In vivo evidence of altered skeletal muscle blood flow in chronic tension-type headache

M. Ashina,1 B. Stallknecht,3 L. Bendtsen,1 J. F. Pedersen,2 H. Galbo,3 P. Dalgaard4 and J. Olesen1

1Departments of Neurology and 2Radiology, Glostrup Hospital, University of Copenhagen and Copenhagen Headache Center, 3Departments of Medical Physiology and 4Biostatistics, The Panum Institute, University of Copenhagen and Copenhagen Muscle Research Centre, Rigshospitalet, Copenhagen, Denmark.

Summary
Painful impulses from tender pericranial muscles may play a major role in the pathophysiology of chronic tension-type headache. Firm evidence for peripheral muscle pathology as a cause of muscle pain and chronic headache is still lacking. Using a microdialysis technique, we aimed to estimate in vivo blood flow and interstitial lactate concentrations in the trapezius muscle at rest and during static exercise in patients with chronic tension-type headache and in healthy subjects. We recruited 16 patients with chronic tension-type headache and 17 healthy control subjects. Two microdialysis catheters were inserted into the trapezius muscle (on the non-dominant side) of subjects, and dialysates were collected at rest, 15 and 30 min after the start of static exercise (10% of maximal force) and 15 and 30 min after the exercise was completed. All samples were coded and analysed blind. The primary endpoints were to detect a difference between patients and controls in changes of muscle blood flow and the interstitial lactate concentration from baseline to exercise and post-exercise periods. The increase in muscle blood flow from baseline to exercise and post-exercise periods was significantly lower in patients than controls \((P = 0.03)\). There was no difference in resting blood flow between patients and controls \((P = 0.43)\). Resting interstitial concentration of lactate did not differ between patients \((2.51 \pm 0.18 \text{ mM}; \text{mean} \pm \text{standard error of the mean})\) and controls \((2.35 \pm 0.23 \text{ mM}, P = 0.57)\). There was no difference in change in interstitial lactate from baseline to exercise and post-exercise periods between patients and controls \((P = 0.38)\). The present study provides in vivo evidence of decreased blood flow in response to static exercise in a tender muscle in patients with chronic tension-type headache. We suggest that, because of increased excitability of neurones in the CNS, the central interpretation and response to normal sensory input are altered in patients with chronic tension-type headache. This may lead to enhanced sympathetically mediated vasoconstriction and thereby a decreased blood flow in response to static exercise.

Keywords: tension-type headache; muscle blood flow; microdialysis; tenderness; exercise

Abbreviation: RR = relative recovery

Introduction
Chronic tension-type headache is one of the most common and important types of primary headaches (Rasmussen et al., 1991) and represents a considerable health and socioeconomic problem (Rasmussen et al., 1992). Increased tenderness of pericranial myofascial tissues to manual palpation is the most prominent abnormal finding in patients with chronic tension-type headache (Bendtsen et al., 1995; Lipchick et al., 1997; Jensen et al., 1998). Painful impulses from these tissues may be referred to the head and perceived as headache; myofascial mechanisms may, therefore, play a major role in the pathophysiology of tension-type headache (Olesen, 1991). However, extensive research of the tender areas in tension-type headache and in other myofascial pain disorders (Bendtsen, 2000) has failed to demonstrate relevant pathology in muscles.

Microdialysis is a unique technique for investigating and monitoring local muscle blood flow and metabolism in vivo within a tissue volume of \(<1 \text{ cm}^3\) (Hickner et al., 1994). Using a microdialysis technique, we aimed to estimate blood flow and interstitial lactate concentrations in the trapezius...
muscle at rest and in response to static exercise in patients with chronic tension-type headache and in healthy control subjects.

**Material and methods**

**Subjects**

We recruited 16 patients (Table 1) with a diagnosis of chronic tension-type headache according to the criteria of the International Headache Society (headache frequency >15 days per month for >6 months) (Headache Classification Committee, 1988) from the out-patient headache clinic at Glostrup University Hospital, Denmark. All patients completed a diagnostic headache diary (Russell et al., 1992) during a 4-week run-in period to confirm the diagnosis. At screening, we undertook a full physical and neurological examination. Patients were included if they had increased tenderness of the trapezius muscle to manual palpation [scored >2 on a 4-point (0–3) scale]. None of the patients had ongoing pain in the trapezius muscle or widespread pain, tenderness or other symptoms characteristic of fibromyalgia. Exclusion criteria were: a history of migraine or any other type of primary headache; use of any kind of daily medication including prophylactic headache therapy but not oral contraceptives; excessive alcohol use; and serious somatic or psychiatric disorders including depression (Hamilton Depression Score >17) (Hamilton, 1960). Seventeen healthy volunteers served as controls (Table 1). They were included if they had no more than mild tenderness of trapezius muscle to manual palpation [scored ≤1 on a 4-point (0–3) scale]. They had never had migraine and suffered <12 days per year with tension-type headache. The study was approved by the Scientific-Ethical Committee of the County of Copenhagen, and was undertaken in accordance with the Helsinki Declaration of 1975, as revised in 1983. All subjects gave informed consent to participate in the study.

**Experimental design**

All subjects reported to the laboratory at 08.00 following an overnight fast (12 h) including abstinence from tobacco, coffee and tea. All procedures were performed in a quiet room at a temperature of 22–24°C. The patients were examined during a typical day of tension-type headache, i.e. they had pain characteristic of tension-type headache with no more than one associated symptom (Headache Classification Committee, 1988). Headache intensity was measured on a visual analogue scale (0–100 mm: 0, no pain; 100, worst imaginable pain). Subjects were not allowed to take analgesics 24 h prior to examination. After a brief rehearsal session, we recorded local tenderness and then the maximal voluntary force during isometric contraction of the trapezius muscle. The subjects were seated upright in a dental chair with arms and legs hanging. Thus, the subjects could not use arms and legs for support during the exercise session. Adjustable force transducers were placed over the shoulders and connected to a force monitor. On command, the subjects were encouraged to elevate both shoulders with maximal force for 3 s. The maximal voluntary isometric contraction force of the trapezius muscle was defined as the average of three recordings. The resting intervals between the contractions lasted ~2 min. The subjects were not allowed to look at the force monitor during this session. Fifteen minutes after the test for maximal voluntary isometric contraction, two microdialysis catheters were inserted in parallel ~1.5 cm apart into the trapezius muscle at a standard anatomical point on the non-dominant side. The point was located on the centre of the descending part of the trapezius muscle midway between the processus spinosus of the seventh cervical vertebra and the acromion. During the insertion of catheters, we used ultrasound imaging (Acuson XP 10 ultrasound unit equipped with a 7 MHz linear transducer; Acuson, Mountainview, Calif., USA) to ensure that the membrane part of the catheters was placed in the muscle in a direction parallel to the muscle fibres (Fig. 1). We did not use a local anaesthetic during the insertion of catheters. After insertion of catheters, the perfusion was started and the catheters were allowed to stabilize for 60 min. Baseline dialysates were collected during the next 60 min. During this period, the subjects were sitting in a dental chair with a headrest and were allowed to watch a video or listen to music. After the resting period, the subjects performed a voluntary isometric contraction of the trapezius muscle at 10% of the maximal voluntary isometric force for

### Table 1 Clinical data for headache patients and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Females/males</td>
<td>10/6</td>
<td>12/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42 (18–60)</td>
<td>42 (24–60)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25 (20–30)</td>
<td>25 (18–37)</td>
</tr>
<tr>
<td>Frequency of tension-type headache (days/4 weeks)</td>
<td>22 (15–28)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Amount of used analgesics (g/4 weeks)</td>
<td>5 (0–42)</td>
<td>–</td>
</tr>
<tr>
<td>Local tenderness of trapezius muscle (mm)</td>
<td>34 (8–81)</td>
<td>3 (0–16)</td>
</tr>
<tr>
<td>Maximal voluntary force (kg)</td>
<td>51 (30–86)</td>
<td>45 (21–76)</td>
</tr>
<tr>
<td>Hamilton Depression Score</td>
<td>5 (1–9)</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are mean (range).
30 min. Visual inspection of the monitor by the investigator and the subjects ensured that the desired exercise intensity was performed within ±200 g. Dialysates were collected 15 and 30 min after the start of exercise and 15 and 30 min after the end of the exercise. During the post-exercise period, the subjects were seated in the same position as during rest.

Microdialysis

Two microdialysis catheters (CMA/60; CMA Microdialysis, Stockholm, Sweden) with a membrane length of 30 mm (diameter of 0.6 mm) and a molecular weight cut-off of 20 kDa were used to estimate the nutritive skeletal muscle blood flow and the interstitial lactate concentration. Because the perfusate (the fluid going into the catheter) perfused slowly along the dialysis membrane, the concentration of substances in the dialysate (the fluid coming out of the probe) mirrored the concentration of substances in the interstitial fluid. Nutritive skeletal muscle blood flow was estimated using the microdialysis ethanol technique described previously by Hickner et al. (1994). We used \(^3\)H\(_2\)O instead of ethanol (Hickner et al., 1994) as indicator substance because \(^3\)H\(_2\)O is easier to measure and yields results similar to ethanol (Stallknecht et al., 1999). The ratio between concentration of indicator substance in dialysate and perfusate (the outflow-to-inflow ratio) varies inversely with blood flow in the tissue (Hickner et al., 1994; Stallknecht et al., 1999). A microdialysis catheter was perfused with fluid containing the indicator substance (\(^3\)H\(_2\)O), which diffused out of the catheter into the surrounding tissue. The rate at which the indicator substance diffuses out of the catheter depends on blood flow. In the present study, the catheters were perfused with Ringer acetate containing 3 mM glucose, 5 kBq/ml \(^{14}\)C lactate (specific activity 5.7 GBq/mmol; Amersham, Bucks, UK) and 5 kBq/ml \(^3\)H\(_2\)O (specific activity 37 MBq/ml; NEN, Du Pont, Belgium) at a rate of 2 ml/min using a high-precision syringe pump (CMA/100; CMA Microdialysis, Stockholm, Sweden). The volume of catheter after the membrane was 3 μl. Dialysate was collected in 200 μl capped microvials (CMA Microdialysis, Stockholm, Sweden). Dialysate sampling was delayed by 1 min relative to the experimental protocol to compensate for the transit time in the outlet tubing. Immediately after sampling, 5 μl of dialysate (single) [and in one case, 5 μl of perfusate (quadruple)] were pipetted into counting vials and scintillation fluid (High-Flash Point LSC Cocktail UCH maGold Packard Bioscience B.V., Gronningen, The Netherlands) was added. The samples were kept at 4°C and counted in a liquid-scintillation counter (2200 CA Packard; Packard Instrument, Ill., USA). Counts were corrected to disintegrations per minute on the basis of a quench curve. The rest of the microdialysis sample was kept at –20°C before being analysed for lactate on a CMA 600 microdialysis analyser (CMA Microdialysis, Stockholm, Sweden). The degree of equilibration between the dialysate and the interstitial fluid was not total at the perfusion rate used in the present study. Accordingly, the degree of equilibration, termed the in vivo relative recovery (RR), was determined for lactate using \(^{14}\)C lactate as internal reference (Scheller and Kolb, 1991). The RR was calculated as (dpm\(_p\) – dpm\(_d\))/dpm\(_p\), where dpm\(_p\) is disintegration per minute in perfusate and dpm\(_d\) is disintegration per minute in dialysate. Interstitial concentration of lactate was calculated as (C\(_d\) – C\(_p\))/RR + C\(_p\), where C\(_d\) is dialysate concentration, C\(_p\) is perfusate concentration and RR is the relative recovery. All samples were coded and analysed blind with respect to patients and controls.

Local tenderness

Local tenderness was defined as pressure-induced pain at a standard anatomical point on the trapezius muscle on the non-dominant side. Thus, local tenderness was measured at the same anatomical point where two microdialysis catheters were later inserted (the site of dialysate sampling). Using a palpometer (Bendtsen et al., 1994), we applied a standardized pressure (160 U) on this point and the subjects scored pressure-induced pain on a visual analogue scale.

Force transducer

Two force transducers (Bisco Vaegte A/S, Denmark) were used to measure force during voluntary isometric contractions of trapezius muscle. The contact area of the force transducer was 10 cm × 10 cm and the exerted force was displayed on a monitor.
Data analysis and statistics
Calculation of sample size was based on detection of a difference between patients and controls in changes of the nutritive skeletal muscle blood flow and the interstitial lactate concentration from baseline to exercise and post-exercise periods at 5% significance with 80% power. We intended to calculate the sum of differences between the baseline values and the values at 15 and 30 min after the start of exercise and 15 and 30 min after exercise was completed to obtain a summary measure within each group and to test a difference between groups. We assumed that the analyses of variables would show 30% inter-individual variation. A 30% difference between two groups was taken to be clinically significant. We estimated that 16 subjects should be included in each group.

To assess changes in the nutritive skeletal muscle blood flow, the relative recovery for lactate and the interstitial lactate concentration from baseline to exercise and post-exercise periods, we used the following linear model for normally distributed data: \( Y = \alpha(\text{subject}) + \beta(\text{group} \times \text{time}) + \text{error} \), i.e. there is an individual patient level and an additive time effect of arbitrary shape, which can be different between the two groups. The model was then simplified to \( Y = \alpha(\text{subject}) + \beta(\text{time}) + \gamma(\text{group} \times \text{time}^2) + \text{error} \), where \( \text{time}^2 \) is ‘time not equal to baseline’, which corresponds to an assumption that there is a constant difference between the time courses after baseline. If \( \gamma \) is zero in this model, then there is no difference between the groups (except for the individual levels), so the test for significance of \( \gamma \) is of primary interest. If the data had been complete, this test would correspond to a simple Student’s \( t \)-test on the sums of differences from baseline; the adoption of a formal statistical model allows us to use all available data. To adjust for the possible influence of relative recovery on muscle blood flow, we extended the model for this variable by adding a linear term, \( \theta \times \text{recovery} \). The \( \gamma \) coefficient could also be tested in the extended model. The error terms were assumed to be independent and normally distributed with a constant variance. Model assumptions were checked using graphical methods and the response variables were transformed, if necessary, to meet the assumptions. These analyses were performed using R\(^\text{®} \), version 1.0.1 software (The R language and environment for statistical computing and graphics, www.r-project.org). We used unpaired and paired samples Student’s \( t \)-tests to assess difference in resting blood flow and the interstitial lactate concentration, local tenderness of trapezius muscle, body mass index and maximal voluntary force between patients and controls, and local tenderness of trapezius muscle within the groups. Results are presented as mean $\pm$ standard error of mean. These analyses were performed using SPSS\(^\text{®} \), version 10.0.5 software (SPSS Inc., Chicago, Ill., USA).

Results
All subjects completed the study. Due to technical problems, some samples were missed in both patients and controls. In patients, the following samples were missing: out/inflow ratio and RR for lactate at exercise 1 (Patient 1) and post-exercise 1 (Patient 15); RR for lactate at exercise 1 (Patient 9) and post-exercise 1 (Patient 15). In controls, the missing samples were: out/inflow ratio at baseline (Subject 7), exercises 1 and 2 (Subject 6), and post-exercise 2 (Subject 12); RR for lactate at
baseline (Subject 7), exercises 1 and 2 (Subject 9), and post-
exercise 2 (Subject 12).

The mean headache intensity in terms of the visual
analogue scale in patients on the experiment day was
32 ± 4 mm. There was no difference between patients
(25 ± 1 kg/m²) and controls (25 ± 1 kg/m²) in body mass
index ($P = 0.85$). Maximal voluntary force also did not differ
between patients (51 ± 5 kg) and controls (45 ± 4 kg)
($P = 0.37$). Before the insertion of catheters, local tenderness
at the standardized point was significantly higher in patients
(34 ± 5 mm) than in controls (3 ± 1 mm) ($P = 0.0001$).

**Local muscle blood flow**

There was no difference in resting blood flow, as reflected
by the outflow-to-inflow ratio of $^{3}$H$_2$O, between patients
(0.07 ± 0.01) and controls (0.08 ± 0.01) ($P = 0.43$).

However, the change (increase) in muscle blood flow
(reflected by a decrease in $^{3}$H$_2$O outflow-to-inflow ratio)
from baseline to exercise and post-exercise periods was
significantly lower in patients than controls ($P = 0.03$) (Fig.
2).

**Local interstitial concentration of lactate**

During the rest period, we found no difference in the relative
recovery for lactate between patients (0.54 ± 0.03) and
controls (0.51 ± 0.02) ($P = 0.56$) (Fig. 3). Interstitial
concentrations of lactate values were log-transformed prior to
statistical testing. We found that resting interstitial concentra-
tion of lactate did not differ between patients (2.51 ± 0.18 mM)
and controls (2.35 ± 0.23 mM) ($P = 0.57$) (Fig. 4). However, interstitial concentration of lactate increased significantly over time in response to
exercise in both groups ($P = 4.6 \times 10^{-14}$). There was no difference in change in the relative recovery for lactate ($P = 0.11$) (Fig. 3) and in interstitial concentration of lactate ($P = 0.38$) (Fig. 4) from baseline to exercise and post-exercise periods between patients and controls.

**Local tenderness**

After removal of the catheters from the muscle at the end of the experiment, the mean local tenderness had increased significantly from $34 \pm 5$ to $53 \pm 6$ mm in patients ($P = 0.001$) and from $3 \pm 1$ to $25 \pm 4$ mm in controls ($P = 0.0001$).

**Discussion**

It is a common experience that individuals who have been exposed to static or repetitive work for a long period may develop tender areas in the pericranial muscles and tension-type headache. It has been hypothesized that local muscle ischaemia, disturbances in metabolism, microcirculation and mitochondria function in the tender areas may explain myofascial pain in tension-type headache and in other myofascial pain disorders such as trapezius myalgia (Henriksson et al., 1993). Various in vitro and in vivo methods such as muscle biopsy, single-fibre laser-Doppler and magnetic resonance spectroscopy have been used to explore the mechanisms responsible for myofascial pain. The results of these studies have been conflicting. While open studies suggested abnormalities in microcirculation (Larsson et al., 1990, 1999) controlled and blind studies have failed to find firm evidence for peripheral abnormalities (Yunus et al., 1989; Simms, 1994). More sensitive techniques are needed to answer the question of whether tension-type headache and other myofascial pain disorders are associated with peripheral pathology in tender points.

We used a microdialysis technique that enables the study of in vivo muscle metabolism in humans. We were particularly interested in investigating the relationship between tension-type headache, muscle tenderness and muscle blood flow. The major finding of the present study was a decreased blood flow in response to static exercise in a tender point in patients with chronic tension-type headache. Thus, the increase in muscle blood flow from baseline to exercise and post-exercise periods was significantly lower in patients than controls. Furthermore, we found that resting blood flow tended to be higher (the $^{3}$H$_{2}$O outflow-to-inflow ratio tended to be lower) in the tender point of patients compared with resting blood flow in the same anatomical point in controls, but the difference was not statistically significant. One previous study measuring temporal muscle blood flow by $^{133}$Xe clearance reported no difference in resting blood flow or relative flow increase during isometric work between patients with chronic tension-type headache and controls (Langemark et al., 1990). However, in that study, muscle blood flow was measured from a large muscle area and not in a tender point.

How can we explain the reduced blood flow response to exercise in the tender point? Difference in the static load between patients and controls is unlikely because there was no difference between groups, in either absolute force or relative to maximal voluntary force. It could be suggested that patients develop a relative ischaemia in the tender point during static exercise. If so, one would expect that the increase of interstitial lactate concentration would be higher in patients than in controls. However, in the present study, we observed no difference in local increase of interstitial lactate between patients and controls. Thus, our results seem to rule out the presence of ischaemia in the tender point of patients with chronic tension-type headache during rest and static exercise.

The altered blood flow might be secondary to chronic muscle pain. Recent studies strongly indicate that chronic tension-type headache may be caused by prolonged painful input from pericranial myofascial tissues, e.g. tender points, resulting in central sensitization (i.e. increased excitability of neurones in the CNS) (Bendtsen et al., 1996; Ashina et al., 1999a, b; Bendtsen and Ashina, 2000). The pathophysiological basis for the painful input from the periphery remains unknown. Once the central sensitization had been established, chronic tension-type headache might be an entirely central process without further or only minimal input from the periphery (Bendtsen, 2000). Because of the central sensitization, the central interpretation and response to normal sensory input are altered—possibly mainly when input is increased as during exercise. This may lead to enhanced sympathetically mediated vasoconstriction and thereby a decreased blood flow in response to static exercise. This is supported by studies in animals and humans showing that static exercise produces a one-to-one synchronization of activation of the muscle nociceptors (group IV) and muscle nerve sympathetic activity (Mitchell and Victor, 1996). Furthermore, it has been shown that in patients with fibromyalgia a complete sympathetic blockade produced a marked reduction of the number of tender points suggesting an improvement in microcirculation (Bengtsson and Bengtsson, 1988). Moreover, it has been proposed that central sensitization may maintain increased efferent sympathetic outflow, which in turn maintains sensitization of sensory afferents (Roberts, 1986). These data suggest that the sympathetic outflow may influence or maintain afferent activity in nociceptors or altered blood flow regulation in tender points of patients with chronic tension-type headache.

In conclusion, the present study provides in vivo evidence of altered blood flow regulation in tender skeletal muscle during static work in patients with chronic tension-type headache. The results indicate that the increased excitability of neurones in the CNS may affect the regulation of peripheral mechanisms and thereby lead to increased tenderness and chronic headache.
Acknowledgements
We wish to thank Hanne Andresen, Lisbeth Kall and Regitze Kraunsøe for skilful technical assistance and Dr Rigmor Jensen for valuable comments during the preparation of the manuscript. The Danish Medical Association Research Fund, the Danish Hospital Foundation for Medical Research, Region of Copenhagen, the Faroe Islands and Greenland, the Foundation for Research in Neurology, the Novo Nordisk Foundation, the Gerda and Aage Haensch’s Foundation, the Mauritzen La Fontane’s Foundation the Foundation of Jacob Madsen and his wife Olga Madsen provided financial support for the study.

References


