Differences in the mitochondrial and lipid droplet morphology in female office workers with trapezius myalgia, compared to healthy controls.

A muscle biopsy study.

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Abstract

Objective

Trapezius myalgia, or more specific, myofascial dysfunction of the upper trapezius mainly affects women performing jobs requiring prolonged low level activation of the muscle. This continuous low muscle load can be accompanied by a shift to a more anaerobic energy metabolism, causing pain. To investigate whether morphological signs of an impaired aerobic metabolism are present in female office workers with trapezius myalgia.

Design

Muscle biopsy analysis, using electron and light microscopy, was performed to compare mitochondrial and fat droplet morphology, and irregular muscle fibers, between female office workers with (n=17) and without (n= 15) work-related trapezius myalgia.

Results

The patient group showed a significantly higher mean area ($P=0.023$) and proportion ($P=0.029$) for the subsarcolemmal and intermyofibrillar mitochondria respectively, compared to the control group. A significantly lower mean area of subsarcolemmal lipid droplets was found in the patient group ($P=0.015$), which also displayed a significantly higher proportion of lipid droplets touching the mitochondria ($P=0.035$). A significantly higher amount of muscle fibers with COX deficient areas were found in the patient group ($P=0.030$).

Conclusion

The results of the present study may be indicative for an impaired oxidative metabolism in work-related trapezius myalgia. However, additional research is necessary to confirm this hypothesis.

Key words: mitochondria, lipid droplets, irregular fibers, neck pain, myofascial pain

Introduction

Work-related neck pain is a prevalent musculoskeletal disorder, which mainly affects women and is associated with a high socio-economic burden ⁽¹⁻³⁾. In a study of Juul-Kristensen et al. (2006) on self-reported neck complaints in a population of screen workers, work-related trapezius myalgia (TM) was the most common clinical diagnosis ⁽¹⁾.

Trapezius myalgia, or more specific, myofascial dysfunction of the upper trapezius muscle (UT) is often present in jobs requiring prolonged static postures and repetitive upper limb movements, such as office work ^(2, 4). Also psychological stress plays an important role in the development and maintenance of myofascial dysfunction ⁽⁵⁻⁷⁾.

The pathophysiology of myofascial pain is still not fully understood. It is hypothesized that continuous activation of the low threshold motor units (type I fibers), during prolonged low level muscle activity, leads to abnormal motor endplate activity, an excessive release of acetylcholine and a subsequent continuous local muscle contraction ⁽⁸⁻¹⁰⁾. This persistent contraction can restrict the local muscle blood flow, causing tissue hypoxia. The imbalance between the limited local blood flow and thus reduced oxygen supply, and the increased energy need due to the continuous muscle activity, may lead to an energy crisis. This causes muscle distress and a release of sensitizing substances, which activate the nociceptors ⁽¹⁰⁾. Besides this, the local tissue hypoxia may lead to a decreased pH, activating specific pain receptors. It can be hypothesized that due to the local hypoxia, the aerobic energy metabolism is impaired in this patient population, causing a shift to a more anaerobic energy metabolism ⁽⁹⁾. Since mitochondria play a key role in the aerobic energy metabolism, it can be hypothesized that disturbances in the morphology or function of the mitochondria may contribute to this myofascial pain condition. Morphological signs of mitochondrial

dysfunction have already been found in muscle tissue of fibromyalgia patients ^(11, 12). As mitochondria also play an important role in lipid oxidation, the morphology of lipid droplets can also provide relevant information about the aerobic energy metabolism⁽¹³⁾. Structural and functional abnormalities of mitochondria (e.g. a decline in mitochondrial density) are present in several diseases such as obesity and diabetes and are often associated with an increase in the amount and size of lipid droplets ^(14, 15). This storage of lipids can be a consequence of an impaired oxidative phosphorylation ⁽¹⁴⁾.

In several studies about TM, irregular fibers have been identified in both patient and healthy control groups ⁽¹⁶⁾. “Irregular fibers” is a collective term for muscle fibers which show signs of an aberrant mitochondrial morphology or function, when stained for a particular enzyme or part of the respiratory chain, and may serve as indirect evidence for a lack of mitochondria in these regions ⁽¹⁴⁾. Several attempts for unravelling the underlying pathophysiology of TM have already been done by means of muscle biopsy analysis. However, to our best knowledge, no studies have been performed yet about the potential role of mitochondria and lipid droplets in this myofascial pain condition.

Therefore, the aim of the present study was to compare the mitochondrial and lipid droplet morphology, and the presence of irregular fibers between female office workers with and without TM.

Methods

Subjects

For this case-control study, female office workers between 20 and 50 years old were recruited from January to November 2016. E-mails and flyers were distributed among several workplaces with predominant computer-based tasks. Interested volunteers were asked to fill

out an online questionnaire to check eligibility and to register relevant demographic information such as the perceived pain in the last week, as measured by the numeric rating scale (NRS), and their perceived disability caused by neck pain, as measured by the Neck Disability Index (NDI). The NRS was used to measure the mean, minimal and maximal neck/shoulder pain during the past week. Subjects had to score their pain on a scale from zero (no pain) to ten (worst pain)⁽¹⁷⁾. The NDI (Dutch language version) is a valid questionnaire to measure self-reported neck-pain related disability⁽¹⁸⁾. In- and exclusion criteria were evaluated based the online questionnaire by the main researcher of this study (KDM) and are thoroughly described in De Meulemeester et al. (2019)⁽¹⁹⁾. All subjects signed an informed consent and the study was approved by the Local Ethics Committee of Ghent University Hospital. This study conforms to all STROBE guidelines and reports the required information accordingly (see Supplemental Checklist, Supplemental Digital Content 1, <http://links.lww.com/PHM/A803>).

Muscle biopsies

Muscle biopsy sampling

The biopsy location was marked at the palpated MTrP region at the midpoint between the spinous process of C7 and the lateral edge of the acromion. Details about the biopsy sampling are described in De Meulemeester et al.(2019)⁽¹⁹⁾. Each biopsy sample was cut into two pieces, one piece was stored in a solution of 4% paraformaldehyde and 5% glutaraldehyde in 0,1 M cacodylate buffer for electron microscopy and another piece was mounted with Tissue-Tek (OCT compound), frozen in isopentane, precooled with liquid nitrogen and stored in a freezer at -80 °C until they were stained to identify irregular muscle fibers. All biopsy samples were given a unique identification number and were blinded by an independent researcher.

Muscle tissue preparation for electron microscopy

Biopsy samples were cut into smaller pieces (1 to 2 mm) and stained with the fixative osmium tetroxide. Afterwards, the biopsy samples were dehydrated with ethanol and propylene oxide. Thereafter, the samples were placed in beam embedding capsules, in which Epon, an embedding medium, was added. After hardening the tissue overnight in an oven at 60°C, the tissue was cut into longitudinal semi-thin sections of 4µm, using a glass microtome system (Ultramicrotomy System, Pyramitome, LKB (Stockholm-Bromma, Sweden)). After a tissue orienting staining with toluidine blue, the semi-thin sections were cut with a diamond microtome (Reichert Supernova, Leica) at 90 nm and afterwards at 70 nm. Then a staining with uranylacetate (to identify RNA, ribosomes, mitochondria and membranes) and lead nitrate (to mark filaments and the lipid droplets) was performed. Last, the muscle sections were covered with pulverized carbon powder. This EM procedure was performed by a skilled lab technician at the EM laboratory at the Pathology Department of Ghent University Hospital.

Analysis of electron microscopy images

Samples were viewed at 3000x magnification (for the analysis of lipid droplets) and at 12000x magnification (for the analysis of mitochondria) using a JEOL 1200EX transmission electron microscope. For each subject, 10 micrographs were acquired from 2 randomly sampled longitudinal sections of muscle fibers at 3000x magnification and 15 micrographs were selected of 3 randomly longitudinal sections at 12000x magnification. One micrograph was taken near the cell surface to visualize the subsarcolemmal region, the other micrographs were taken of parallel bundles of myofibrils, representing the intermyofibrillar region. The

reference for subsarcolemmal space quantification was the cytoplasmic space between the sarcolemma and the first layer of myofibrils.

Specific criteria were set in advance for the identification of mitochondria and lipid droplets. Mitochondrial fragments and lipid droplets were circled and converted to actual size using a scale bar. For each set of 25 images, the mean mitochondrial and lipid droplet area (μm^2), the proportion of lipid droplets touching mitochondria (%), the proportion of mitochondrial and lipid droplet area (%), and the number of mitochondria and lipid droplets per square micrometer of muscle tissue ($\#/\mu\text{m}^2$) were calculated in the subsarcolemmal and intermyofibrillar compartments by digital imaging software (ImageJ 1.50i). Calculations were performed as follows: mean mitochondrial/lipid droplet size (μm^2) = sum of all mitochondrial or lipid droplet sizes / # mitochondria or lipid droplets, proportion of lipid droplets touching mitochondria (%) = (# lipid droplets touching mitochondria / total # lipid droplets) x 100 %, proportion of mitochondrial or lipid droplet area (%) = (sum of all mitochondrial or lipid droplet sizes / total tissue area) x 100 %, # of mitochondria or lipid droplets per μm^2 of muscle tissue ($\#/\mu\text{m}^2$) = # mitochondria or lipid droplets / total tissue area (μm^2). Important to note is that only mitochondria and lipid droplets with an area that was visualized completely on the image, were taken into account. The total tissue area consists of the area of the image that is covered with muscle tissue.

Histological and histochemical staining of muscle tissue

Transverse serial muscle cryosections (4 μm) were cut by means of a freezing microtome (Microm GmbH, type HM 550 OMVP, Germany) at a temperature of -15°C and mounted on glass slides.

Afterwards the muscle cryosections were histologically stained with Gomori Trichrome to visualize subsarcolemmal or intermyofibrillar accumulation of mitochondria, called ragged

red fibers. Besides, irregular muscle fibers were identified using histochemical reactions for Nicotinamide Adenine Dinucleotide (NADH), and oxidative enzymes Cytochrome C Oxidase (COX) and Succinate Dehydrogenase (SDH). This was performed by skilled lab technicians in the clinical laboratory at the Pathology Department of Ghent University Hospital.

Normally, a regular ordered intermyofibrillar network is seen with these oxidative enzyme stains ⁽¹⁴⁾. A common change or abnormality that can be visualized with these stains, is a disruption of the intermyofibrillar network with a patchy staining pattern or larger areas devoid of staining. This may reflect a loss of myofibrils and a disturbed mitochondrial distribution.

Analysis of histochemical staining images

Light microscopic images of the histochemical stainings were captured by means of a 3D Histech Panoramic 250 Flash III (CaseViewer 2.0 RTM and CaseCenter 2.7 of 3D Histech) in 20x Brightfield modus. The images of the transverse sections were digitalized by means of 3D Histech Panoramic 250 Flash III in collaboration with Bimetra Biobank, Ghent, Belgium (ID: BE 71067049). For each subject, at least three areas were randomly selected by an independent researcher and used for the determination of ragged red fibers and muscle fibers with an aberrant staining for NADH, COX, and SDH. Image analysis was performed using ImageJ 1.50i, muscle fibers with an abnormal staining pattern were manually counted with the Cell Counter Plugin.

Statistical analysis

Data were analysed using SPSS Version 20.0 (IBM Corporation, Armonk, NY) software. Mean and standard deviation (in case of normal distribution), median and interquartile range (in case of non-normal distribution) or proportions were estimated for demographics. Group

differences for demographics were assessed using unpaired T-tests in case of normal distribution, Mann-Whitney U test in case of non-normal distribution and Chi squared tests in case of categorical data. Descriptive statistics (mean $\pm$ standard deviation and 95 % confidence interval or median (IQR) and min-max) were estimated for all outcome measures. A multiple linear regression model was applied for each outcome measure with “group” (2 levels: myalgia group and control group) as a categorical predictor. Relevant covariates (age, hours of computer work per week, and performing upper limb sports) were added to the model for each outcome measure. The model was checked for multicollinearity. The residuals of each model were checked for normality and homoscedasticity. Statistical significance was accepted at $\alpha < 0.05$.

Results

The online questionnaire was completed by 111 women. After screening of the questionnaire and performing the clinical examination, 32 female office workers, of which 17 patients and 15 healthy controls, were found eligible to be included in the study. Descriptive statistics of demographic features and outcome measures are shown in Table 1 and 2.

Mitochondria and lipid droplets

For the electron microscopic analysis, the muscle sample of one patient was excluded due to problems during the tissue preparation. Fifty images of 20 different subjects were excluded due to low image quality. Results of the linear regression analysis are presented in Table 3. In all subjects (both patients and controls), ultrastructural changes were seen in the muscle tissue such as focal loss of myofibrils, splitting and/or narrowing myofibrils, areas of disorganized myofibrils, irregular Z-lines and Z-line loss.

Mitochondria

A significantly higher mean area (μm^2) was found for the subsarcolemmal mitochondria in the patient group, compared to the healthy control group ($P=0.023$). The intermyofibrillar mitochondria cover a significantly higher area (%) in the patient group, compared to the healthy control group ($P=0.029$). No significant differences were found for the other outcome parameters ($P>0.05$). A borderline missed significantly higher intermyofibrillar mean mitochondrial area was found for the patient group ($P=0.051$). As an example, an image of subsarcolemmal and intermyofibrillar mitochondria in a patient is shown at a 12000 x magnification in Figure 1.

Lipid droplets

A significantly smaller mean area (μm^2) was found for the subsarcolemmal lipid droplets in the patient group, compared to the control group ($P=0.015$). The percentage of intermyofibrillar lipid droplets, touching the mitochondria, was significantly larger in the patient group, compared to the control group ($P=0.035$). No significant differences were found for the other outcome parameters ($P>0.05$). As an example, an image of subsarcolemmal and intermyofibrillar lipid droplets in a patient is shown at a 3000 x magnification in Figure 2.

Irregular fibers

For the morphometric analysis of irregular fibers, several samples were excluded due to freezing artefacts, limited amount of muscle tissue or a failed staining. The Gomori Trichrome staining was performed on 10 patients and 10 controls. The amount of included subjects for COX, NADH and SDH stains are shown in Table 2.

Despite the occurrence of several muscle fibers with a disorganized mitochondrial pattern, the Gomori Trichrome staining revealed no ragged red fibers in none of the groups. A significantly higher proportion of muscle fibers with an irregular COX staining was found in the patient group, compared to the control group ($P=0.030$). Despite the finding that muscle fibers with areas of irregular COX staining also showed areas with irregular NADH and SDH staining, no significant differences between both groups were found for NADH and SDH ($P>0.05$). An image of a COX, NADH and SDH staining in a patient and a healthy control is shown in Figure 3, 4 and 5 respectively.

When interpreting the adjusted R^2 and P-values of the model (Table 3), we can conclude that the independent variable “group” only has a small contribution to the variance of the outcome measure. So, there must be several other factors contributing to the outcome measures apart from the group to which a subject belongs.

Discussion

To our best knowledge this is the first study in which mitochondrial and lipid droplet morphology is studied in patients with TM. The present study revealed a significantly higher mean area and proportion in the patient group for SS and IMF mitochondria respectively. Mitochondrial area and proportion are usually positively correlated with mitochondrial function and aerobic capacity. An increased mitochondrial area is for example seen in response to endurance training⁽²⁰⁻²²⁾. In a previous study on the same study sample was shown that significantly more type IIA and significantly more type IIAX (although not clinically meaningful), and significantly less type IIX fibers were present in the TM group⁽¹⁹⁾. It can be hypothesized that a higher load on these mixed aerobic/anaerobic type IIA muscle fibers⁽²³⁾ in the patient group, could have triggered mitochondrial proliferation to increase

oxidative capacity, explaining the observed difference in mitochondrial density. Since we could not distinguish fiber types on EM, we can however not confirm this hypothesis. It can also be hypothesized that the larger mitochondrial mean area and proportion is a compensatory mechanism for an impaired mitochondrial function. This potential compensatory mechanism was found in a study on mouse models for mitochondrial myopathy, where an increased mitochondrial volume density together with decreased respiratory chain enzyme activities was found in skeletal muscle tissue ⁽²⁴⁾. Besides, an increased mitochondrial number and size was also found in patients with mitochondrial disorders ⁽²⁵⁾.

The significantly higher amount of muscle fibers with COX deficient areas found in the patient group, compared to the healthy control group, contributes to this hypothesis. However, it must be noted that this was the only respiratory complex that showed significant differences so this hypothesis should be approached with caution. Cytochrome C oxidase is an enzyme in the mitochondrial respiratory chain which corresponds to complex IV and is essential for the reduction of molecular oxygen to water ⁽²⁶⁾. These COX deficient areas can correspond to areas with a loss of mitochondria, which is indicative for a structural myopathy.

In contrast to the results of this study, no significant differences in muscle fibers with COX deficient areas were found between patients with TM and healthy controls in the study of Larsson et al.(2000) ⁽²⁷⁾. In recent studies about patients with specific muscle diseases, muscle fibers with COX deficient areas, together with decreased activities of respiratory complexes were found ^(28, 29). In future studies it may be useful to investigate the mitochondrial respiratory enzyme activities by means of spectrophotometric enzyme analysis ⁽³⁰⁾. This allows to distinguish between true respiratory complex defects or a decreased mitochondrial content ⁽³¹⁾. However, this technique requires more muscle tissue than can be obtained by microbiopsy.

Ragged red fibers, which are a marker for a disturbed mitochondrial metabolism, were found in none of the groups. In the study of Kadi et al. (1998) and Lindman et al. (1991) ragged red fibers were rarely detected in women with work-related TM^(32, 33). In contrast, a significantly higher amount of ragged red fibers was found in cleaners with and without TM, when compared to healthy teachers⁽²⁷⁾. No significant differences were found between both groups for SDH (complex II of the respiratory chain) and NADH. No specific results were reported in previous studies regarding SDH since mostly combined COX/SDH stains were used^(27, 32). Regarding NADH, our results are in line with the findings of Larsson et al. (2004) where no significant differences were found between female cleaners with and without TM⁽³⁴⁾. Larsson et al. (2004) and Lindman et al. (1991) however, did find significant differences in NADH deficient fibers between a patient and control group with a different occupation^(33, 34). It can be hypothesized that changes in NADH are rather work-related instead of pain-related. Irregular muscle fibers usually have a segmental appearance and their occurrence is also dependent on biopsy location and size, which can also explain the variable results in different studies^(14, 27).

In this study also a significantly lower mean area of SS lipid droplets was found in the patient group, compared to the healthy control group. From a metabolic point of view, it is more beneficial to have more and smaller lipid droplets instead of fewer and larger droplets because of the greater surface area for proteins, responsible for lipid droplet turnover⁽²²⁾. It can be hypothesized that this is also a mechanism to compensate for a decreased mitochondrial function. Another possible explanation is that in the patient group a significantly lower amount of type I fibers was found⁽¹⁹⁾. Since lipid droplets are more abundantly present in type I fibers^(20, 35), it is possible that this difference in fiber type proportion is also reflected in lipid droplet density. Again, we were not able to distinguish between fiber types so this hypothesis cannot be confirmed based on this study.

The patient group of this study displayed a significantly higher proportion of lipid droplets touching the mitochondria. First, it can be hypothesized that due to the significantly larger mitochondria in the patient group, there is also a higher probability of the larger mitochondria to make contact with more lipid droplets ⁽²²⁾. On the other hand, it can also reflect a structural-functional relationship to optimize the oxidative metabolism and thus aerobic capacity. For example, endurance leads to a higher amount of lipid droplets in direct contact of mitochondria in response to endurance exercise ⁽²²⁾. Also, in obese or diabetic people, it is found that an inability to oxidize lipid droplets, can lead to an accumulation of lipid by-products which is reduced in case of a close proximity between lipid droplets and mitochondria ⁽²²⁾. It can be hypothesized that in the patient group, the lipid droplets, which are dynamic organelles, are being triggered to move towards the mitochondria in order to optimize lipid substrate utilization and thus energy production.

It can be hypothesized that the differences between both groups can be explained by a higher stress on the trapezius muscles in the patient group. Several studies have already shown that the EMG activity of the UT is significantly higher in a myalgia group, compared to a healthy control group ⁽³⁶⁻³⁸⁾. Besides this, several studies have already demonstrated that the capillarisation is significantly lower in the patient groups with TM, compared to healthy controls ^(33, 34). It is possible that office workers who develop TM, have a lower capillarisation by genetic predisposition, making them more vulnerable for the development of this condition. So another possible hypothesis is that an increased mitochondrial mean area and proportion, and a mobilization of the lipid droplets towards the mitochondria, are a consequence of the higher demands where the mitochondria are subjected to because of the muscle overload and restricted oxygen supply during office work.

Lastly, it has already been shown that mental stress plays an important role in the development and maintenance of neck and shoulder pain ⁽³⁹⁻⁴¹⁾. Since we did not monitor the mental stress level in both groups, it may be possible that the patient group experiences more mental stress than the control group, leading to significant morphological differences.

Strengths and limitations

A strength of this study is the use of electron microscopy as an investigation technique for the quantification of mitochondrial and lipid droplet morphology. Electron microscopy enables a direct and detailed imaging of lipid droplet and mitochondrial size, number and location ^(20, 21, 42, 43). Another strength is that muscle biopsy samples were taken from the dominant side in both the patient and control group, which excludes differences in muscle morphology due to hand preferences.

A first limitation of the present study is that no distinction was made in the location of mitochondria and lipid droplets regarding fiber types. Mitochondria and lipid droplets are usually more abundant in oxidative type I fibers, compared to type II fibers ^(20, 35). Since mitochondrial and lipid droplet content is dependent on the fiber type distribution of the muscle tissue ^(14, 44), it might be relevant to compare the distribution of mitochondria and lipid droplets between the patient and control group. It is also important to mention that the analysis of two-dimensional electron microscopic images does not reflect the fact that every visible mitochondrion is actually a fragment of the three-dimensional mitochondrion ⁽⁴⁵⁻⁴⁷⁾. A second limitation is that the enzymatic activity of the respiratory chain complexes was not investigated. However, the analysis of enzymatic activity requires a much higher amount of muscle tissue than obtained by means of microbiopsy, thus another, more invasive biopsy technique is then required. A third limitation is that no combined COX /SDH staining was

performed. A combined COX/SDH staining allows a clearer identification of fibers with a mosaic pattern of COX deficient and COX normal fibers, which is indicative for a mitochondrial DNA defect ⁽¹⁴⁾. A last limitation is the loss of muscle tissue due to freezing artefacts, which had a negative impact on our included sample size for the evaluation of irregular muscle fibers and may have negatively influenced the reliability and sensitivity of the present results.

Considerations for further research

In future studies more attention could be paid on the clinical presentation of the patients with TM. A more thorough examination of clinical features such as range of motion, strength and postural awareness could for example be correlated to the morphological outcome measures. It would also be of added value to monitor lifestyle factors such as sleep, nutrition, smoking, physical activity and stress and to investigate the influence of these factors on the development and maintenance of TM. It would be interesting to conduct longitudinal follow-up studies in which office workers without neck pain are followed over time and clinical and morphological features are monitored at several time points. In addition, the effectiveness of several treatment techniques such as dry needling ⁽⁴⁸⁾, manual compression techniques ⁽⁴⁸⁾ and exercise therapy ⁽⁴⁹⁻⁵¹⁾ could be studied by evaluating the effect on morphological features of the UT.

Conclusion

In this muscle biopsy study on female office workers with and without work-related TM, several morphological differences in mitochondria, lipid droplets and COX deficient fibers were found. Whether these differences indicate an improved aerobic capacity or reflect a

compensatory mechanism for a decreased mitochondrial function, which may be associated with TM, remains elusive and requires further research.

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Figure legends

Figure 1 Subsarcolemmal and intermyofibrillar mitochondria (12000 x magnification) in a healthy control

White asterisks indicate subsarcolemmal mitochondria, black asterisks indicate intermyofibrillar mitochondria

Figure 2 Subsarcolemmal and intermyofibrillar lipid droplets (3000 x magnification) in a patient

White asterisks indicate subsarcolemmal lipid droplets, black asterisks indicate intermyofibrillar lipid droplets

Figure 3 Enzymohistochemical staining for COX of muscle fibers in the upper trapezius muscle

A: muscle tissue of a healthy control, B: muscle tissue of a patient

Figure 4 Enzymohistochemical staining for NADH of muscle fibers in the upper trapezius muscle

A: muscle tissue of a healthy control, B: muscle tissue of a patient

Figure 5 Enzymohistochemical staining for SDH of muscle fibers in the upper trapezius muscle

A: muscle tissue of a healthy control, B: muscle tissue of a patient

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1 Demographic data of the patient group and healthy control group

	Patient group (n=16)		Control group (n=15)		P-value [§]
	Mean/median or frequencies	SD or IQR	Mean/median or frequencies	SD or IQR	
Age [*]	29.87	5.98	27	9	0.052
BMI (kg/m ²) [*]	23.10	2.80	21.93	1.95	0.111
NDI (0-50) [*]	9.33	5	2.33	2.06	P < 0.001
NRS (0-10) [†]	5	4	NA	NA	NA
Duration neck pain (months) [†]	12	25	NA	NA	NA
Hours computer work per week (21-30 h/31-40 h/> 40h) [‡]	3/9/4		5/8/2		0.685
Upper limb sports (yes/no) [‡]	5/11		3/12		0.474

n: number of subjects, SD: standard deviation, IQR: interquartile range, yr: years, BMI: body mass index, NDI: Neck Disability Index, NRS: Numeric Rating Scale, h: hours, NA: not applicable

^{*}Values are mean ± standard deviation, [†] Values are median (IQR), [‡] Values are frequencies,

[§] P-values for continuous data were calculated using unpaired T-tests in case of normal

distribution (BMI and NDI) or Mann-Whitney U Test in case of non-normal distribution (age), P-values for categorical data were calculated using Chi squared tests (hours computer work per week and upper limb sports)

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Table 2 Descriptive statistics for mitochondrial morphology, lipid droplets and irregular fibers

	Mean	SD	95%CI	Mean	SD	95%CI
Mitochondrial morphology	Patient group (n = 16)			Control group (n=15)		
SS mean mitochondrial area (μm^2)	0.076	0.018	0.066 to 0.085	0.061	0.025	0.047 to 0.075
SS proportion mitochondrial area (%)	14.323	4.882	11.721 to 16.925	10.379	7.089	6.454 to 14.305
SS # mitochondria/total area ($\#/\mu\text{m}^2$)	1.981	0.740	1.587 to 2.376	1.578	0.986	1.032 to 2.124
IMF mean mitochondrial area (μm^2)	0.062	0.001	0.057 to 0.067	0.055	0.014	0.048 to 0.063
IMF proportion mitochondrial area (%)	3.900	0.759	3.496 to 4.304	3.467	0.833	3.006 to 3.929
IMF # mitochondria/total area ($\#/\mu\text{m}^2$)	0.631	0.069	0.594 to 0.667	0.616	0.114	0.553 to 0.679
Lipid droplets	Patient group (n = 16)			Control group (n=15)		
SS proportion	80.046	15.44	71.817 to	70.911	21.344	59.091 to

lipid droplets touching mitochondria (%)		3	88.275			82.730
SS mean lipid droplet area (μm^2)	0.244	0.106	0.188 to 0.301	0.368	0.137	0.292 to 0.444
SS proportion lipid droplet area (%)	4.075	2.086	2.964 to 5.186	4.758	2.198	3.541 to 5.975
SS # lipid droplets/total area ($\#/\mu\text{m}^2$)	0.168	0.071	0.130 to 0.205	0.145	0.083	0.099 to 0.190
IMF lipid droplets touching mitochondria (%)	78.396	8.054	74.104 to 82.687	69.050	12.961	61.872 to 76.228
IMF mean lipid droplet area (μm^2)	0.221	0.041	0.199 to 0.243	0.223	0.069	0.184 to 0.261
IMF proportion lipid droplet area (%)	0.502	0.310	0.337 to 0.667	0.555	0.296	0.392 to 0.719
IMF # lipid droplet/total area ($\#/\mu\text{m}^2$)	0.021	0.013	0.015 to 0.028	0.024	0.012	0.017 to 0.031
	Median	IQR	Min-max	Median	IQR	Min-max
Irregular fibers	Patient group (n = 10)			Control group (n=12)		
Fibers with	13.905	4.580	5.190 -	7.691	6.510	4.750 -

irregular COX staining (%)			26.250			22.030
Irregular fibers	Patient group (n = 11)			Control group (n=11)		
Fibers with irregular NADH staining (%)	6.601	14.210	0.740 - 23.200	6.794	5.140	3.270 - 11.920
Irregular fibers	Patient group (n = 11)			Control group (n=11)		
Fibers with irregular SDH staining (%)	6.404	7.940	0.000 - 11.530	2.083	5.550	0.260-12.960

n: number of subjects, SD: standard deviation, 95% CI: 95% confidence interval, IQR: interquartile range, SS: subsarcolemmal, IMF: intermyofibrillar, COX: cytochrome c oxidase, NADH: nicotinamide adenine dinucleotide, SDH: succinate dehydrogenase

TABLE 3 Results from multiple linear regression analysis: mitochondrial morphology, lipid droplet and irregular fibers

Mitochondrial morphology	F	Slope (95 % CI)	Adjusted R ²	P-value of model	P-value for group*
SS mean mitochondrial area (μm ²)	1.729	0.021 (-0.030 to 0.039)	0.089	0.174	0.023
SS proportion mitochondrial area (%)	2.595	3.894 (-0.842 to 8.630)	0.175	0.060	0.103
SS # mitochondria/total area (#/μm ²)	1.774	0.344 (-0.350 to 1.038)	0.094	0.164	0.318
IMF mean mitochondrial area (μm ²)	1.791	0.009 (-0.000 to 0.019)	0.095	0.161	0.051
IMF proportion mitochondrial area (%)	2.352	0.700 (0.078 to 1.321)	0.153	0.080	0.029
IMF # mitochondria/ total area (#/μm ²)	0.445	0.025 (-0.054 to 0.105)	-0.080	0.775	0.521
Lipid droplets	F	Slope	Adjusted R ²	P-value of model	P-value for group*
SS proportion lipid droplets touching mitochondria (%)	0.867	8.754 (-7.009 to 24.517)	-0.018	0.497	0.264
SS mean lipid droplet area	2.020	-0.135 (-0.240 to -	0.120	0.121	0.015

(μm^2)		0.029)			
SS proportion lipid droplet area (%)	1.305	-0.419 (-2.157 to 1.319)	0.039	0.294	0.624
SS # lipid droplet /total area ($\#/\mu\text{m}^2$)	1.852	0.034 (-0.026 to 0.094)	0.102	0.149	0.260
IMF lipid droplet touching mitochondria (%)	2.280	9.620 (0.752 to 18.493)	0.146	0.088	0.035
IMF mean lipid droplet area (μm^2)	1.077	-0.008 (-0.054 to 0.038)	0.010	0.388	0.723
IMF proportion lipid droplet area (%)	1.669	-0.010 (-0.248 to 0.228)	0.082	0.187	0.933
IMF # lipid droplet /total area ($\#/\mu\text{m}^2$)	2.240	0.000 (-0.009 to 0.009)	0.142	0.092	0.984
Irregular fibers	F	Slope	Adjusted R^2	P-value of model	P-value for group*
Fibers with irregular COX staining (%)	1.696	5.861 (0.652 to 11.070)	0.117	0.197	0.030
Fibers with irregular NADH staining (%)	1.796	3.998 (-1.305 to 9.300)	0.132	0.176	0.130
Fibers with irregular SDH staining (%)	0.629	1.909 (-2.057 to 5.874)	-0.076	0.649	0.324

95% CI: 95% confidence interval, SD: standard deviation, 95% CI: 95% confidence interval, SS: subsarcolemmal, IMF: intermyofibrillar, COX: cytochrome c oxidase, NADH: nicotinamide adenine dinucleotide, SDH: succinate dehydrogenase

Statistical analyses were performed using multiple linear regression analysis. Age, hours of computer work/week and upper limb sports were included as covariates.

* Statistical significance was accepted when $P < 0.05$

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