Effects of Low-Load Repetitive Work and Mental Load on Sensitising Substances and Metabolism in the Trapezius Muscle

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To My Family

“If you would be a real seeker after truth, it is necessary that at least once in your life you doubt, as far as possible, all things”

— Descartes
ABSTRACT

Low-load repetitive work (LLRW) and mental load are important risk factors for the development of work-related muscle pain. The link between these risk factors and the development of pain is still not understood, but stimulation of chemo-sensitive receptors in the muscle probably plays an important role. It has been suggested that sensitising substances may accumulate in the muscle during LLRW, especially when combined with mental load.

The overall purpose of this thesis was to try to shed some light on the effects of LLRW on the concentration of sensitising substances (glutamate, prostaglandin E₂ (PGE₂), norepinephrine (NE)) and on metabolism (lactate, pyruvate and oxygenation) in the trapezius muscle of healthy controls (CON) and subjects with trapezius myalgia (TM).

A first step was to investigate whether females with TM exhibit higher absolute concentrations of glutamate and PGE₂ in the affected muscle during rest. Using Microdialysis (MD) females with TM and asymptomatic controls were studied during four hours of rest. [Glutamate] and [PGE₂] during rest did not differ between groups.

A second step was to investigate, in a simulated occupational setting, the effects of LLRW on the concentration of sensitising substances and metabolism in the trapezius muscle of TM and CON, and whether increased work duration resulted in a progressive effect. Asymptomatic females were studied during baseline rest, 30 versus 60 min work and recovery, using MD and near infrared spectroscopy (NIRS). Subjects with TM were studied during baseline rest, 30 min work and recovery. [Glutamate] and [lactate] increased in response to work, but not progressively with increased work duration. [Glutamate] was at all time points significantly lower in TM. [PGE₂] and oxygenation remained unchanged during work for CON, while for TM oxygenation decreased significantly during work. In TM [pyruvate] increased during both work and recovery, and a significant interaction between groups was found for [pyruvate] during recovery; while moderately increased in CON it increased progressively in TM.

The effects of LLRW with and without superimposed mental load on intramuscular [NE], muscle activity and oxygen saturation in the trapezius were also investigated and compared. Using MD, electromyography and NIRS, healthy females were studied on two occasions; during 30 min LLRW and during 30 min LLRW with superimposed mental load. During work [NE], and muscle activity, were increased, while oxygenation decreased, but no differences between occasions. However, recovery of [NE] to baseline was slower after LLRW with superimposed mental load.

The findings of the present thesis suggest: (i) no inflammation, or increased interstitial [glutamate] in TM; (ii) LLRW causes an increased anaerobic metabolism in both TM and CON; (iii) no effect of work duration was found; (iv) a significant difference in the effects of LLRW on the interstitial milieu of the trapezius muscle in TM as compared to CON; (v) LLRW causes a significant increase in [NE], but superimposed mental load does not cause a further increase; (vi) LLRW with a superimposed mental load may result in a slower recovery to baseline [NE] as compared with LLRW alone.

Key-words: work-related muscle pain, microdialysis, near-infrared spectroscopy, electromyography, glutamate, lactate, pyruvate, prostaglandin E₂, norepinephrine
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### ABBREVIATIONS

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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>ECF</td>
<td>Extra cellular fluid</td>
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<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
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<tr>
<td>HPLC-ED</td>
<td>High-precision liquid chromatography with electro-chemical detection</td>
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<td>IMMD</td>
<td>Intramuscular microdialysate</td>
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<td>IMP</td>
<td>Intramuscular pressure</td>
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<td>LLRW</td>
<td>Low-load repetitive work</td>
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<td>MA</td>
<td>Mental arithmetic</td>
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<td>MD</td>
<td>Microdialysis</td>
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<tr>
<td>MPF</td>
<td>Mean power frequency</td>
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<tr>
<td>MU</td>
<td>Motor unit</td>
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<td>NE</td>
<td>Norepinephrine</td>
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<td>NIRS</td>
<td>Near infrared spectroscopy</td>
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<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>REP 30</td>
<td>Repetitive work for 30 minutes</td>
</tr>
<tr>
<td>REP 60</td>
<td>Repetitive work for 60 minutes</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
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<tr>
<td>RPE</td>
<td>Rating of perceived exertion</td>
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<td>RPP</td>
<td>Rating of perceived pain</td>
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<tr>
<td>RPS</td>
<td>Rating of perceived mental stress</td>
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<tr>
<td>RR</td>
<td>Relative recovery</td>
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<tr>
<td>RM ANOVA</td>
<td>Repeated measures analysis of variance</td>
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<td>RW</td>
<td>Repetitive work</td>
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<td>Repetitive work – mental load</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SCWT</td>
<td>Stroop colour word test</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SFL</td>
<td>Skin-fat-layer</td>
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<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
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<tr>
<td>%StO₂</td>
<td>Percent oxygen saturation</td>
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<tr>
<td>TC</td>
<td>Test contraction</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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<td>WRMP</td>
<td>Work-related muscle pain</td>
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This thesis is based on the following papers, referred to in the text by their Roman numerals.


III. **Flodgren GM**, Crenshaw AG, Hellström F, Fahlström M. Effects of low-load work on sensitising substances and muscle metabolism in trapezius myalgia - determined with microdialysis and near infrared spectroscopy. *(submitted)*

IV. **Flodgren GM**, Crenshaw AG, Gref M, Fahlström M. Effects of low-load work on interstitial norepinephrine, muscle activity and oxygen saturation. *(manuscript)*

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INTRODUCTION

General background

Epidemiological research strongly suggests that both physical and psychosocial factors interact in the development of work-related chronic muscle pain, e.g. trapezius myalgia (Buckle & Devereaux 2002; Punnet & Gold 2003; Bongers et al. 2006). Complaints related to physical exposure are increasing in the European Union (Bernard 1997). In Sweden, which is undeniably among the forefront of ergonomic development, factors related to physical exposure are still the largest work environmental problems. During 2006, self-reported musculoskeletal complaints due to physical as well as psychological exposure were 28.2 % in the female working population in Sweden. Approximately 50% of these complaints constituted neck and shoulder disorders (Swedish Government 2006).

Unfortunately, the aetiology of chronic work-related muscle pain (WRMP) is poorly understood. It is acknowledged that the genesis of WRMP, e.g. trapezius myalgia, is multi-factorial, and that known risk factors like prolonged low-load repetitive and/or static work and mental stress probably interact, but the causal links between risk factors and WRMP are still not fully understood. Many researchers today believe that stimulation of chemo-sensitive receptors in muscle plays an important role (Mense 1993; Knardahl 2002; Johansson et al. 2003). It has been suggested that metabolites and inflammatory substances may accumulate in the muscle during low-load repetitive and/or static work, causing pain and disturbed motor control, and that increased activity in the sympathetic nervous system (SNS) may worsen the situation (Johansson et al. 2003; Roatta et al. 2003; Passatore & Roatta 2007).

Knowledge about the mechanisms behind the development of WRMP is crucial for successful prevention, diagnosis and for treatment of muscle pain once it has evolved. This thesis is an attempt to contribute to the contemporary knowledge of WRMP by investigating the effects of low-load repetitive work (LLRW) and mental load on changes in sensitising substances and muscle metabolism in the trapezius muscle of females suffering from trapezius myalgia and healthy controls.
Muscle pain and peripheral sensitisation

Pain is, according to the definition by I.A.S.P. (International Association for the Study of Pain), “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (I.A.S.P. 1979). The physical part of muscle pain results from activation of the terminal endings of small-diameter afferent fibers (Mense 1993). These muscle nociceptors are free nerve endings supplied by the group III (myelinated A-delta) and IV (unmyelinated C) muscle afferents that are sensitive to strong mechanical, thermal, and chemical stimuli i.e. to pain producing substances that are released from various tissues. The nociceptors are typically located to the walls of arterioles or the connective tissue (Mense 1993).

The sensitivity of muscle afferents to stimuli is not fixed. Repeated stimulation may cause a reduction in threshold and an increase in responsiveness, i.e. peripheral sensitisation, which contributes to the pain hypersensitivity found at sites of tissue damage and inflammation. It arises due to the actions of inflammatory mediators released round the site of tissue damage or inflammation, and other chemical mediators produced in the affected tissues (Mense 1993).

Possible pathophysiological mechanisms in chronic work related myalgia

Many hypotheses have been suggested concerning possible mechanisms involved in the genesis of work related myalgia (Johansson et al. 2003). However, none of the hypotheses seem to be singularly able to completely account for the development of WRMP. On the contrary, they seem to be, on the whole, complementary rather than competing hypotheses. Indeed, a serious attempt has been made by a large group of researchers to produce a model explaining the way individual mechanisms may interact in various ways during different times in the disease process (for a detailed account of the so called Brussels Model, see Johansson et al. 2003). Below is a brief account of the mechanisms of particular relevance for the present thesis, and the ways they may interact.

There is some consensus among researchers that stimulation of chemosensitive receptors in muscle plays an important role in the development of chronic muscle pain (Mense 1993; Knardahl 2002; Johansson et al. 2003). It is believed that metabolites and inflammatory substances may accumulate in the muscle during prolonged low-load repetitive and/or static work, causing pain
and disturbed motor control, and that increased activity in the sympathetic nervous system (SNS) may worsen the situation (Johansson et al. 2003; Roatta et al. 2003; Passatore & Roatta 2007).

The SNS may be involved in the development of WRMP through several actions exerted at the muscle level, through local release of catecholamines. Firstly, norepinephrine (NE) has direct nociceptive effects and increases the sensitivity of nociceptors to other stimuli (Shyu et al. 1989; Roatta et al. 2003). Secondly, NE has a vasoconstrictory effect, suggested to be most evident at low contraction levels (< 10%), which may impair metabolic removal and oxygen delivery by overriding the vasodilatory actions of metabolites (Hansen et al. 1996; Roatta et al. 2003). Thirdly, by decreasing the contraction force and thereby increasing the fatiguability of Type I fibres (Bowman 1980; Roatta et al. 2003; Passatore & Roatta 2007), which are predominantly activated during low-force contractions (Henneman et al. 1965). Finally, it has been suggested that SNS may have direct effects on the muscle-spindle system, thereby negatively influencing motor control by reducing the quality of proprioceptive information. This may cause suboptimal movement strategies such as increased co-contractions of antagonist muscles (Johansson et al. 2003; Roatta et al. 2003; Passatore & Roatta 2007).

The activation of chemo-sensitive muscle afferents may have a negative influence on functions of the CNS, such as motor control (muscle activation and coordination). This may occur through different mechanisms: (i) disturbances of the muscle spindle system due to activation of chemosensitive afferents, resulting in disturbed proprioception, and maybe also in increased co-contractions; (ii) decreased inhibition of α-motoneuron input by Renshaw cells, resulting in less precise coordination of muscle activation (for a more detailed description, see Johansson et al. 2003).

According to the Brussels Model, once a pain condition is established, the above mentioned factors may interact to perpetuate the condition, thus constituting a vicious circle. For a review, and an evaluation of the empirical support of the Brussels Model, see Johansson et al. (2003) and Visser & van Dieën (2006).

Other mechanisms that have been suggested, and which may be complementary to the Brussels Model, include, among others: (i) the hypertrophy of type I fibers (Kadi et al. 1998) which in combination with reduced microcirculation (Larsson et al. 1999) in subjects with trapezius myalgia, may limit metabolic removal and oxygen delivery (ii) disturbed metabolism due to mitochondrial changes in trapezius myalgia (Kadi et al. 1998;
Larsson et al. 2004); (iii) overloading of type I motor units due to stereotypic and/or unfavourable motor unit recruitment patterns i.e. the “Cinderella-hypothesis” (Hägg 2000), the “rotation-hypothesis” (Westgaard & de Luca 1999) and the “gaps-hypothesis” (Veiersted et al. 1990); (iv) $\text{Ca}^{2+}$ accumulation due to sustained motor unit activity may cause muscle-fiber injury (Gissel 2005); (v) mechanical effects due to shear-forces between muscle fibers, that, if sustained during a prolonged period of time, may activate pain receptors in the muscle (Vøllestad & Røe 2003); (vi) vessel-nociceptor interaction causes pain independently of muscle activation (Knardahl 2002).

Most of the hypotheses so far mentioned, suggest that, in one way or the other, WRMP is associated with some form of chemical or structural change that occurs in the muscles during prolonged exposure to physical and/or mental risk factors. However, the empirical evidence of what actually occurs in the muscles does not yet provide enough to reliably draw any conclusions about which hypotheses lie closer to the truth than others. The present thesis aims to further add to the empirical findings describing the chemical changes that actually occur in the trapezius muscle during LLRW, with and without superimposed mental load, in females with WRMP and asymptomatic controls.

Investigated sensitising substances

Substances known to stimulate and sensitise chemo-sensitive afferents are, among others: prostaglandin E$_2$ (PGE$_2$) (Mense 1993), glutamate (Hargreaves et al. 1994; Cairns et al. 2001; Carlton 2001), norepinephrine (NE) (Mense 1993), and lactic acid (Rotto & Kaufman 1988; Sinoway et al. 1993).

The pro-inflammatory substance PGE$_2$ is a substance with known sensitising effects on muscle nociceptors (Mense 1993). In human studies, increased PGE$_2$ concentration has been found in response to intense dynamic leg muscle contractions, but not to static contractions (Karamouzis et al. 2001), while in an animal study, static contractions were reported to increase PGE$_2$ (Symons et al. 1991). Very little has been done to investigate PGE$_2$ concentrations in pain subjects and in response to low-load work.

The excitatory neuro-transmitter glutamate is well-known for its pain-mediating function in the central nervous system (CNS), where it acts on the ionotropic glutamate receptor N-methyl-D-Aspartate (NMDA) (Hargreaves et al. 1994), but is also suggested to play a role in pain transmission in the periphery (Carlton et al. 2001). Indeed, both increased concentrations of glutamate, as well as the occurrence of NMDA-receptors in tendons of individuals with chronic tendon pain have been reported (Alfredson et al. 1999; Alfredson et al. 2000;
Alfredson et al. 2001a+b). Injections of glutamate into the masseter muscle have been reported to cause significantly higher levels of peak pain and duration of pain than placebo injections (Cairns et al. 2001), to sensitize rat muscle afferent fibers (Cairns et al. 2002), and to evoke pain in part through activation of peripheral NMDA receptors (Cairns et al. 2003). Glutamate is suggested to be released from peripheral afferent fibers when nociceptors are activated, e.g. due to inflammation (Svensson et al. 2003). However, Ashina et al. (2005) found no signs of increased release of glutamate from nociceptors during or after experimentally induced pain and tenderness. In trapezius muscle pain, the findings are conflicting, and increased glutamate levels have only rarely been related to pain. Increased levels of glutamate have been seen in subjects with trapezius myalgia in response to work (Rosendal et al. 2004b), but not in subjects with chronic tension-type headache (Ashina et al. 2003). Consequently more research is needed to illuminate the role of glutamate in trapezius muscle pain.

The catecholamine NE, which is released from the adrenal medulla as a hormone into the blood, is also a neurotransmitter in the central and the sympathetic nervous system, where it is released from noradrenergic neurons during synaptic transmission. There is evidence that NE has direct nociceptive effects, and also increases the sensitivity of nociceptors to other stimuli (Shyu et al. 1989; Roatta et al. 2003). Increased [NE] in urine has been reported in response to prolonged low-load work (Garde et al. 2003) and repetitive work with superimposed mental load (Lundberg et al. 1994). Increased intramuscular NE during rest has been reported in subjects with neck pain (Shah et al. 2005), but little is known about the effects of low-load work and/or mental load on intramuscular NE.

Lactate is a product of anaerobic metabolism, and has in its acidic form been shown to stimulate chemosensitive afferents in the muscle (Rotto & Kaufmann 1988, Sinoway et al. 1993). Increased lactate concentration has been reported to correlate with the development of muscle fatigue during intense muscle contractions (Grassi et al 1999; Miura et al. 2000), but the role of lactate in muscle fatigue is not fully investigated (Gladden 2004; Cairns 2006). In studies of low-load work both higher lactate levels (Rosendal et al. 2004b) as well as similar lactate levels in the trapezius muscle of pain subjects and controls have been found (Ashina et al. 2002). These few and conflicting results call for further research.
Microdialysis

Microdialysis (MD) is an in-vivo sampling technique that provides a way of directly assessing chemical changes in the muscle interstitium over time, with minimal trauma. Bito et al. (1966) were first to describe the possibility of using a semi-permeable membrane to sample free amino acids in the extracellular fluid of brain in dogs, and Delgado et al. (1972) then invented the “dialytrode”, a forerunner of todays MD-probes, for long-term intra-cerebral perfusion in conscious monkeys. But it was Ungerstedt & Pycock (1974) that reported the first in vivo MD-set-up. Since the early applications in the neurosciences, MD has been performed in almost every organ or bodily tissue (for details see reviews by Ungerstedt 1991 and Chaurasia 1999), and in this thesis in the trapezius muscle.

The basic MD system of today consists of a MD-probe, connective tubing, a syringe pump and a sample collector.

MD (see Fig. 1) is performed by implanting a small tubular membrane in the tissue of interest, and slowly perfusing it with a physiological liquid that equilibrates with the extra cellular fluid (ECF) by diffusion in both directions (Ungerstedt 1991). Small solutes in the interstitial space diffuse through the semi-permeable membrane into the perfusion fluid, which is collected for analysis. The diffusion coefficient (i.e. the concentration gradients between the perfusate and the ECF) dictates the migration of the solute. The dialysis purifies the sample by preventing macro-molecules from entering the probe, which simplifies the chemical analysis (Ungerstedt 1991).

In the beginning of the sampling, a rapid fall in the concentration of many substances is seen, due to tissue trauma causing a release of substances from cellular storage compartments, and also due to the drainage of substances from the ECF into the probe that occurs directly after probe insertion (Amberg & Lindefors 1989; Beneviste 1989). A steady state in the muscle must be re-
established, and therefore it is vital to allow enough time for stabilisation of the interstitial milieu after the trauma of probe insertion (Ungerstedt 1991). The time to steady state varies depending on the matrix tortuosity of the investigated tissue, the degree of the initial trauma and on the size of the investigated organ, with a longer stabilisation time in tissues with high tortuosity and for smaller organs (Ungerstedt 1991; Chaurasia 1999).

The degree of equilibration between the ECF and perfusion fluid (i.e the degree of recovery) is also to a great extent dependent on the size of the membrane and the perfusion flow rate (Rosdahl et al. 1998), i.e. the recovery of substances is greater with larger membranes and lower perfusion flow. Other factors, that affect probe recovery are; diffusion of the substance in the ECF, diffusion rate in dialysis membrane, molecular weight cutoff of the membrane, chemical interaction between the analyte and the membrane, but also other factors like blood flow rate, metabolism rate, uptake into cells and the extent of tissue vascularisation (Chaurasia 1999).

The perfusion flow rate should preferably be low, between 0.1-5 µL min\(^{-1}\) in order to remove as little as possible of the extracellular content, thereby causing as little disturbance as possible from normal physiology (Ungerstedt 1991).

It is generally considered necessary to calibrate the probe in vivo (for review see Ungerstedt 1991 or Chaurasia 1999) to assess changes in the in-flux of substances into the probe (relative recovery, RR), since RR is suggested to increase with exercise in the absence of true interstitial changes (MacLean et al. 1999). RR of lactate (MacLean et al. 1999) and PGE\(_2\) (Karamouzis et al. 2001) have been reported to increase considerably (100-600\%) with increased intensity of dynamic leg muscle contractions, due to changes in intramuscular pressure (IMP) and blood flow, according to the authors. However, in MD-studies of low-load work investigating the trapezius muscle, no change (Ashina et al. 2002; Ashina et al. 2003; Rosendal et al. 2004a) or a minimal (2\%) increase (Rosendal et al. 2004b) in RR of lactate were reported during work. This is probably due to the low intramuscular pressure (Järvholm et al. 1991) and small changes in blood flow.
AIMS OF THE THESIS

General aim

Even though epidemiological research have provided strong evidence for the notion that low-load work and mental load are important factors in the genesis of WRMP, the causal link between these risk factors and the development of muscle pain is still not understood. A fruitful approach to this problem is to measure the effects of standardised physical and mental stimuli in controlled laboratory settings, with methods that allow local measurements in muscle, like MD and NIRS. Accordingly, the general aim of this thesis was to contribute to the contemporary knowledge of WRMP by investigating the effects of low-load repetitive work and mental load on interstitial changes in sensitising substances (glutamate, PGE₂, NE) and muscle metabolism (lactate, pyruvate and oxygenation) in the trapezius muscle of females suffering from trapezius myalgia and healthy controls, using MD and NIRS.

Specific aims

Paper I

To determine, and to compare, the interstitial concentrations of PGE₂ and glutamate in the trapezius muscle of females with trapezius myalgia, with those in pain-free controls. A further objective was to employ a systematic approach to determine the equilibration times for these substances, in order to determine when samples should be collected.

Paper II

To investigate the effects of low-load repetitive work and the duration of work on intramuscular concentrations of lactate, pyruvate, glutamate, and PGE₂, in the trapezius muscle of healthy females, and also to investigate whether changes in lactate concentration in response to low-load work were correlated to local muscle oxygen saturation.

Paper III

To investigate and compare the effects of low-load repetitive work on intramuscular concentrations of lactate, pyruvate, glutamate, and PGE₂, in the trapezius muscle of females with trapezius myalgia and healthy controls. Also,
to investigate whether changes in lactate concentration in response to low-load work were correlated to local muscle oxygen saturation.

**Paper IV**

To determine and compare the effects of low-load repetitive work with and without superimposed mental load on intramuscular NE concentration, trapezius muscle activity and oxygen saturation in the trapezius muscle of healthy young females. Further to investigate whether NE concentration during work was related to increased muscle activity, and/or signs of localised muscle fatigue.
METHODS

Materials and experimental designs

Paper I
Nine females with chronic trapezius myalgia (TM) (mean age 44.1 (35-53)) and nine healthy age-matched controls (CON) (mean age 42.2 (35-52)) participated in the study. The inclusion criteria for the pain group were non-specific localised muscle pain in the shoulder, sustained for a period of > 3 months, and no history of trauma to the neck or shoulder. The subjects in the pain group were otherwise subjectively healthy, i.e. they had no signs of any generalised inflammatory joint- or muscle disease or arthrosis. Microdialysate samples for glutamate and PGE2 analyses were obtained from the upper trapezius muscle every 30 min during 4 hours of rest.

Paper II
This study included 20 healthy female subjects (mean age 40.3 (31-51)) with no reported upper extremity musculoskeletal disorders. Subjects were randomised into groups performing either 30 or 60 min low-load repetitive work, and studied during 120 min initial rest, 30 or 60 min work and 60 min recovery. Microdialysate samples, for determination of lactate, pyruvate, glutamate and PGE2, were obtained.

Paper III
Fourteen female subjects (mean age 40.0 (± 8.0)) with trapezius myalgia participated in the study. The pain subjects were matched in age to a group of healthy asymptomatic females, who had participated in a previous study (paper II) at our laboratory. The mean (±SD) duration of complaints from the neck-shoulder were 70.5 (± 74.7) months.

Inclusion criteria were, that during the clinical examination subjects were required to (i) report pain of a duration of at least 3 months from the neck-shoulder region, (ii) verify pain in the upper part of the trapezius muscle with a pain drawing, (iii) have reason to believe that the pain had been caused by their work, and (iv) have the most pronounced complaints on the side subjected to the greatest workload. Exclusion criteria were (a) previous trauma to the neck or shoulder, (b) signs of shoulder tendonitis or shoulder joint affection, (c) signs of nerve affection (d) pronounced pain from more than three body regions, and (e)
other diseases, and lastly (f) medication. None of the subjects were on sick-leave. The same experimental design was used as in study II.

**Paper IV**

This cross-over design study included 15 healthy female subjects (mean age 24 (±2.0)), with no reported upper extremity musculoskeletal disorders. Each subject was individually exposed twice; once to repetitive work only (LLRW) and once to repetitive work with superimposed mental load (LLRW+ML), during two different experimental days. The order of sessions was randomised. The subjects were at each occasion studied during 135 min rest, 30 min work and 60 min recovery. Microdialysate samples, for determination of noradrenaline were obtained.

All the female subjects that participated in the studies were Caucasians, right-handed non-smokers. Also, they had normal body weight, were not pregnant or allergic to local anaesthesia (for further details on personal characteristics and experimental designs, see respective paper).

**Methods**

The methods used in the thesis are summarised in Table 1 (see page 23).

**Microdialysis (Paper I, II, III and IV)**

MD permits measurements of changes in substance concentration in muscle in response to work, and with a minimal trauma (Ungerstedt 1991). In paper I, II, III and IV, microdialysis was performed in the upper trapezius muscle.

After local anaesthesia, a commercially available MD-probe (CMA 60, 20 kDa molecular cut-off, membrane length 30 mm, 0.5 mm outer diameter) was implanted in parallel with the muscle fibre direction in the middle third of the upper trapezius muscle. The MD-probe was perfused with a physiological solution at the following flow-rates; 0.3 μL min⁻¹ in paper I, 2 μL min⁻¹ in paper II and III, and 5 μL min⁻¹ in paper IV. Samples were obtained every 30th minute during the whole 4 hours resting period in paper I, during the initial two hours of rest in paper II, III and IV, and every 15th minute during work and the subsequent recovery time. The samples were immediately frozen and kept at -70°C until analyses were performed. In study IV, the microvials were prepared with hydrochloric acid (0.03 times the expected volume) and they were kept on ice during the sampling of dialysate to prevent degradation of NE.
All samples were coded by the authors and analysed blindly by an independent laboratory assistant.

PGE\textsubscript{2} was analysed with a radioimmunoassay kit (Du Pont, Boston, Mass, USA) and lactate, glutamate and pyruvate with a CMA 600 Microdialysis Analyser (CMA Microdialysis, Solna, Sweden). NE was analysed with high precision liquid chromatography with electrochemical detection (HPLC-ED).

**Near infrared spectroscopy - NIRS (Paper II, III and IV)**

With near infrared spectroscopy (NIRS), non-invasive measurements of percent tissue O\textsubscript{2} saturation (\%StO\textsubscript{2}) (and blood volume) can be performed (Boushel & Piantadosi 2000; Ferrari et al. 2004). The NIRS-technique is based on the principle of differential absorption properties of oxygenated and deoxygenated forms of haemoglobin (and to a lesser extent, myoglobin) in the near infrared range (Boushel & Piantadosi 2000; Ferrari et al. 2004).

Measurements of local tissue oxygen saturation (StO\textsubscript{2}\%) were performed in study II, III and IV using a near infrared spectrometer, NIRS (InSpectra Tissue Spectrometer – model 325, Hutchinson Technology Inc, the Netherlands). A self-adhesive O\textsubscript{2}-shield, connected to the optical cable, was placed on the skin overlying the upper part of the trapezius muscle, and in close proximity to the MD-probe. Before placement of the optical probe, the system was calibrated according to instructions from the manufacturers, i.e. inserted in a light scattering calibrator for capturing reference light intensities of all wavelengths. All tissue measurements were related to the reference measurement, thereby converting light intensity measurements to optical absorbance. Optical absorbance values were further processed into a scaled second derivative absorbance spectrum, whereby a measure of oxygen saturation was obtained (\%StO\textsubscript{2}). The software supplied with the InSpectra device allows for absolute values \%StO\textsubscript{2} In study II and III, the distance between the light transmitter and the detector was 12 mm, and in study IV 24 mm. The sampling frequency was 0.3 Hz.

**Electromyography-EMG (Paper IV)**

Bi-polar surface electrodes (SEMG) were used in study IV to determine and compare the degree of global trapezius muscle activity during repetitive work with and without superimposed mental load, and to study possible EMG-signs of muscle fatigue (\downarrow\text{RMS}, \uparrow\text{MPF}). For details on the electrode placement, EMG-registration, and calculations, see methods section of paper IV. Three test-contractions (TC) were performed by the subjects; before the work, directly after
work and after the recovery period. The TCs were performed with the right arm elevated to 90 degrees, the elbow straight, semi-pronated arm and a 1 kg dumbbell in the hand. All RMS-data was referred to as % of the first reference contraction. The second and third reference contraction was used to investigate possible signs of muscle fatigue during the experiment. For the EMG-data obtained during work, root mean square (RMS) and mean power frequency (MPF) were calculated.

**Blood sampling (Paper II, III and IV)**

In paper II and III, capillary blood samples for lactate analysis were obtained (at baseline, directly after work and recovery). The coded samples were analysed with a lactate analysis device YSI 2300 STAT plus (Clandon Scientific, Famborough, UK) by an independent laboratory technician.

In paper IV, venous blood samples for NE analysis were obtained (at baseline, after work with and without mental stress, and after the recovery period). The NE concentration in the venous blood was determined with HPLC-ED.

**Repetitive work model (Paper II, III and IV)**

The work model was designed to simulate an occupational work task, and consisted in alternatively pushing in a piston and pressing down a button, with the use of a hand-held manipulandum (130 grams), while seated at a table (see Fig. 1). The subjects maintained, with the aid of a metronome, a pace of 30 work cycles per minute, and a single piston push followed by a button press constituting one work cycle. The workstation consisted of a height-adjustable table and office chair, to provide optimal ergonomics for each subject.

![Fig. 2. The low-load repetitive work consisting in a) pushing in a piston and b) pressing down a button on the table with a handheld manipulandum (130 g), at a pace of 30 work cycles per minute.](image-url)
In a pilot study from our laboratory including healthy subjects (unpublished data), we used electromyography to assess the trapezius muscle activity during the same repetitive work as has been used in these studies, and found the mean electrical activity to be 9.3% of maximal voluntary contraction, which is similar to muscle activity measured during low-load work at a real work place (Jensen et al. 1993).

**Mental tasks (Paper IV)**

Stroop colour word task (Stroop 1935), SCWT and the so called Norinders test, which is a mental arithmetic test, were used as stressors during the repetitive work stress sessions (see Frankenhaeuser & Lundberg 1977). These tests have been used before to evaluate the effects of mental load during static muscle contractions (Lundberg et al. 1994). See paper IV for a detailed description of the tests.

**Subjective ratings**

In study II, III and IV, subjects rated their perceived muscle fatigue in the right shoulder according to the Borg CR-10 scale (Borg 1982) with “0” representing no fatigue and “10” severe fatigue. Ratings were obtained at baseline, at the end of both work and recovery period, and (in study IV only) also in the middle of the recovery period.

In study IV, subjects rated their perceived mental stress according to the Borg CR-10 scale with 0 = no stress and 10 = severe stress. Ratings were obtained at base-line, at the end of the work period, and in the middle and at the end of recovery period.

In study III, subjects rated their perceived pain in the right shoulder at baseline, and at the end of both work and recovery period, using the non-hatched visual analogue scale (VAS), marked at one end as “no pain at all” and at the other “worst pain imaginable” (Melzack & Katz 1999).

**Blood pressure and heart rate**

In study IV, measurements of blood pressure and heart rate were obtained with an automatic measuring device (Omron M4, OMRON Corporation, Japan), at baseline, after work, after 30 min recovery and at the end of the recovery period. The blood pressure was measured in the left arm.
**Ultrasonography**

In paper III and IV an ultrasonography apparatus (Aloka SSD-2000, Aloka Co, Ltd, Japan) was used to facilitate the probe insertion into the trapezius muscle and to verify the placement of the probe. It was also (paper II, III and IV) used to estimate the skin-fat layer and the trapezius muscle thickness at the site of the probe (see paper III for a more detailed description).

**Table 1.** Summary over stimuli and methods used in studies.

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**Nordic Ministry Council Questionnaire & Clinical examination**

In study I and III, The Nordic Ministry Council Questionnaire (NMCQ) (Kourinka et al. 1987) and the visual analogue scale (VAS) were used to survey pain during the last 12 months and at the time of participation. All participants were clinically examined by a physiotherapist specialised in rehabilitation. For further details concerning inclusion and exclusion criteria, see respective papers.

**Statistics**

The level of significance was for all analyses set to P<0.05. For details on the statistics used, see the respective papers.
RESULTS

Paper I

Equilibration (i.e. steady-state) for PGE$_2$ was reached at 180 and 150 min (see Fig. 3), and for glutamate at 150 and 120 min (see Fig. 4) after probe insertion for TM and CON respectively. At equilibration, the mean [PGE$_2$] (± SE) was 0.71 (± 0.11) ng mL$^{-1}$ for TM and 0.97(±0.35) ng mL$^{-1}$ for CON. For glutamate, the mean concentration was 66.3 (±13.3) µmol L$^{-1}$ for TM and 60.6 (±22.9) µmol L$^{-1}$ for CON. The study showed no differences between TM and CON in absolute concentrations of PGE$_2$ and glutamate in the trapezius muscle.

![Fig. 3. PGE$_2$ concentrations (ng mL$^{-1}$) at different time-points after probe insertion. For each time-point the mean ± SE and the sample size are presented. Filled bars represent the values for the pain-group and unfilled bars those for the control group. The arrows indicate the time-point of equilibration for each group.](image-url)
Fig. 4. Glutamate concentrations (μM L⁻¹) at different time-points after probe insertion. For each time-point the mean ± SE and the sample size are presented. Filled bars represent the values for the pain-group and unfilled bars those for the control group. The arrows indicate the time-point of equilibration for each group.
**Paper II**

The main results of the study were significantly increased interstitial [lactate] and [glutamate] in the trapezius muscle of healthy asymptomatic females in response to LLRW. However, no progressive increase with increasing work duration was found. Further, PGE$_2$ did not accumulate in the muscle during work (see Fig. 5 for all MD results). Local muscle oxygen saturation (% StO$_2$) did not change significantly in response to LLRW and was not correlated to the increased [lactate]. Thus, we found no indications that the increased [lactate] was due to a locally decreased or insufficient muscle oxygenation in healthy asymptomatic females.

![Fig. 5. Intramuscular microdialysate concentration (mean ± SEM) of (mM L$^{-1}$), pyruvate (mM L$^{-1}$), glutamate (µM L$^{-1}$), and PGE$_2$ (µg mL$^{-1}$) at baseline, in response to work and recovery for REP 30 and REP 60 respectively (healthy subjects). Significant differences with respect to baseline are indicated with *.
](image-url)
The main results of this paper were the significantly increased interstitial [lactate] and [pyruvate], and decreased %StO₂ found in the trapezius muscle of TM in response to LLRW, and the progressive increase in [pyruvate] found during recovery. No accumulation of [PGE₂] was found in response to LLRW in TM, and significantly lower [glutamate] in TM, as compared to CON (see Fig. 6 for all MD results). There were no correlations between [glutamate] and pain intensity in TM. Also, the effects of LLRW on [lactate] tended to be significantly correlated to the effect on muscle oxygen saturation in TM.

Fig. 6. Intramuscular microdialysate concentration (mean ± SEM) of lactate (mM L⁻¹), pyruvate (mM L⁻¹), glutamate (µM L⁻¹), and PGE₂ (µg mL⁻¹), at baseline, in response to work and recovery for TM and CON. Significant differences with respect to baseline are indicated with * for a p-value <0.05, ** for a p-value<0.01, and significant differences between groups with #.
Paper IV

The main findings of the study were that interstitial [NE] increased significantly in the trapezius muscle of healthy females in response to LLRW (see Fig. 7). There was no significant additional increase when mental load was superimposed onto the LLRW (LLRW = 96%; LLRW+ML = 126%). However, a significant interaction was found between occasions for [NE] in muscle during recovery, with a slower recovery of [NE] after LLRW+ML, as compared to LLRW.

Fig. 7. Mean (± SEM) interstitial NE concentration (nM L⁻¹) during the stabilisation period, at baseline, in response to work and recovery. Filled circles represent data for LLRW and unfilled circles LLRW+ML. Significant differences with respect to baseline are indicated with * for a p-value<0.05, ** for a p-value<0.01 and *** for a P-value<0.001.

There were no firm EMG-signs of muscle fatigue during work with, and without mental load (i.e. no frequency changes), only slightly increased RMS with increasing work duration. The RMS was still significantly increased after recovery. No differences were found between occasions, when comparing RMS of the whole work periods. However, during LLRW+ML, when mental load was superimposed on the repetitive work, the RMS was significantly increased as compared to periods involving repetitive work only (see Fig. 8).
Fig. 8. Mean (± SEM) RMS (root mean square) EMG during 30 min LLRW+ML. Filled bars represent work periods with mental load, and unfilled bars work periods without mental load.

For LLRW, but not for LLRW+ML, interstitial [NE] was significantly correlated to EMG-activity during work. The %StO₂ decreased significantly and to a similar extent both during LLRW and LLRW+ML (see Fig.9).

Fig 9. Mean (± SEM) local muscle oxygen saturation (%StO₂) at baseline, in response to work and recovery. Filled circles represent data for LLRW and unfilled circles LLRW+ML. Significant differences with respect to baseline are indicated with * for a p-value<0.05, ** for a p-value<0.01 and *** for a p-value<0.001.
DISCUSSION

Main findings
The main findings of the thesis are: (i) no accumulation of [PGE$_2$] in response to LLRW nor increased levels in TM compared to CON even at rest; (ii) no increased [glutamate] in TM compared to CON; (iii) increased anaerobic metabolism in response to LLRW in both pain subjects and healthy subjects; (iv) no difference in either metabolism or accumulation of sensitising substances with increased work duration in healthy subjects; (v) during recovery there was a progressive increase in [purvivate] for TM, and a significant interaction between [purvivate] in TM and CON; (vi) increased interstitial [NE] in response to LLRW, but no additionally increased interstitial [NE] with superimposed mental load; (vii) significant interaction between [NE] during recovery after LLRW vs. after LLRW with superimposed mental load, in healthy subjects; (viii) at least 120-150 min. stabilisation time after probe insertion is required to achieve steady state for glutamate and PGE$_2$.

PGE$_2$
There were no differences in [PGE$_2$] between TM and CON in any of the studies, nor could we find any effect of LLRW on [PGE$_2$]. This may be taken as an indication that there is no ongoing inflammation in the chronic phase of trapezius myalgia. The findings and conclusion are in general agreement with another human study on the effects of low-load static work on [PGE$_2$] in muscle (Ashina et al. 2003).

However, it is not clear at what time point in the disease process an accumulation of inflammatory mediators such as PGE$_2$ may occur in the muscle. It is possible that increased [PGE$_2$] is only present in the acute phase of the development of WRMP. Indeed, Larsson et al. (2000) found no signs of inflammation when investigating muscle biopsies from subjects with chronic trapezius myalgia. It has been suggested that later in the disease process, when the pain has become chronic, a shift occurs from pain mechanisms in the periphery to mechanisms in the central nervous system (Windhorst 2003). This would be in accordance with the hypothesis of initial tissue damage and inflammation as an initiating factor to the development of WRMP proposed by Barbe et al. (2006).
There is some evidence from animal studies for muscle inflammation in response to performance of LLRW (see review by Barbe et al. 2006). In a rat model of low-load repetitive work, signs associated with inflammation (e.g. increased concentrations of interleukins and macrophages) in muscle biopsies were reported by Barbe et al. (2003). In humans, increased levels of PGE\(_2\) have been reported in tenosynovial tissues collected from patients with carpal tunnel syndrome (Freeland et al. 2002; Hirata et al. 2005), but in the study by Hirata et al., the increase was only evident in the early stages (between 4-7 months duration). On the other hand, no pathological intratendinous [PGE\(_2\)] was found in chronic Achilles tendinopathy (Alfredsson et al. 1999). Furthermore, in subjects with chronic trapezius myalgia, interstitial cytokine Interleukin 1\(\beta\) concentrations (IL-1\(\beta\)) have been reported to be below detection level (Rosendal 2004b), indirectly indicating no increased PGE\(_2\) synthesis. IL-1\(\beta\) enhances the expression of COX 2, a pro-inflammatory enzyme with an important role in the synthesis of PGE\(_2\) and other prostanoids (see Barbe et al. 2006).

It remains to discuss the lack of effect of LLRW on [PGE\(_2\)] in paper II and III. Increased intramuscular [PGE\(_2\)] in response to high-intensity contractions have been reported both from animal (Symons et al. 1991) and human studies (Karamouzis et al. 2001). However, this is not seen during intermittent static contractions (Karamouzis et al. 2001). Karamouzis et al. (2001) suggested that [PGE\(_2\)] may only be affected when the blood velocities are high, i.e. during high intensity dynamic contractions.

In conclusion, the findings in paper II and III do not support an accumulation of PGE\(_2\) during low-load work or the presence of inflammation in the chronic stage of trapezius myalgia.

**Glutamate**

There was a significant and equal increase in [glutamate] in response to LLRW, for both TM and CON. This is in accordance with the studies of low-load work by Ashina et al. (2003) and Rosendal et al. (2004b), while Axelsson et al. (2002) reported unchanged [glutamate] in response to work. Since [glutamate] was not specifically increased in TM, nor correlated to pain intensity, these findings may be interpreted as signs of normally increased metabolism, in response to work, not to increased peripheral pain signalling in TM.

It is exceedingly difficult to provide an unambiguous interpretation of the differences and changes in interstitial [glutamate], within or between groups, because glutamate is not only the most abundant neurotransmitter in the human body it is also a key metabolite in cellular metabolism. It is well-known for its
pain-mediating function in the CNS (Hargreaves et al. 1994), while its role in pain transmission in the periphery is not fully investigated. In subjects with chronic tendinopathy, increased intratendinous \[\text{glutamate}\] during rest has been reported. However, increased vascularisation was also found, which may be indicative of an increased metabolism (Öhberg & Alfredson 2001).

Injections of glutamate into the masseter muscle have been reported to cause significantly higher levels of peak pain and duration of pain than placebo injections (Cairns et al. 2001), to sensitise rat muscle afferent fibres (Cairns et al. 2002), and to evoke pain in part through activation of peripheral NMDA receptors (Cairns et al. 2003). However, it is questionable whether the mechanisms for experimentally induced acute muscle pain and chronic work-related pain are the same.

Glutamate is suggested to be released from peripheral afferent fibers when nociceptors are activated, e.g. due to inflammation (Svensson et al. 2003). However, Ashina et al. (2005) found no signs of increased release of glutamate from nociceptors during or after experimentally induced pain and tenderness. Hence, it seems that peripheral pain per se does not induce higher levels of glutamate.

A somewhat problematic result of paper III is that \[\text{glutamate}\] was at all time points significantly lower in TM compared to CON, even during rest. Other studies have either found no differences between groups during rest (Axelsson et al 2002; Ashina et al. 2003), or higher levels in pain subjects (Rosendal et al. 2004b). There is no clear explanation to this mismatch. A suggestion is, that too few studies have so far been conducted with too few subjects of different study groups, to establish a consensus on what is to count as a normal vs. pathological glutamate concentration, and that the role of glutamate in peripheral muscle pain needs to be further investigated.

In conclusion, the findings in paper III do not support a greater accumulation of glutamate in TM during low-load work, or higher baseline levels, as compared to controls.

**Lactate, pyruvate and oxygenation**

Increased \[\text{lactate}\] in response to LLRW was found in both TM and CON, but no differences between groups, and \[\text{lactate}\] was still increased at the end of the recovery period. Furthermore, there was no progressive increase in \[\text{lactate}\] due to work duration. These findings indicate that low intensity work causes an increase in anaerobic metabolism in both TM and CON. This may be interpreted as a normal response to increased metabolic demands.
The findings of studies investigating the effects of LLRW on interstitial [lactate] in pain subjects and controls are conflicting. Ashina et al. (2002) reported, in accordance with paper III, increased [lactate] in the trapezius muscle to low-load static work but no differences between pain subjects and controls. Rosendal et al. (2004b) reported increased [lactate] in response to LLRW in female pain subjects but not in controls. The different results may perhaps partly be a consequence of the difference in the experimental work-models and the exposure times used in various studies.

There was a significant increase in [pyruvate] during work for TM, but not for CON. During recovery [pyruvate] was significantly increased as compared to baseline in both groups. These findings may be signs of increased metabolism in general in response to LLRW (Wasserman et al. 1985). However, while [pyruvate] remained the same for CON during the whole recovery period, it showed a progressive increase for TM, which is more difficult to interpret. It is recognised that oxidation of lactate into pyruvate is enhanced during exercise, and that muscles revert from net lactate release to net uptake during prolonged low-intensity exercise (Gladden 2004). One may speculate whether the progressive increase in [pyruvate] in TM may be caused by an enhanced oxidation of lactate into pyruvate caused by a greater production of lactate, which provokes an early reversion from net release to net uptake of lactate, thus masking the higher lactate production in TM.

Another possible explanation is that TM activate fatigueable type II fibers to a greater extent than CON, possibly because of metabolic insufficient type I muscle fibers in TM (Kadi et al. 1998; Larsson et al. 2004). That could explain the greater production of pyruvate already during work in TM. This would be in accordance with the greater contribution of type II fibers during low-intensity work reported for chronic pain cases (Kallenberg & Hermens 2006), and with the higher perceived exertion reported by TM in paper III. However, this remains a speculation, since EMG-activity was not measured.

In conclusion, the progressive increase in [pyruvate] during recovery in TM constitutes a significant difference in the effects of work on the interstitial milieu of the trapezius muscle in TM and CON. This finding may possibly reflect an altered metabolism in TM. However, more research is needed to establish the precise nature of this possible alteration.

In paper III there was a small but significant decrease in local trapezius muscle oxygenation in response to LLRW in TM but no for CON, but no differences between groups. In TM a tendency to a significant correlation between the effects of work on oxygenation and IMMD [lactate] was found.
This could be interpreted as an indication of a small but significant increase in metabolic demands in TM. However, the work intensity as well as the training level may influence the effect of oxygenation (see Boushel and Piantadosi 2000 for review). Since the work was standardised, the work intensity was controlled for, but not for training level. Hence the increased metabolic demand in TM may merely reflect a lower capacity, and not necessarily an altered metabolism or impaired delivery.

**Norepinephrine, muscle activity and oxygen saturation**

Study IV showed significantly increased interstitial [NE] in the trapezius muscle of healthy females in response to LLRW. This is in line with a number of occupational and exercise studies showing an increased [NE] in urine and plasma in response to work and exercise (for an overview, see Sluiter et al. 2000). However, it has been estimated that only 20% of the NE found in plasma at rest is derived from muscle (Esler et al. 1984), and that plasma and urine NE are closely correlated (Sluiter et al. 2000). Therefore MD must be considered a better measure of what happens locally in muscle. To my knowledge, this is the first MD study of the effects of LLRW on trapezius interstitial [NE]. Other human MD studies have found increased [NE] in response to high-intensity exercise in muscle (Costa et al. 2002) and in adipose tissue (Stallknecht et al. 2001). The findings in paper IV show that low-load repetitive work causes a significant and measurable increase in intramuscular [NE] as well.

There was a small but not statistically significant additional increase in interstitial [NE] when mental load was added to the physical load. Lundberg et al. (1994) reported increased [NE] in urine in response to low-load static contractions with superimposed mental load, while Garde et al. (2003) found a progressive increase in [NE] in urine during four hours intermittent static hand-grip contractions with superimposed mental load. On the other hand no increase in [NE] in urine was found in response to physical and mental demands during computer work (Garde et al. 2002).

It is difficult to compare these results due to differences in experimental designs. The physical demands in paper IV were high compared to Lundberg et al. (1994) and Garde et al. (2002), and the periods of superimposed mental loads were shorter. In addition, increased [NE] in urine reflects a systemic effect of increased sympathetic outflow and not just local effects in muscle.

It is known that arm work per se has a large effect on the SNS-activity (Saltin et al. 1998), and this might perhaps make a small effect of an intermittent superimposed mental load on interstitial [NE] difficult to distinguish. A
drawback in paper IV was that the effect of mental load per se was not evaluated. In conclusion, low-load repetitive work caused a significant increase in intramuscular [NE], but the addition of mental load did not elicit any further increase.

On the other hand there was a somewhat slower recovery to baseline interstitial [NE] after LLRW+ML as compared to LLRW, and it may be speculated whether this may be caused by a sustained activity of SNS during recovery due to the effects of the mental load. It has been suggested that insufficient recovery could be an important factor in the development of WRMP (Lundberg 1999; Sluiter et al. 2000; Lundberg 2005).

In addition, the exposure time to low-load work and to mental load in paper IV was short as compared to the exposure during a whole working day. More studies, with longer exposure to both LLRW and mental load, and including pain subjects, are needed to further elucidate the effects on intramuscular NE and its possible role in the development of WRMP.

In paper IV, a small but significant progressive increase in EMG-activity during work was found on both occasions, and EMG-activity and interstitial [NE] were significantly correlated for LLRW. These findings are in line with results from many occupational and exercise studies, in which [NE] in plasma and urine have been reported to increase in healthy subjects in response to exercise and with exercise intensity (for review, see Sluiter et al. 2000).

Even though no significant differences were found in EMG-activity during work between occasions, EMG-activity was not, surprisingly, correlated to interstitial [NE] during LLRW+ML. This may be explained by the fluctuating RMS found during LLRW+ML. EMG-activity was significantly increased during the intermittent periods of superimposed mental load, as compared to periods involving repetitive work only. This indicates that superimposed mental load has an effect on muscle activity, but that the intermittent periods of mental load may have been too few and/or too short to produce a measurable increase in muscle activity. Several studies have shown increased muscle activity in response to mental load (see Wærsted (2000) for review).

In paper IV, there were no firm EMG signs of localised muscle fatigue (unchanged MPF) in response to work on any occasion. However, the RMS was still increased during test-contractions at the end of the recovery period on both occasions, which indicates that the subjects were not unaffected by the work.

On both occasions %StO₂ was significantly and similarly decreased during work. Bias due to training level can be excluded, because the subjects were their own controls. This is probably a sign of normally increased oxidative
metabolism in response to work. Since no difference between occasions was found, it does not seem as if the superimposed mental load gave rise to increased vasoconstriction, and thereby impaired oxygen delivery, in the healthy females.

**Methodological considerations I – Microdialysis**

To avoid biased results when performing MD, there are some important technical aspects to consider, notably concerning the: (i) stabilisation period, (ii) trauma caused by the probe, (iii) possible draining of the interstitial milieu, (iv) relative recovery, and (v) probe calibration.

**(i) Stabilisation period**

It is important to allow enough time for stabilisation of the interstitial milieu after the initial trauma caused by the probe insertion (Ungerstedt 1991). According to Ungerstedt (1991) the degree of initial tissue trauma determines how rapidly baseline levels are reached. To minimise the trauma, and thereby possibly shortening the stabilisation time, a commercially available probe was used, which requires only one penetration of the muscle fascia, and not two as the custom made probes often used. Baseline samples were obtained at 120 min (Paper II and III) and at 135 min (Paper IV) after probe insertion, which has been suggested to be sufficient for reaching a steady state for lactate (Rosdahl et al. 1998), and for glutamate in paper I, at least for healthy subjects. For NE, the stabilisation time is more uncertain.

There were no significant differences between the two last samples obtained during the stabilisation time in study II and III, for [lactate], [pyruvate], or [glutamate], while for [PGE₂] a significant decrease was found, which indicates a sufficiently long stabilisation time for most but not all substances. Also, for NE, a significant decrease in concentration between the two last samples obtained before work was found, which could possibly indicate an insufficient stabilisation. This, however, should not have affected the reliability of the increase found in [NE] in response to work. An insufficient stabilisation time may, however, affect the reliability of comparisons between baseline and recovery, as is discussed in paper III concerning PGE₂.

It is possible that in general, stabilisation times are longer for pain-subjects. In paper I, the estimated stabilisation time for PGE₂ and glutamate was found to be somewhat longer for the pain subjects. It may be speculated that individuals with pain are tenser than healthy controls during probe insertion, thereby suffering a higher degree of trauma, or maybe it is due to increased pain sensitivity of the tissues, giving rise to a greater reaction to the trauma.
(ii) Trauma during work

It should also be considered whether the changes in substance concentrations found in response to work are partly due to trauma caused by the presence of the probe in the active muscle and not a result of the work per se. The risk for trauma during work is presumably smaller in the trapezius muscle as compared to large dynamic muscles, e.g. vastus lateralis, since the contraction force is much lower. Furthermore, the flexible small-diameter probe is implanted in parallel with the muscle fiber direction, which should minimise the trauma. This is a possible source for bias in all microdialysis studies on the effects of work or exercise, which is difficult to control for. However, comparisons between groups and occasions should not be as much affected.

(iii) Draining of the interstitial space

It has been suggested, that there is a risk for drainage of substances from the interstitial space, thereby disturbing the interstitial milieu, and that glucose and lactate should therefore be added to the perfusate to minimise the risk (Lönnroth 1987). In the current studies, no lactate or glucose were added to the perfusion fluid. According to Rosdahl et al. (1993), glucose need not be included in the perfusion fluid in order to obtain normal dialysate lactate values. Further, the authors report that the dialysate lactate level in muscle is not affected by large changes in the interstitial glucose concentration. Glucose concentration in dialysate is dramatically affected by (relatively large) changes in blood flow, while lactate is less affected by changes in blood-flow (Rosdahl et al. 1993). Furthermore, according to Ungerstedt (1991) the risk of draining the interstitial space is small when using flow rates between 0.1 - 5 µL min⁻¹. Therefore, during low-load work (with small blood flow changes) there is little risk for draining of the interstitial space.

(iv) Relative recovery

It is generally considered necessary to calibrate the probe in vivo to assess changes in the in-flux of substances into the probe (relative recovery, RR), since RR is suggested to increase with exercise in the absence of true interstitial changes (MacLean et al. 1999). RR of lactate (MacLean et al. 1999) and PGE₂ (Karamouzis et al. 2001) has been reported to increase considerably (100-600%) with increased intensity of dynamic leg muscle contractions, due to changes in intramuscular pressure (IMP) and blood flow, according to the authors.

However, it is questionable whether an estimation of RR really is necessary in studies of low-load work. In MD studies of the trapezius muscle, no change
or a minimal (2%) increase (Rosendal et al. 2004b) in RR of lactate was reported in response to low-load exercise. The lack of change in RR in these studies may be explained by the fact that IMP in the trapezius muscle does not increase significantly during low-load work (Järholm et al. 1991). Further, while MacLean et al. (1999) reported a 20-fold increase in blood flow in response to leg muscle contractions (50 W), only small increases in blood flow have been reported in the trapezius muscle in response to low-load work (Ashina et al. 2002; Rosendal et al. 2004b). Small changes in blood flow, as during low-load work, are suggested not to affect RR of substances in vivo (Scheller & Kolb 1991; Rosdahl et al. 1993). In the current studies, RR was not assessed, and therefore a bias cannot be excluded, although it is unlikely that this bias is significant for the reasons given above.

(v) Probe calibration

Most MD studies are performed during non-equilibrium conditions, and absolute recovery of substances into the probe is strictly speaking only possible to achieve using very slow flow rate (0.3 µL min⁻¹). However, slow-flow methods, including the extrapolation to zero-flow (Jacobsson et al. 1985), and zero-net flux (Lönnroth et al. 1987) methods, yield very little sample volume per time unit, and therefore have poor temporal resolution (for details, see Ungerstedt 1991; Chaurasia 1999). They are therefore unsuitable for studying effects of relatively short periods of work or exercise.

The retro-dialysis method (internal standard method) (Larsson 1991) does not yield absolute recovery, but it is assumed that the absolute concentration of the substance in the tissue can be calculated. The method of calculation is based on the assumption that the recovery of the analyte is equivalent to the delivery of the internal standard. However, the accuracy of probe calibration can be questionable if the MD characteristics of the analyte and internal standard differ significantly in terms of physical properties (such as diffusion characteristics and method of analysis), and biological behaviour (such as metabolism, protein binding, receptor uptake and release etc.), of which the latter is often overlooked (Chaurasia et al. 1999). Furthermore, calculating small losses from the dialysate, when using retro-dialysis, is more prone to mathematical errors than the gain of molecules into the dialysate (Chaurasia et al. 2007).

Papers II-IV aimed at investigating the effects of LLRW by looking at changes in substance concentrations in the muscle relative to work, and not to assess absolute tissue concentrations. The flow-rates used (2-5 µL min⁻¹), may
not give a complete recovery of substances, but a steady state is known to be achieved which makes the relative changes independent of the degree of recovery (Chaurasia 1999). Further, in comparison with the substance concentrations reported in the literature, e.g. the reported lactate concentrations (see Rosdahl et al. 1998), the recovery in papers II-IV is relatively high.

**Methodological considerations II – Near infrared spectroscopy**

During exercise, skeletal muscle deoxygenate to varying degrees depending on work intensity and level of training (see review by Boushel & Piantadosi, 2000). In studies II-IV, the work intensity was standardised with the use a metronome. In studies II and III, the training level was not controlled for, which may constitute a possible bias. In study IV, however, the subjects were their own controls.

When evaluating changes in muscle oxygenation, it is also important to consider the contribution of the skin and subcutaneous fat layer to the NIRS-signal. In individuals with a high BMI, changes in the NIRS-signal may be blunted (McCully & Hamaoka 2000). However, when subjects are lean, as in papers II-IV, the contribution of skin overlying muscle has been shown to constitute less than 5% of the signal (Boushel & Piantodosi 2000).

Yet another factor of importance is the measuring depth of the O₂-probe. The muscle volume measured by NIRS is still controversial. NIRS signals are presumed mostly to be obtained from a tissue depth of 60% of the distance between the transmitter and detector, although a banana-shaped region of sensitivity extends both above and below this depth (Ferrari et al. 2004). In papers II and III, a 12 mm probe was used, to obtain measurements from the trapezius muscle and not from the underlying supraspinatus muscle, which means that NIRS data were obtained from a region above and below a tissue depth of around 7.2 mm. The estimated mean skin-fat-layer (SFL) of pain subjects in study III, over the upper trapezius muscle, was 4.7 mm, while it was 6.5 mm for the healthy subjects in study II. This means that there is a risk that the data for the healthy subjects may have been blunted due to some sampling of non-muscle tissue. However, the mean %StO₂ for the healthy subjects in that study was 80.4, which is in good agreement with the results reported by Ferrari et al. (2004) of around 80% StO₂ in the brachioradialis muscle. This can be compared with the mean value of 43.4 %StO₂ obtained over the trapezius muscle of an excluded subject with a skin-fat-layer of 15 mm.
In study IV, a 24 mm probe was used, which means that signals were obtained mostly from a depth above and below 14.2 mm, but with a maximal depth of about 24 mm. The combined mean SFL and trapezius muscle thickness was 16 mm, which means that there is a risk that the measurements derive partly from the underlying supraspinatus muscle.
CONCLUSIONS

I. The findings of the present thesis do not suggest the presence of inflammation in chronic trapezius myalgia.

II. There were no signs that chronic trapezius myalgia is associated with increased peripheral glutamate levels.

III. Short periods of LLRW were shown to cause increased anaerobic metabolism in both pain subjects and healthy subjects. However, during the relatively short exposure times, no sure indication was found that either metabolism or accumulation of sensitising substances would be affected with increased work duration.

IV. The progressive increase in [pyruvate] in TM constitutes a significant difference in the effects LLRW on the interstitial milieu of the trapezius muscle in TM as compared to CON. This finding may possibly reflect an altered metabolism in trapezius myalgia.

V. It was demonstrated that interstitial [NE] increases significantly in the trapezius muscle of healthy subjects in response to short periods of LLRW. However, no additionally increased interstitial [NE] due to intermittent superimposed mental load could be detected, even though there were signs of delayed recovery to baseline [NE] after LLRW with superimposed mental load.
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