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Brief Research Report

Effects of Two Different Specific Neck Exercise Interventions on Palmitoylethanolamide and Stearoylethanolamide Concentrations in the Interstitium of the Trapezius Muscle in Women with Chronic Neck Shoulder Pain

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Abstract

Purpose. Chronic neck/shoulder pain (CNSP) is one of the most common pain conditions. The understanding of mechanisms, including the peripheral balance between nociceptive and antinociceptive processes, is incomplete. *N*-acylethanolamines (NAEs) are a class of endogenous compounds that regulate inflammation and pain. The aim of this study was to investigate the levels of two NAEs: the peroxisome proliferator-activated receptor type-α ligand palmitoylethanolamide (PEA) and stearoylethanolamide (SEA) in the muscle interstitium of the trapezius muscle in women with CNSP randomized to two different neck specific training programs and in a healthy pain-free control group (CON).

Materials and Methods. Fifty-seven women with CNSP were randomized to strength + stretch or stretch alone exercise programs. Twenty-nine subjects underwent microdialysis procedure before and after 4–6 months of exercise. Twenty-four CON subjects underwent microdialysis procedure before and after 4–6 months without any intervention in between. Microdialysate samples were collected from the trapezius muscle and analyzed by mass spectrometry for PEA and SEA levels.

Results. PEA and SEA levels were significantly higher in CNSP patients compared with CON. PEA was significantly higher in CNSP than in CON after both training programs. SEA was significantly higher in CNSP than in CON after stretch alone but not after strength + stretch training. A significant positive correlation was found between changes in pain intensity and in SEA levels in the strength + stretch group, but not in the stretch alone group.

Conclusion. Our results indicate that exercise interventions differentially affect the levels of the bioactive lipids PEA and SEA in the interstitium of the trapezius muscle in women with CNSP.

Key Words. *N*-Acylethanolamines; Palmitoylethanolamide; Stearoylethanolamide; Exercise; Chronic Muscle Pain; Microdialysis

Introduction

Chronic neck/shoulder pain (CNSP) is one of the most common pain conditions. There is good evidence that female gender, older age, high job demands, low social/work support, being an ex-smoker, as well as a history of low back and neck disorders, are risk factors for onset of nonspecific neck pain [1]. Repetitive work tasks, forceful exertions (with hand/wrists), and awkward postures have also been reported as risk factors [2]. Thus, the literature concerning risk factors suggest a multifactorial aetiology with both non-modifiable and modifiable factors [3]. Physical activity is considered as a protective modifiable factor [3]. However, evidence of therapeutic efficacy for many of the standard exercise treatments used in clinical practice in this patient group is lacking. Randomized controlled trials have demonstrated that specific strength training of neck/shoulder muscles, in contrast to stretching or general fitness training, decreases pain and increases range of movement and muscle strength significantly, in women with CNSP [4,5]. The understanding of the mechanisms behind chronic muscle pain and the potential molecular changes in muscle resulting from different exercise programs are incomplete. The trapezius muscle has been used as a model in several studies investigating peripheral alterations in CNSP by a microdialysis technique [6–10]. Most studies have investigated metabolites and algesic substances and in a recent systematic review serotonin, glutamate, pyruvate, and lactate were identified as potential biomarkers of chronic myalgia [11]. As pointed out in [11], very little is known about pain-inhibitory substances in CNSP.

N-acylethanolamines (NAEs) are a family of endogenous lipid mediators that have a diversity of actions including the regulation of inflammation and pain [12]. The two most well-characterized of these are the endogenous cannabinoid receptor ligand arachidonoylethanolamide (anandamide, AEA) [13,14] and the peroxisome proliferator-activated receptor type- ligand palmitoylethanolamide (PEA) [15,16]. Although the exact mechanisms involved needs to be further investigated, endogenous PEA has been suggested to have a protective function and a key role in maintaining cellular homeostasis in response to different stressful events such as tissue trauma and inflammation [17,18]. Results from clinical trials suggest that exogenous administration of PEA decreases pain intensity in patients with different pain conditions such as temporomanibular joint osteoarthritis [19], carpal tunnel syndrome [20], as well as diabetic neuropathy, herpes zoster infection, failed back surgery syndrome, and osteoarthritis [21].

To date, little is known as to the potential role of NAEs in human chronic muscle pain. In an initial small study, we investigated the levels of NAEs by microdialysis in of the trapezius muscle in women with CNSP and in healthy controls [22]. During the microdialysis session, subjects conducted a 20-minute repetitive low-force exercise, which induces pain. Higher levels of PEA and stearoylethanolamide (SEA) were seen in CNSP compared with controls before, during, and after the brief exercise. These results were recently confirmed in a larger study [23]. The effects of longer exercise interventions—over several weeks or months—upon PEA and SEA levels in CNSP are not known.

Thus, the aim of this study was to investigate the levels of PEA and SEA in the interstitium of the trapezius muscle in women with CNSP randomized to two different types of exercise programs. The programs comprised of neck-specific stretch alone or stretch together with strength training, based on a trial conducted by Ylinen et al. [4]. Microdialysis was conducted before and after 4–6 months of training. The levels of PEA and SEA were also investigated in a healthy pain-free control group (CON) who did not receive any intervention.

Materials and Methods

Study Design and Recruitment

This study was conducted as part of a randomized controlled trial undertaken with women with CNSP. The overall aim of the trial was to evaluate two home exercise concepts comprising 1 year of strength exercises or stretching exercises, with pain intensity and function as primary outcomes, in women with chronic neck pain who were recruited from a general population. The trial was performed in the county of Östergötland, Sweden, between September 2009 and February 2011 [24]. All participants in the trial were recruited through advertisements in the local newspapers. Respondents to the advertisements were informed about the study and interviewed by phone to preliminary check inclusion and exclusion criteria. Detailed information about the study and a questionnaire about present pain and function due to neck pain were sent to the respondents. A clinical examination of the neck and shoulders preceded inclusion to the study. Participants were included in a consecutive manner. All subjects were randomized to strength + stretch (denoted STRENGTH) or stretch alone (denoted STRETCH) exercise interventions (see below for details). The group start-up sequence (either STRENGTH or STRETCH) was randomly selected beforehand using Minitab v. 15 (Minitab Pty. Ltd., Sydney, NSW, Australia). In each group, subjects were randomly selected a priori (Minitab v. 15) to undergo the microdialysis procedure. Healthy pain free subjects (CON) also undertook the microdialysis procedure before and after 4–6 months, without any intervention in between. This article reports pain intensity, PEA, and SEA levels in CNSP subjects (and controls) who undertook the microdialysis procedure before (denoted pretreatment MD) and after (denoted posttreatment MD) 4–6 months of training.

A detailed report of the sample size calculation (Power and Sample Size Calculations [v. 3.0.43, http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize]) for the large trial has been reported elsewhere [24] and in consequence only a brief summary is given here. Estimation of sample size for analyzing changes in pain intensity (one of

the primary outcomes) *within the groups* revealed a sample size of 20 pairs of subjects with pairs of data (power: 0.80, probability: < 0.05 [two tailed], mean improvement of pain intensity on a numeric rating scale [NRS]: 2, and standard deviation of mean difference: 3). The sample size estimation *between the groups* resulted in 36 subjects in each group (power: 0.80, probability: < 0.05 [two tailed], mean improvement on the NRS: 2, and standard deviation of mean difference: 3).

Based on data presented in our earlier study [23] we also have estimated the sample sizes based on SEA and PEA. Estimation of sample size for analyzing changes in SEA or PEA within the groups revealed a sample size of seven subjects with pairs of data (before and after) (power: 0.80, probability: < 0.05 [two tailed], mean change in SEA or PEA: 2, and standard deviation: 1.5). The sample size estimation between the groups resulted in 10 subjects in each group (power: 0.80, probability: < 0.05 [two tailed], mean difference: 2, and standard deviation: 1.5).

Subjects

Women with CNSP

Inclusion criteria were female sex, age range 20–65 years, and pain in the neck/shoulder area that had lasted more than 6 months. Exclusion criteria were widespread pain such as fibromyalgia, upper extremity bursitis, tendonitis, capsulitis, postoperative conditions in the neck/shoulder area, prior neck trauma, disorder of the spine, neurological disease, rheumatoid arthritis or any other systemic diseases, metabolic disease, malignancy, severe psychiatric illness, pregnancy, and difficulties understanding the Swedish language. The results from the first microdialysis session in four of the subjects have been reported previously [22].

Pain-Free Subjects

Inclusion criteria were female sex, age range 20–65 years and pain-free. The exclusion criteria were as above, and additionally pain that had lasted more than 7 days during the past 12 months. The results from the first microdialysis session in 11 of the subjects have been reported previously [22].

Ethical Considerations

After receiving verbal and written information about the study, all subjects signed a consent form that was in accordance with the Declaration of Helsinki. The study was granted ethical clearance by the Linköping University Ethics Committee (Dnr: M10-08).

The study qualified for registration in ClinicalTrials.gov Id: NCT01876680.

Methods

Self-Reported Pain Questionnaire

The Nordic Ministry Council Questionnaire (NMCQ) was used in order to ascertain that inclusion criteria were fulfilled [25]. This is a well-established self-reported pain questionnaire allowing assessment of low back, neck, shoulder, and general complaints. It includes symptoms (ache, pain, and discomfort) arising from nine well-defined anatomical regions over the past 12 months and during the previous 7 days.

Standardized Clinical Neck and Shoulder Examination

In order to confirm eligibility for inclusion in the study, all participants were examined by a standardized and validated clinical examination according to Ohlsson et al. [26]. The examination includes questions on pain, tiredness, and stiffness on the day of examination, as well as physical tests including range of motion and tightness of muscles, pain threshold and sensitivity, muscle strength and palpation of tender points. Patients with trapezius myalgia that were included in the study had neck pain at the examination day, tightness of the trapezius muscle, i.e., a feeling of stiffness in the descending region of the trapezius muscle reported by the subject at examination of lateral flexion of the head, and palpable tender parts in the trapezius muscle. For inclusion the range of motion of the cervical columna had to be normal or only slightly decreased.

The Two Exercise Interventions

The exercise programs are described in detail elsewhere [24]. In summary, the training session in the strength + stretch training program (denoted STRENGTH) started with specific strength training with focus on the neck and shoulder muscles. The strength training included arm abduction, upright row, biceps curls, reverse flyes, flyes and pullovers. Dumbbells were used in all above-mentioned exercises. Lifting the head up from supine position was also incorporated into the training program. Stretching exercises for the neck, that is retraction of the neck, shoulder and upper limb muscles, i.e., stretching of m. trapezius upper and middle portion ended each training session in the STRENGTH program and constituted the complete training session for the stretch alone training program (denoted STRETCH). Stretching exercises for both groups also included m. sternocleidomastoideus, m. rhomboids, m. pectoralis major and the flexors and extensors of the wrist.

Both groups were asked to undertake the training session three times a week and additionally they were encouraged to perform optional aerobic exercise for 30 minutes with the same frequency. The STRENGTH group was instructed to give priority to the specific strength exercises if they did not manage to perform the complete session.

Training support was provided by skilled physiotherapists by phone or e-mail every 4–8 weeks. The support was more frequent in the beginning of the training period.

Completers

We defined a *completer* of any of the two exercise programs as a participant who reported at least 8 unbroken weeks of the specific exercise with a frequency of at least 1.5 times weekly preceding the outcome measurement time point (4–6 months).

Pain Intensity

Pain intensity ratings refer to the ratings by the participants during each microdialysis procedure. Subjects were asked to rate pain intensity on a numeric rating scale (NRS) with numbers (0–10; 0 = no pain and 10 = worst possible pain) provided for guidance. All pain ratings concerned pain intensity in the trapezius muscle for the most painful side in CNSP (generally the dominant side) and the dominant side in CON. A change of at least two points between the two microdialysis sessions with 4–6 months interval on the NRS was considered as clinically relevant.

Microdialysis Procedure

Microdialysis (MD) was performed as previously described in detail [27]. The participants were asked not to take nonsteroidal anti-inflammatory drugs for the last 7 days and/or paracetamol medication for the last 12 hours before the experiment. In addition, they were asked not to consume coffee, tea, cigarettes or nicotine agents for the last 8 hours before the experiment. They were also instructed not to perform any shoulder- or neck-straining exercises for the last 48 hours before the study, except for ordinary daily work and/or leisure activities. The participants reported to the laboratory in the morning. The examiner went through a checklist in order to assess whether or not participants had followed the instructions, and their current health status as well as current medication was confirmed. The most painful side in CNSP (generally the dominant side and the same at both microdialysis sessions) and the dominant side in CON of the trapezius muscle were used for the microdialysis procedures. To guide the placement of catheters, ultrasonographic measurements of the trapezius muscle area were conducted. The B-scan application was used measuring skin/fat/muscle thickness of the trapezius muscle area with a 7 MHz linear probe. Tissue thickness was determined as the distance between the skin surface and the initial hypo-echoic structure (skin), the distance within the hypo-echoic structure (fat) and the distance between the fascias (muscle). The skin and the subcutaneous tissues above the area where the catheter entered the trapezius muscle were anesthetized with a local injection (0.5 mL) of Xylocaine (20 mg/mL) without adrenaline, and care was taken not to anesthetize the underlying muscle. Two commercially available microdialysis catheters (cut-off points of 20 and 100 kDa; CMA Microdialysis AB, Solna) were inserted with a catheter into the pars descendens of the trapezius muscle at half the distance between the processus spinosus of seventh cervical spine and the lateral end of the acromion. Typically, a brief involuntary contraction and change of resistance were perceived when the tip of the insertion needle of the catheter entered the fascia and muscle. The catheters were placed in the trapezius muscle parallel to the muscle fibers and perfused at 5 l/min with a solution (perfusate) resembling the muscle interstitial fluid. The perfusion fluid consisted of Ringer acetate solution containing 3 mM glucose and 0.5 mM lactate and 0.3 l/mL [^{14}C]-lactate (specific activity: 5.77 GBq/mmol; GE Healthcare, Uppsala, Sweden) as well as 0.3 l/mL $^{3}H_2O$ (specific activity: 37 MBq/gram) were added to the perfusate used in the MD catheters. After the insertion of catheters, participants rested comfortably in an armchair for a 120 minutes period to allow the tissue to recover from possible changes in the interstitial environment induced by the operative procedure. After this period, participants continued to rest for a 20-minute period ("baseline"). This was followed by a 20-minute period of standardized repetitive low-force exercise performed on a peg-board (PEG, described below). The experiment ended with a recovery period of 120 minutes during which participants rested. Immediately prior and after catheter insertion subjects were asked to rate their pain intensity. They continued to do so every 20 minutes throughout the experiment that lasted 4 hours. At 20 minutes (i.e., beginning of trauma period), 120 minutes (i.e., after recovery from the operative procedure), 140 minutes (i.e., baseline), 160 minutes (i.e.,; low-force exercise, PEG), 180, 200, and 220 minutes (i.e., recovery period) after the start of the experiment, microdialysate was collected in glass vials (CMA Microdialysis, Sweden). Each vial was weighed before and immediately after sampling in order to confirm that sampling was working according to the perfusion rate set. All samples were controlled for visible signs of hemolysis that would result in the discarding of the sample. The samples were stored at 4°C throughout the experiment and then stored as aliquots 70°C until analysis.

Participants had a standardized light meal at the 100 minutes time point of the microdialysis session. No food or beverages except for water were otherwise allowed during the experiment.

In our previous study, we found no significant variations of the microdialysis concentrations in CNSP and healthy controls between the different time points [22]. Thus, samples from the 20 kDa catheter at 140 minutes (baseline) were used for the NAE analysis in the present study.

Standardized Low-Force Repetitive Exercise (PEG)

PEG consisted of a repetitive arm movement task that was performed unilaterally using the arm on the same side as the microdialysis catheter had been inserted in the trapezius. The subjects moved short wooden sticks (11.8 g) back and forth between standardized positions 30 cm apart on a pegboard at a frequency of 1.3 Hz for 20 minutes, the frequency being given by an electronic

metronome (Korg Inc., Tokyo, Japan). The participants performed the exercise in a seated position with the pegboard placed 30 cm in front of them, measured from the elbow with the upper arm hanging vertically and the elbow in a 90° flexion. The exercise was supervised by qualified personnel (research nurse or physiotherapist).

Analysis of NAE Levels

Analysis of NAE levels was performed as described previously [22] with slight modifications. On the day of analysis, 50 l samples were dried by SpeedVacc, redissolved in methanol, vortexed and centrifuged. The supernatants were separated by nano-flow HPLC system (EASY-nLC fromBruker Daltonics, Bremen, Germany) and data were acquired using an HCTultra PTM Discovery System on-line electrospray ionization ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). The EASY-nLC was equipped with column 5 m C18 (Nano Separations, 360 m × 75 m i.d., 10 cm). The compounds were eluted isocratically with 75% acetonitrile in water with 0.1% formic acid at a flow rate of 0.0006 mL/min. The mass spectrometer was operated in the positive ion multiple reaction monitoring mode and the selected ion pairs used were 300.2/283.1 and 328.3/311.1 m/z for PEA and SEA, respectively.

Statistics

The GraphPad Prism computer programme (GraphPad Software Inc, San Diego, CA, USA) and IBM SPSS (version 20.0; IBM Corporation, Route 100 Somers, New York, USA) were used for the statistical analyses. In text are reported median values with min and max values in brackets. Wilcoxon's signed rank test and Kruskal–Wallis test (Dunn's multiple comparison test when approriate) were used when comparing two or more groups of subjects with respect to age, anthropometric data, PEA, SEA, and pain intensity. Mann–Whitney *U*-test was used when testing changes in PEA, SEA and pain intensity variables within a group of subjects. Spearman's rank order correlation test was used when correlating pain intensity changes with changes in SEA and PEA. in order to determine significance levels in non-parametric tests (Kruskal–Wallis test, Dunn's multiple comparison test, Mann–Whitney *U*-test, Wilcoxon's signed rank test and Spearman's rank order correlations, as appropriate). Results are reported in the text as median values with min and max values in brackets. $P < 0.05$ was considered significant in all statistical tests.

Results

Subjects

Women with CNSP

Fifty-seven women with pain in neck/shoulder area comprised the CNSP group in the larger trial (Figure 1). As stated in the method section, in the initial phase of the study subjects randomized to the different training regimes were also randomized to the MD procedure. However, due to difficulties in recruitment, a decision was made to perform MD on all subjects once they were included. Hence, 12 out of 34 subjects in STRENGTH and 4 out of 23 subjects in STRETCH did not perform MD due to the initial MD randomization (Figure 1). In total, twenty nine subjects conducted both pre- and posttreatment MD (18 in the STRENGTH and 11 in the STRETCH group). Medians (min-max) of age and body mass index (BMI) noted in the records (N = 29) were 45 (29–60) years and 24 (20–42) kg/m^2, respectively. In total, 24 / 29 Twenty four out of 29 subjects were completers in which samples were analyzed from both MD sessions. For the non-completers (N = 5) medians (min-max) of age and body mass index (BMI) were 43 (31–49) years and 24 (23–29) kg/m^2, respectively.

Dropouts Between Pre- and Posttreatment Microdialysis

In the STRENGTH group 3 subjects left the study (one subject due to pregnancy and two subjects for unknown reasons).

In the STRETCH group 5 subjects left the study. Discomfort from the MD procedure (N = 1), knee pain (N = 1), and starting with anticoagulant therapy (N = 1) were reported as reasons for leaving. Two subjects left the study for unknown reasons.

Pain-Free Subjects

Twenty-four pain-free women comprised the healthy control group (CON). Medians (min-max) of age and BMI were 44 (27–56) years and 23 (20–31) kg/m^2, respectively.

Age and BMI of the Two Groups of Subjects

Data concerning age and BMI are given in the section of subjects above; there were no significant differences in age or BMI between CNSP and CON ($P = 0.31$ and $P = 0.40$, respectively).

Pain Intensity in CON

Median pain intensities in CON were zero at all time points during both first and second MD sessions. The ranges (min-max) in pain intensities for CON were: 0 minute: 0–2 vs 0–1, 140 minutes: 0–1 vs 0–1, 160 minutes: 0–5 vs 0–3, 180 minutes: 0–1 vs 0–1, 200 minutes: 0–1 vs 0–0, and 220 minutes: 0–1 vs 0–1.

Pain Intensity Ratings in CNSP Randomized to STRENGTH

The pain intensity throughout the MD sessions before and after 4–6 months exercise in subjects who were completers (N = 13) are shown in Figure 2A. Pain intensity increased significantly between 140 and 160 minutes time points at the pretreatment but not posttreatment MD session ($P < 0.01$ vs $P > 0.06$). The median values were

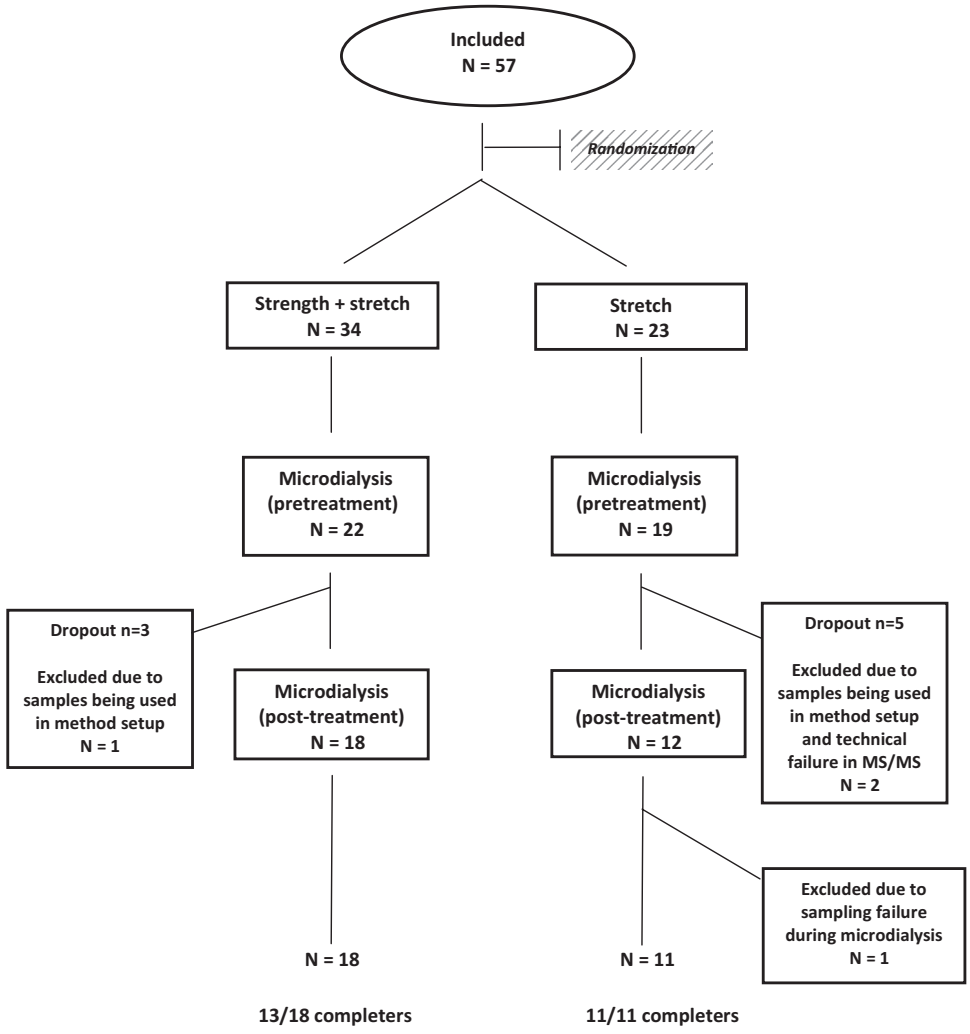

Figure 1 Flow chart of the recruitment and study process of women with chronic neck /shoulder pain (CNSP). * In the initial phase of the study subjects were randomized to the microdialysis procedure. However, due to difficulties in recruitment, a decision was made to perform microdialysis on all subjects once they were included. Hence, 12 out of 34 subjects in STRENGTH and 4 out of 23 subjects in the STRETCH did not perform microdialysis. Eighteen subjects in STRENGTH and 11 subjects in STRETCH performed pre-and post microdialysis in which samples were analyzed. All 11 subjects in STRETCH and 13 out of 18 subjects in STRENGTH were completers (i.e., at least eight unbroken weeks of the specific exercise with a frequency of at least 1.5 times weekly preceding the outcome measurement time point).

lower after the intervention than before (Figure 2A), but the differences only reached statistical significance at the 160 minutes time point ($P < 0.05$) at the posttreatment compared with the pretreatment MD session.

When the data for the STRENGTH intervention were analyzed in terms of a clinically relevant decrease in pain (ΔNRS ≥2), 4/13 patients reached this level at the 0 and 140 minutes; 8/13 at 160 minutes and 5/13 at 180, 200, and 220 minutes time points.

For the non-completers (N = 5) of STRENGTH, medians (min-max) of pain intensities at pretreatment MD were 140 minutes: 3 (3–7) and 160 minutes: 4 (3–8). Corresponding figures at the second MD session were 140 minutes: 2 (1–5) and 160 minutes: 3 (2–8).

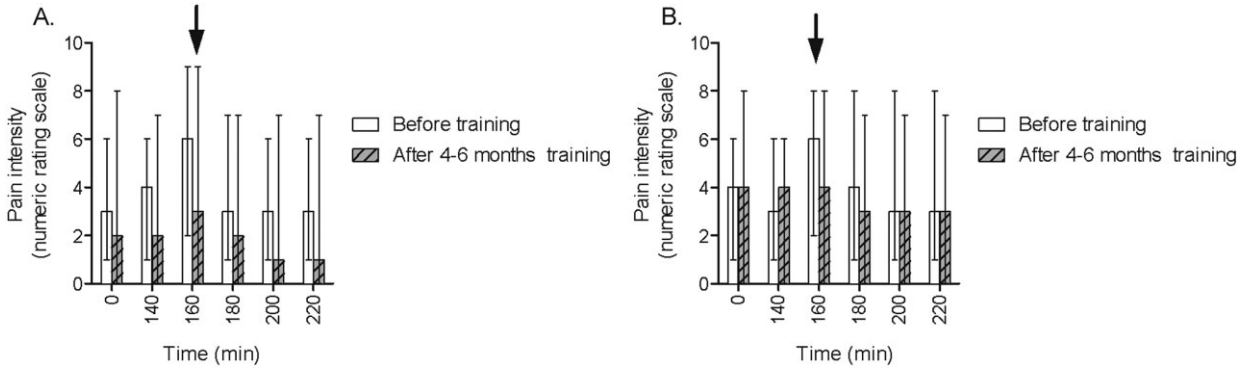

Figure 2 Pain intensity ratings (median (min-max)) during microdialysis (MD) procedures in women with chronic neck shoulder myalgia (CNSP) randomized to either of two training programmes: A: strength + stretch (STRENGTH, N = 13) or B: stretch alone (STRETCH, N = 11). The x-axes show the times after catheter insertion. The 140 minutes time point which was after a 120-minute acclimation period served as baseline. The arrow at 160 minutes denotes pain intensity immediately after a 20-minute repetitive low force exercise (PEG).

Pain Intensity Ratings in CNSP Randomized to STRETCH

The pain ratings during the MD sessions before and after 4–6 months training in subjects who were completers in the STRETCH group (N = 11) are shown in Figure 2B. Pain intensity increased significantly between 140 and 160 minutes time points at both MD sessions ($P < 0.005$ vs $P < 0.05$). The effect of the STRETCH intervention upon the median values was less marked than for the STRENGTH intervention (Figure 2B). No significant differences were found between the two MD sessions ($P > 0.2$ at all time points).

The pain intensities in the STRETCH group were unchanged or increased (ΔNRS ≥2) in 9/11, 10/11, 6/11, 9/11, 10/11, and 10/11 patients at 0, 140, 160, 180, 200 and 220 minutes time points, respectively, when comparing post- and pretreatment MD session. The corresponding values for the STRENGTH were 9/13, 9/13, 5/13, and 8/13, 8/13, 8/13, respectively.

SEA and PEA Levels in CON

PEA and SEA levels were measured in the MD samples at the baseline (i.e., 140 minutes time point) during two separate MD sessions with 4–6 months between the two sessions.

There was no significant difference in SEA levels in the 24 subjects of CON between the two MD sessions; 0.6 nM (0–2.70) vs 0.65 nM (0.10–4.20).

The level of PEA was significantly lower at the second MD session; first MD median [min-max]): 0.65 nM (0–3.70) vs second MD 0.15 nM (0.10–2.50); $P < 0.05$.

SEA And PEA Levels in All CNSP Subjects Compared with CON

The levels of SEA were significantly higher for CNSP (N = 29) than for CON (N = 24): pretreatment MD session: 2.0 nM (0.1–8.0) vs 0.6 nM (0.0–2.7), $P < 0.0001$; posttreatment second MD session: 3.7 nM (0.1–19.8) vs 0.65 nM (0.1–4.2), $P < 0.001$. The difference between the posttreatment MD session and the pretreatment treatment MD session (SEAdiff) was calculated but no significant difference was found between CON and CNSP: 0.0 nM vs 1.57; $P = 0.231$).

A similar result was seen for PEA; pretreatment MD session: 1.4 nM (0.1–8.6) vs 0.65 nM (0–3.7), $P < 0.05$; posttreatment MD session: 2.4 nM (0.1–27.6) vs 0.15 nM (0.1–2.5), $P < 0.0001$. The difference between the posttreatment MD session and the pretreatment treatment MD session (PEAdiff) was calculated but no significant difference existed between CON and CNSP (0.25 nM vs 0.51 nM; $P = 0.214$).

SEA Levels in CNSP Completers

Eighteen subjects comprised the STRENGTH intervention group, of whom 13 subjects were completers (Figure 1). Eleven subjects comprised and completed the STRETCH intervention (Figure 1).

For the completers of the STRETCH group, the median SEA level after the exercise regime was significantly increased ($P < 0.005$). In contrast the median SEA level for the completers of the STRENGTH group was not significantly changed by the exercise regimen ($P > 0.9$).

At the pretreatment MD session, the median values for CON, and the completers of STRETCH and STRENGTH

groups were 0.6, 1.1 and 2.5 nM, respectively. Only the completers of the STRENGTH group was significantly different from CON ($P < 0.001$). At the posttreatment MD session, the corresponding median values were 0.65, 5.0 and 2.4 nM, respectively. In this case, the median values for the completers of the STRETCH group ($P < 0.001$), but not the completers of the STRENGTH group was significantly different from CON. For SEAdiff was found a significant difference between the completers of the STRETCH and the STRENGTH interventions (4.1 nM vs 0.20 nM; $P = 0.05$).

For the non-completers (N = 5) of STRENGTH medians (min-max) of SEA levels at pretreatment MD and at the second MD session were 1.1 (1.0–2.4) and 0.5 (0.1–10.8) nM, respectively.

PEA Levels in CNSP Completers

In the STRETCH group ($P = 0.041$) but not in the STRENGTH group ($P = 0.88$) were seen a significant difference in PEA levels between the two MD sessions; the PEA level was significantly higher at the posttreatment MD session in STRETCH.

In view of the difference in PEA levels between the two MD sessions in CON, it is difficult to be able to make firm conclusions concerning the effects of the interventions upon PEA levels. However, comparisons between different interventions can be made at a given time point. At the pretreatment MD session, the median values for CON, STRETCH and STRENGTH groups were 0.65, 0.4 and 2.0 nM, respectively; the individual variations in PEA levels are given in Figure 3A and C. Only the STRENGTH group was significantly different from CON ($P < 0.05$). At the posttreatment second MD session, the corresponding median values were 0.15, 3.3 and 2.0 nM, respectively. In this case, the median values for both intervention groups were significantly different from CON ($P < 0.001$ and $P < 0.011$ for STRETCH and STRENGTH groups, respectively), but the two exercise groups did not differ significantly from each other. For PEAdiff no significant difference existed between the completers of the STRETCH and the STRENGTH interventions (2.7 nM vs 0.15 nM; $P = 0.104$).

For the non-completers (N = 5) of STRENGTH medians (min-max) of PEA levels at pretreatment MD and at the second MD session were 2.5 (1.4–2.7) and 0.1 (0.1–9.0) nM, respectively.

Changes in SEA and PEA Levels vs Pain Intensity Increase During PEG at the Second MD

The data above allow investigation into whether there was any relation between the change in SEA or PEA levels (the differences between the posttreatment MD session and the pretreatment MD session, SEA_{diff} and PEA_{diff}) and the increase in pain intensity as a consequence of PEG at the posttreatment MD session (i.e., the difference in pain intensity between 160 minutes and 140 minutes, ΔNRS). When all completers in CNSP—regardless of exercise intervention—were included there was a positive correlation between ΔNRS and SEA_{diff} (Spearman's rho: 0.52, $P = 0.011$) but no significant correlation between ΔNRS and PEA_{diff} (Spearman's rho: 0.11, $P = 0.603$). Similar correlations were found in the STRENGTH group ΔNRS vs SEA_{diff} (Spearman's rho: 0.67, $P = 0.018$) and ΔNRS vs PEA_{diff} (Spearman's rho: 0.03, $P = 0.938$). In the STRETCH group, no significant correlations were found (ΔNRS vs SEA_{diff} (Spearman's rho: 0.321, $P = 0.336$) and ΔNRS vs PEA_{diff} (Spearman's rho: 0.177, $P = 0.603$).

Changes in SEA and PEA Levels vs Changes in Pain Intensity During PEG (Between First and Second MD)

The difference in pain intensity increase during PEG between the two MD sessions (labelled ΔNRS-MDsessions) was calculated. No significant correlations between ΔNRS-MDsessions and PEA_{diff} (P: 0.510–0.987) or SEA_{diff} (P: 0.495–0.996) were found for either all completers in CNSP or in the two intervention groups.

Discussion

The present study was designed to determine whether two exercise interventions differentially affected trapezius PEA and SEA levels in patients with CNSP. Due to drop-outs, subjects not meeting completer criteria, and samples being excluded due to technical failure, the two exercise groups were small, which is a major study limitation. Nonetheless, this pilot study is the first investigation into the long-term effect of different exercise regimes on PEA and SEA concentrations in women with CNSP, and the findings are worthy of discussion.

Exercise therapy forms to date a natural part of rehabilitation of patients with chronic muscle pain, although the mechanism leading to decrease in pain are still unknown [28]. In this study, subjects in both intervention groups performed a brief repetitive low-force exercise (PEG), which is known to increase pain intensity in myalgic subjects, during each MD session. The result showed that only in the STRENGTH group there was a significantly less increase in pain intensity after the PEG at 160 minutes at the posttreatment MD session compared with the pretreatment MD session. Also, in this group, there was a significant increase in pain intensity between 140 minutes (baseline) and 160 minutes time point during the pre- but not the posttreatment MD session. Thus, in this small sample, the primary effect of the STRENGTH intervention appears to be to reduce the deleterious effect of the PEG, rather than to reduce the baseline pain levels. However, the number of non-completers were higher in STRENGTH than in STRETCH (5 vs 0) and future research should—as pointed out elsewhere (Karlsson et al, under revision)—include analyses of how to improve adherence for unsupervised specific strength training in subjects with chronic pain.

For all 29 CNSP subjects, the levels of PEA and SEA were significantly higher than the corresponding values for the

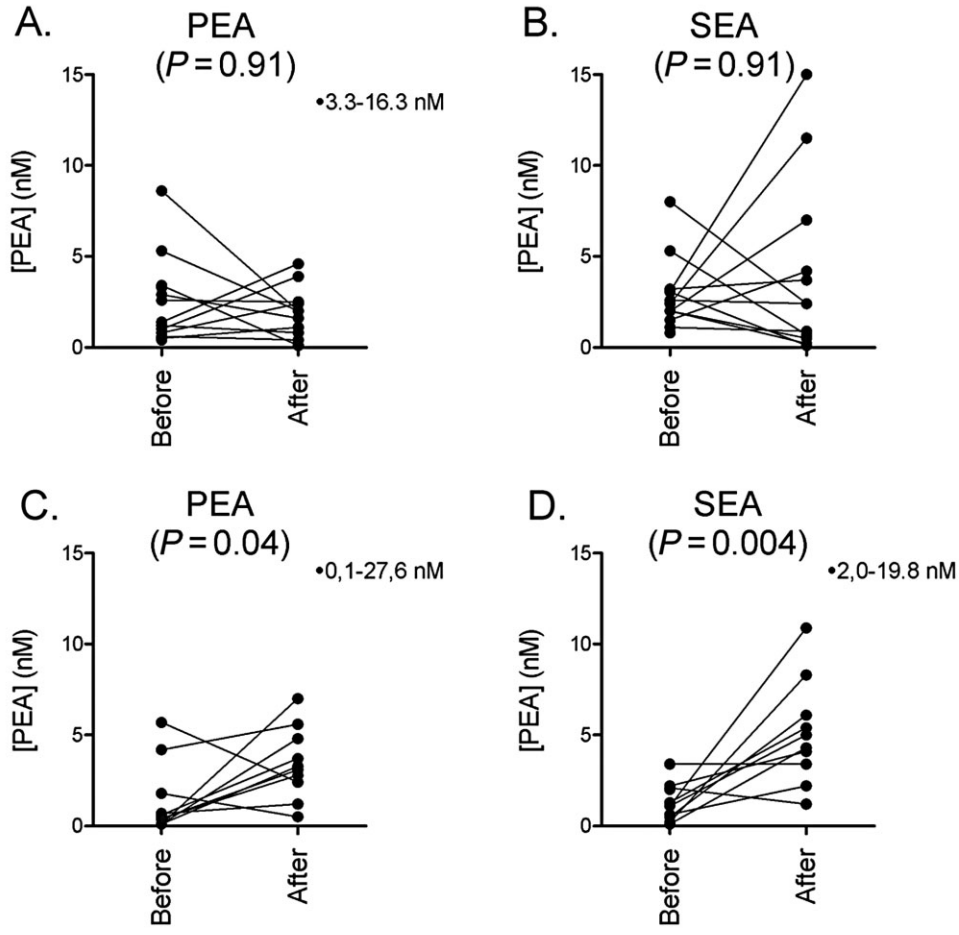

Figure 3 PEA and SEA levels in women with chronic neck shoulder myalgia (CNSP) randomized to strength + stretch (STRENGTH, N = 13, A and B) or stretch alone (STRETCH, N = 11, C and D) interventions. In the present study baseline data (i.e., 140 minutes time point) concerning PEA and SEA is used. Data for the completers of each intervention (see text) are shown. The x-axes show mesurements before (i.e., pretreatment MD session) and after 4–6 months training (i.e., posttreatment MD session). For enhancement of the figure 0–15 nM was chosen for the Y-axes. $P < 0.05$ was considered significant.

24 healthy subjects in CON, confirming our previous data [22,23]. This differs from the situation for patients with chronic widespread pain, where levels of PEA and SEA are not increased [23].

The median pain intensity in CON was zero at all time points during both MD sessions, which is in agreement with our earlier MD studies [11]. PEA level was significantly lower at the second MD session in CON. This might be due to seasonal variations but that is somewhat speculative since it has not been investigated in humans previously, although there is some data suggestive of temporal variations in circulating NAE levels [29]. Also dietary fat intake might affect tissue levels of NAEs, as has been seen in the brains of rodents [30]. At the posttreatment MD session, the median values in PEA for both intervention groups of CNSP were significantly different from CON but the two intervention groups did not differ significantly from each other. Thus, the most conservative conclusion to be made from these data is that neither intervention regimes normalizes PEA levels in CNSP to those seen in healthy controls. However, other types of studies (mechanistic) are necessary in order to elucidate the role of different NAEs in human muscle.

For the concentrations of SEA, the median values for CON between the two microdialysis sessions were not significantly different. The data can thus be analyzed with respect to changes over time for a given treatment. For the STRETCH group of CNSP, the median SEA level after the intervention was significantly increased. In contrast, the median SEA levels for the STRENGTH group were not significantly changed by the exercise intervention. However, even in this group there may be subtle

changes during PEG, since there was a positive correlation between the change in pain intensity following PEG after the STRENGTH intervention (i.e., at posttreatment MD session) and the change in SEA levels.

Focussing upon the within-group changes in the two intervention groups of CNSP, only the STRETCH intervention was associated with significant changes in PEA and SEA. Hitherto, data on the impact of exercise interventions on concentrations of pain-relieving substances (opioids, serotonin, and norepinephrine) in plasma or cerebrospinal fluid in patients with musculoskeletal pain are not conclusive [31]. Recently it was reported that circulating PEA, AEA and oleoylethanolamide (OEA) increases as response to acute exercise in humans [32]. This class of lipids has not previously been investigated in this context in muscle. The strength of the present study is that it provides information on PEA and SEA sampled locally from the muscle interstitium of the trapezius muscle in women with and without neck/shoulder pain. Although several other muscles such as levator scapulae and neck extensors are also affected, showing severe tenderness [33], chronic trapezius myalgia is the most frequent clinical diagnoses in adults with self-reported CNSP [34] and hereby gives validity to our choice of the trapezius muscle for this study.

In conclusion, the present results confirm the difference in PEA and SEA levels between CNSP and pain-free controls. With the major limitation that the sample size is small, the study also provides evidence that the exercise interventions differentially affect the observed levels of these bioactive lipids in the interstitium of the trapezius muscle. Given the anti-inflammatory and/or anti-nociceptive properties of the NAE class of lipids, the present study motivates a larger study investigating the effect of treatment paradigms on interstitial trapezius muscle NAE levels in patients with CNSP.

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