to be more frequent in myalgic trapezius muscle and in muscle exposed to long lasting static work than in healthy non-exposed trapezius muscle (6,8,24,25), as well as in subjects considerably older than the middle-aged subjects of this study (26).

Muscle fibers characterized by focal or multi-focal regular or irregular zones lacking activity of the mitochondrial enzyme nicotinamide adenine dinucleotide tetrazolium reductase [NADH-TR] were characterized as moth-eaten fibers (27). One specimen was omitted due to bad quality of one NADH-TR staining. Previously, a painful trapezius muscle has been found to have more moth-eaten fibers than a non-painful muscle (4,19).

The histochemical examination further included a staining for determination of cytochrome-c-oxidase. Unstained fibers in sections with this staining, which also have been found to be more frequent in painful trapezius muscle, (4,8) were called cytochrome-c-oxidase negative fibers.

Histomorphometric Analysis

Serial cryosections were visualized and analyzed using an Olympus BX40 microscope [Olympus Optical Co., Ltd, Tokyo, Japan], a Sanyo Hi-resolution Colour CCD camera [Sanyo Electronic Co., Ltd., Japan], and an 8-bit Matrox Meteor Framegrabber [Matrox Electronic Systems Ltd., Quebec, Canada], combined with image analyzing software [Tema, Scanbeam, Hadsund, Denmark]. Descriptive computer statistical analysis allowed determination of capillaries in the laminin stainings and of cross sectional areas of fibers and preparations. The numbers of capillaries around muscle fibers were normalized with respect to fiber area, the capillary per fiber area [CAFA]. In mean, 1,162 fibers [range: 197 to 2838 fibers] stained for NADH-TR and 804 fibers [range: 117-1866 fibers] stained with the GT staining were counted using the imaging system Olympus DP-soft [version 3.0].

Pressure-Pain Thresholds

An electronic pressure algometer [Somedic[®], Sweden] was used for measuring PPTs. It consists of a gun-shaped handle with a pressure-sensitive strain gauge at the tip and is connected to a power supply, an amplifier, and a display. The diameter of the contact area was 10 mm and was covered with 1 mm rubber to minimize irritation of the skin. A standardized speed of pressure increase of 40 kPa/s [kiloPascal per second] was used. A scale on the display helped the investigator to keep the rate of the pressure increase fixed. The registered pressure threshold in kPa remained on the display when the subject indicated the PPT by pressing a signal button. The instrument was calibrated at the start of the series and the zero level was balanced before each measuring session. Pressure algometry has been used for a long time with varying precision. Present knowledge, however, regarding the validity of our particular device (28-30), shows that the technique has sufficient reproducibility with proper handling [see below].

All measurements were made by the same person. The subject was comfortably seated in a chair with a low support for the back, and with a pillow in the lap for arm support. The PPT recording points were located and marked with a felt pen bilaterally along a straight line used for surface electromyogram recordings (16) from the spinous process of the seventh cervical vertebra to the lateral edge of the acromion with seven points on each side, three over the trapezius muscle and four over the deltoid muscle, in total fourteen points [Figure 1]. Two test trials, one point over each rhomboid muscle, were performed to make the subject familiar with the procedure. The pressure was applied perpendicularly against the skin over the marked points in a fixed order, starting medially on the right hand side over the trapezius muscle [three points] and continuing laterally over the deltoid muscle [four points]. The subjects were instructed to press the signal button as soon as the sensation of pressure

changed to pain (31). Thereafter, the same sequence was repeated on the left hand side. In total, four measurements were made during the test.

Fig 1

Figure 1: Method line drawing showing the pressure-pain threshold points and the incision placed 2 cm lateral of the midpoint between the seventh process of the cervical spine and the lateral part of the acromion process.

Protocol and Endurance Test

A weight belt of 1 kg was wrapped around the wrist of the hand. In the resting position the subject held her forearms and hands on a pillow in the lap. During the sub-maximal endurance test, the right arm was abducted 90° in the scapular plane, with a slightly flexed [20°] elbow, pronated with the thumb pointing downwards. The PPTs were assessed at five time points with a ten-minute pause between each measurement [two times before the endurance test]. The time allocated to assess the PPT points was three to five minutes depending on the PPT levels. The subject was verbally encouraged during the whole

endurance test to achieve as long a holding time as possible. The total holding time was registered and the pain intensity assessed before and immediately after the endurance test on a 0 to 100 mm visual analog scale [VAS; endpoints were “no pain” and “worst pain possible” (32)]. When the subject could no longer maintain her arm in the static holding position, the test ended immediately followed by a PPT assessment. In the present study only the PPT measurements before and immediately after the sub-maximal endurance test over the exposed trapezius muscle were used for analyzing results. We considered this selection of two points in time to be sufficient as well as adequate since it focused on the intervention. Furthermore, VAS was registered at these two points in time.

Statistical Analyses

All statistics were performed using the statistical package Statistical Package for the Social Sciences for Windows [version 12.0] or SIMCA-P [version 10.02]. For variables under investigation, mean, SD, and median values and range are reported.

Two PPT measurements were used in this study. The measurement before the endurance test denominated “absolute PPT before” and the measurement immediately after the endurance test denominated “absolute PPT after.” An increase in PPTs [+PPT in Table 1] implies a decreased sensitivity to pressure and a decrease in PPTs [–PPT in Table 1] an increased sensitivity to pressure.

In order to explore the multivariate relationships between the variables, a principal component analysis [PCA] (33) using the Statistical Package for the Social Sciences was made. Commonly, multivariate data reduction methods such as factor analysis assumes that a high subject to variables ratio is present (5-10), but such assumption does not exist for PCA; in fact, PCA can handle ratios lower than 1.0. The PCA can be viewed as a multivariate correlation analysis. A component consists of a vector of numerical values between –1 and 1, referred to as loadings. The loading expresses the degree of correlation between the item and

Table 1. Individual [Median and Range] Changes in Pressure Pain Threshold, Pain, Holding Time, and Histopathological Variables from 14 Patients

Subjects	Absolute PPT before	Absolute PPT after	Absolute PPT change after vs. before	Pain VAS before	Pain VAS after	Pain VAS after vs. before	Holding time	Capillaries per fiber area CAFA	Ragged-red fibers number per sample	Cytochrome-c-oxidase negative fibers number per sample	Moth-eaten fibers per sample %
	kPa	kPa	kPa	mm	mm	mm	s	mm ²	mm ²	mm ²	
1	89	105	16	64	65	1	130	1.36	0	0.12	--
2	210	262	52	52	67	15	237	1.22	0	0	0.05
3	258	291	32	68	94	26	179	1.10	0.18	0.41	0.09
4	177	275	97	20	53	33	276	1.42	0.03	0.13	0
5	264	233	- 31	21	48	27	391	1.00	0.15	0.17	0.02
6	110	103	- 7	60	88	28	177	0.92	0.17	0.16	0.03
7	233	329	95	40	87	47	221	1.41	0.35	0	0
8	318	326	9	92	65	- 27	153	0.86	0	0.17	0.04
9	129	184	55	36	80	44	192	0.74	0.1	0.10	0.02
10	454	427	- 28	31	42	11	218	0.98	0.07	0	0.04
11	187	198	11	47	88	41	272	0.95	0.07	0.03	0.03
12	188	230	42	17	45	28	159	0.89	0.16	0.21	0.11
13	316	182	- 134	30	97	67	270	0.89	0	0.21	0.07
14	292	344	52	32	73	41	196	1.25	0.34	0.15	0
Median	222	247	24	38	70	28	207	0.99	0.09	0.14	0.03
Range	89 - 454	103 - 426	- 134 - 97	17 - 92	42 - 97	- 27- 67	130 - 391	0.74 - 1.42	0 - 0.35	0 - 0.41	0 - 0.11

PPT = pressure pain threshold, kPa = kilopascal, VAS = visual analogue scale, s = seconds, CAFA = capillaries per fiber area

the component. A loading is obtained for each variable included in the PCA model. Variables that have high loadings [with positive or negative sign] upon the same component are inter-correlated. Items with high loadings [ignoring the sign] are considered to be of large or moderate importance for the component under consideration. We have considered loadings greater than 0.50 in absolute numbers, i.e., irrespective of sign to be high and therefore of interest. Only significant components are presented in the tables. Components with Eigenvalues less than 1.0 were considered as trivial factors and excluded. Eigenvalues reflect the amount of variance in the data explained by the factor. Generally factors with Eigenvalues greater than 1.0 are considered significant. For only one subject, with respect to prevalence of moth-eaten fibers, one missing value was replaced with the mean value of the group. For each significant component is given the explained variation [R^2]. Outliers were checked for prior to the PCA using two methods available in SIMCA-P: 1. score plots in combination with Hotelling's T^2 [identifies strong outliers] and 2. distance to model in X-space [identifies moderate outliers]. No multivariate outlier was identified. The five percent significance level [$P < 0.05$, two-tailed] was chosen in all statistical tests. Wilcoxon's signed rank test was used for comparison between groups.

RESULTS

Data concerning the variables under investigation have been reported earlier in group form (13,16,19). In the present study, the individual, median and range values of the psychophysical as well as of the histopathological data for the 14 included subjects are presented in Table 1. It can be seen that there is a median PPT increase of 24 kPa, but no significant difference was seen before and after exertion in this limited group [$P = 0.096$]. The median post exertion pain intensity was 70 mm, and was significantly greater than that prior to exertion [$P = 0.003$].

Using PCA, the multivariate correlation pattern between absolute changes in PPT, the histopathological variables, pain intensity [VAS], and holding time were investigated [Table 2]. The PCA analysis showed a model with three statistically significant components [first, second, and third component]. These three components explained 75 percent of the variation in the data matrix [Table 2].

Table 2. *The Loadings of the Three Identified Components in the Principal Component Analysis from 14 Patients*

Variables	First Component	Second Component	Third Component
PPT difference	0.69	-0.53	0.18
Pain difference	0.17	0.70	0.53
Holding time	0.20	0.84	-0.16
CAFA	0.73	-0.24	0.06
Ragged-red fibers	0.50	0.02	0.74
Cytochrome-c-oxidase negative fibers	-0.58	-0.14	0.60
Moth-eaten fibers	-0.82	-0.21	0.31
R²	0.33	0.23	0.19

The explained variance R^2 of is given on the bottom row. The sum of $R^2 = 0.75$.
PPT = pressure pain threshold, CAFA = capillaries per fiber area
Loading >0.50 [irrespective of sign] are given in bold types.

According to the first component, explaining 33 percent of the variation in the data matrix [$R^2 = 0.33$], PPT changes were positively correlated with the capillary density [CAFA], and negatively correlated with the prevalence of moth-eaten fibers and cytochrome-c-oxidase negative fibers.

The second component [$R^2 = 0.23$] reflected the correlations between holding time, changes in pain intensity, and in PPTs, i.e. between various aspects of the perceived pain after exertion

According to the third component [$R^2 = 0.19$], the perceived pain intensity after versus before exertion correlated positively with the prevalence of cytochrome-c-oxidase negative fibers and ragged-red fibers. Hence subjects, with high prevalence of these two fiber types, also experienced increases in pain intensity upon muscle exertion.

DISCUSSION

First component: Pressure-Pain Thresholds Increase, Relatively High Capillarization, and Minor Pathology

According to the first component [Table 2], low prevalence of moth-eaten fibers and cytochrome-c-oxidase negative fibers were associated with high capillary density and high PPT differences. The occurrence of moth-eaten fibers and cytochrome-c-oxidase negative fibers can be interpreted as unspecific disturbances in the microcirculation and oxidative metabolism, respectively (34), and occur to a minor degree also in pain free subjects (9). A high CAFA implies relatively highly vascularized muscle fibers. Thus, the findings in the first component reflect no or minor muscle pathology in well vascularized muscle tissue and clear inhibition of nociceptive transmission upon muscle exertion. This may be interpreted as a relatively healthy situation [Table 3] upon strenuous exercise, with interacting peripheral and central mechanisms.

Interestingly, we found a negative correlation between CAFA and moth-eaten fibers in these patients in our multivariate analysis. This negative correlation is in line with the experimental study of Heffner et al. (34) showing that decreased microcirculation can induce moth-eaten fibers. The correlations in our first component, with a combination of high CAFA, no or minor pathology, and an increase of PPTs, may indicate that this component represents a fairly normal situation of skeletal muscle being subjected to transient strain. A

Table 3. Interpretation of PCA Results Including Explained Variation [R^2] and Conclusions

PCA Results	Explained Variation	Interpretations	Conclusions
First component			
<ul style="list-style-type: none"> • Pressure-pain threshold increase • High capillary density • Few cytochrome-c-oxidase negative fibers and moth eaten fibers 	33 %	Good pain inhibition Good micro circulation Minor muscle pathology	Relatively healthy muscle situation
Second component			
<ul style="list-style-type: none"> • Pressure pain threshold decrease • Increased pain intensity • Holding time increase 	23 %	Increased pain sensitivity Strong pain Inadequate behavioral response to body signals	A cognitive phenomenon of not accepting the pain
Third component			
<ul style="list-style-type: none"> • Increased pain intensity • Many cytochrome-c-oxidase negative fibers and ragged red fibers 	19 %	Strong pain Major muscle pathology	Muscle tissue damage

high CAFA has previously been associated with non-painful trapezius muscles (19). On the other hand, moth-eaten fibers have been associated with chronic myalgia, even if these fibers have also been found in subjects without pain (4). Furthermore, we found that cytochrome-c-oxidase negative fibers correlated negatively with CAFA in the first component. A relationship between the prevalence of cytochrome-c-oxidase negative fibers and the intensity of myalgic pain (35) has been reported, but is not consistent with a study by Larsson and co-workers (8). Taken together, it is reasonable to assume that in cases dominated by our first component, the myalgic condition may be transient.

Second Component: Pressure-Pain Threshold Decrease, Strong Pain, and Longer Holding Time

In the second component [Table 2], a decrease in PPT post-exertion was correlated to a larger increase in pain intensity and a longer holding time. Had the final pain intensity been related to the holding time, a shorter holding time would rather be expected. A central pain modulation mechanism might be responsible for the increased pain intensity found here. For example, Kosek et al. (17) found that after isometric contraction of the quadriceps muscle, PPTs decreased in patients with fibromyalgia syndrome, but increased in healthy subjects. The authors interpreted this reaction as being associated with a disturbed central pain inhibition. Habituation to chronic pain may partly contribute to the longer holding time. Furthermore, a cognitive mindset with difficulties in accepting and responding adequately to bodily pain (36,37) may sustain behaviors that are important maintaining factors in chronic myalgia. It should be pointed out that interdisciplinary cognitive behavioral rehabilitation programs focus on mindset transformations (38), and are among the few interventions found effective in musculoskeletal rehabilitation (39).

Third Component: Strong Pain and Major Pathology

According to the third component [Table 2] a high prevalence of ragged-red fibers and cytochrome-c-oxidase negative fibers was associated with large increases in pain intensity after muscle exertion, indicating that a disturbed metabolism might be linked to the perception of pain. The findings in the third component suggest muscle pathology and strong pain [Table 3], and may indicate muscle tissue damage. This may be a prerequisite for the peripheral sensitization that has been presumed to be related to metabolic alterations in contracting muscles (40).

Increased occurrence of ragged-red fibers has been found in trapezius myalgia (24,25,41,42). Interestingly, ragged-red fibers have been related to insufficient blood supply in experimental studies on ischemic rat muscle (34). The present analysis could not, however, confirm the relationship of ragged-red fibers to structural ischemia since the prevalence of ragged-red fibers loaded mainly on the third component, whereas CAFA loaded strongly (Table 2) on the first component. The prevalence of cytochrome-c-oxidase negative fibers correlated positively with the prevalence of ragged-red fibers [third component]. This positive correlation with ragged-red fibers is consistent with the findings of Larsson and co-workers (8) showing that a high proportion of ragged-red fibers also are cytochrome-c-oxidase negative fibers. Moreover, Banker and Engel (27) have pointed out that the enzyme cytochemical profile of the ragged-red fibers depends on underlying abnormalities.

Pathophysiological Implications

Recently, several biochemical reports concerning trapezius myalgia have been published indicating a disturbed situation in the chronically painful trapezius muscle (43-45). For instance, Shah and co-workers (45) reported significantly higher levels of interstitial protons, bradykinin, calcitonin gene-related peptide, substance P, tumor necrosis factor- α , interleukin 1- β , serotonin, and norepinephrine in subjects with myofascial trapezius pain as compared to healthy subjects. These biochemical changes might be due to the disturbed

pathological and vascular conditions in the muscle tissue and would probably be at play in subjects dominated by our third component. Alternatively, they might be parallel processes and the biochemical changes linked to indirect consequences of pain such as patterns of movement and inactivity.

Methodological Aspects

Projection methods like PCA have the advantage of being robust to outliers, to deal with nonlinear relationships, missing data, and data that are noisy and highly collinear (33). The PCA can handle situations when groups of variables correlate, and when the ratio between number of cases and variables are low, as in the present study. Repeated univariate analyses increase the risk for different types of errors and are therefore not suitable when the number of cases is low as in our study. The low number of cases is due to the fact that biopsy is an invasive method which can be uncomfortable to subjects and is expensive.

The muscle biopsy technique has been used sparsely in follow up studies. Windisch and co-workers (46) studied indurative areas of myalgic trunk muscles including the trapezius muscle, palpable even post mortem in 11 human cadavers. Biopsies from such previously painful areas showed ragged-red fibers, moth-eaten fibers, split fibers, and atrophy of type II fibers. Since indurative painful areas of shoulder and trunk muscles often clinically are found to be strikingly resistant to therapy, these muscle biopsy findings were interpreted to be irreversible. Therefore, the current time interval between the two studies (8,16) would probably influence our results only to a minor degree, particularly since the subjects met the criteria for chronic pain at the first as well as at the second study. The fact that we have functional and structural data from the same patients is unique. Our results can hopefully be a basis for future studies, preferably including healthy controls and larger sample sizes.

In summary, our results suggest that not only capillarization and histopathological findings of the trapezius muscle, but also centrally modulated pain intensity and PPT changes

after muscle exertion are associated. Hence, three clusters of different properties were revealed in women with myalgia, highlighting the highly complex, multifactorial mechanisms responsible. The possible influence of these components and their robustness in relation to the clinical course of myalgic patients will be examined further.

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