From
DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
AND
DEPARTMENT OF CLINICAL SCIENCE AND EDUCATION, SÖDERSJUKHUSET
Karolinska Institutet, Stockholm, Sweden

BLOOD FLOW IN HUMAN SKELETAL MUSCLE – THE EFFECT OF ADRENALINE AND THE INFLUENCE OF A SMALL MUSCLE INJURY

TORBJÖRN VEDUNG

Karolinska Institutet

Stockholm 2010

This thesis is the result of a research collaboration between
the Department of Physiology and Pharmacology, Karolinska Institutet, and
the Department of Hand Surgery, Södersjukhuset, Stockholm.

Published by Karolinska Institutet.
Printed by Larserics Digital Print AB

ISBN 978-91-7409-659-0

Till Kristin, Johannes och Vera

ABSTRACT

A variety of vasoregulatory systems are involved in the complex control of blood flow in human skeletal muscle. The interaction between these systems where one system can override or modify the other makes blood flow regulation complicated. Determinations of muscle blood flow can thus be challenging especially when considering the shortcomings and limitations of the available measurement methods. This thesis focuses on two different topics of which both are dependent on sympathetic tone.

First, the common use of venous occlusion plethysmography (VOP) with only one strain gauge attached per limb is a method with obvious pitfalls. The inherent problems with this simplification of the original VOP method are highlighted in *Study I-II* where small variations in sympathetic tone and venous pressure proved to have a considerable influence on the results. The basic circumstance to this variability is the curvo-linear pressure/volume relationship in the veins and the fact that redistribution of blood can occur between individual limb segments. The results clearly demonstrate that events taking place under one strain gauge cannot be strictly duplicated in adjacent portions of the limb.

Secondly, recent reports have indicated that a minor muscle trauma might change the local blood flow response to adrenaline. Two studies were conducted to test if an acute small muscle injury (*Study III*) and chronic muscle damage (*Study IV*) influences the normal blood flow effect of adrenaline. In support of the hypothesis we found that the microdialysis catheter-induced muscle injury in *Study III* caused a significant vasoconstriction during an i.v. adrenaline infusion, as measured with ^{133}Xenon clearance next to the catheter, whereas no significant change in blood flow was seen with adrenaline-infusion in the absence of the catheter (conventional ^{133}Xe administration). The adverse adrenaline effect is likely to be related to the degree of invasiveness. Hence, it would be expected that any type of invasive measuring device causing a muscle injury could possibly provoke a similar reaction. This finding has a general physiological implication, but has also implications for the use of invasive techniques to study blood flow regulation in skeletal muscle.
Previous studies of tennis elbow (TE) have reported signs of diffuse muscle damage and decreased blood flow in the affected extensor carpi radialis brevis (ECRB) muscle. We conducted a case-control study (*IV*) to test the hypothesis that the muscle damage in the ECRB in TE alters the blood flow response to adrenaline in a vasoconstrictory direction. Muscle blood flow was determined with local clearance of ^{99m}Technetium during an i.v. infusion of adrenaline. In support of the hypothesis, the blood flow reaction to adrenaline was markedly different in the two study groups. Whereas the infusion did not significantly influence ^{99m}Technetium-clearance in the ECRB of controls there was a significantly decreased clearance in the patients. The altered adrenaline effect indicates a vascular dysregulation in TE, which is likely to be of clinical significance by contributing to the development and maintenance of the chronic muscle pain in this large patient group. Whether the vasoregulatory alteration, which would be expected to involve recurring relative muscle ischemia, represents the primary aetiology in TE or is a secondary effect of the muscle injury cannot be determined.
In conclusion, a small muscle injury, acute or chronic, seems to alter the effect of adrenaline on skeletal muscle blood flow in a vasoconstrictory direction.

Key words: adrenaline, blood flow, blood redistribution, ischemia, muscle injury, skeletal muscle, strain-gauge plethysmography, sympathetic tone, transmural pressure, ^{99m}Technetium clearance, tennis elbow, venous compliance, venous occlusion plethysmography, ^{133}Xenon clearance

LIST OF PUBLICATIONS

This thesis is based on the following manuscripts, which will be referred to in the text by their Roman numerals.

I. Lennart Jorfeldt, Torbjörn Vedung, Elisabeth Forsström, Jan Henriksson
Influence of leg position and enviromental temperature on segmental volume expansion during venous occlusion plethysmography
Clin Sci (Lond). 2003 Jun;104(6):599-605

II. Torbjörn Vedung, Lennart Jorfeldt, Jan Henriksson
Alterations in forearm position and environmental temperature influences the segmental volume expansion during venous occlusion plethysmography – special attention on hand circulation
Clin Physiol Funct Imaging (2009) 29, pp376–381

III. Torbjörn Vedung, Lennart Jorfeldt, Jan Henriksson
Intravenous adrenaline infusion causes vasoconstriction close to an intramuscular microdialysis catheter in humans
Clin Physiol Funct Imaging (2010) 30, pp 399-405

IV. Torbjörn Vedung, Michael Werner, Björn-Ove Ljung, Lennart Jorfeldt, Jan Henriksson
Adrenaline causes muscle ischemia in the tennis elbow patient but not in healthy controls
Submitted

CONTENTS

List of abbreviations

α	Alfa (receptor)
β	Beta (receptor)
ADR	Adrenaline
A_{II}	Angiotensin II
ECRB	Extensor Carpi Radialis Brevis
FBF	Forearm blood flow
NADR	Noradrenaline
NO	Nitric oxide
^{99m}Tc	^{99m}Technetium (Pertechnetate)
TE	Tennis elbow
VOP	Venous Occlusion Plethysmography
^{133}Xe	^{133} Xenon

THESIS AT A GLANCE

I) VENOUS OCCLUSION PLETHYSMOGRAPHY (VOP) – LOWER EXTREMITY

Can events taking place under one strain gauge be strictly duplicated in adjacent portions of the limb and be representative as blood flow in the whole limb?

Study design: 6 healthy subjects, 4 strain gauges at different levels of the lower limb, varying room temperature (warm, normal, cold) and varying leg position (up, horizontal, down).

VOP in its modern form, measuring volume expansion rate over an isolated limb segment, is associated with problems due to the curvo-linear pressure/volume relationship in the veins and the fact that redistribution of blood can occur between individual limb segments. The curvo-linear relationship between the transmural pressure (p) and the venous volume (v) furthermore changes with an increasing sympathetic tone. When the veins are in a relaxed state, the curve is S-shaped with a lower slope of the p/v curve at high transmural pressure than at low pressure. In contracted veins, the curve is shifted to the right, resulting in an increased venous pressure and lower venous volume. Depending on the position of the limb and whether the veins are in a relaxed or contracted state at the time of venous occlusion the obtained results will thus differ.

II) VOP – UPPER EXTREMITY, SPECIAL ATTENTION ON HAND CIRCULATION

Does the hand circulation influence the results of VOP with strain gauge technique?

Study design: 6 healthy subjects, 3 strain gauges at different levels of the forearm, varying room temperature (warm, normal, cold) and varying arm position (up, horizontal, down). Half of the measurements with, and half without, hand circulation.

The curvo-linear pressure/volume relationship in the veins causes similar problems to the VOP technique in the upper extremity as shown in the lower extremity (*Study I*). The usage or non-usage of a distal wrist cuff during VOP for exclusion of hand circulation influences the results in several aspects. With included hand circulation the highest expansion rate was found in the distal segment at normal and high temperatures, but in the proximal segment at low temperature. This pattern was found with all arm positions. With excluded hand circulation, there was a significant two factor interaction between arm position – strain gauge position, which was independent of temperature. The highest expansion rate was found in the proximal segment when the arm was elevated, but in the distal segment when the arm was lowered.

Conclusion Study I-II

VOP is frequently used with only one strain gauge attached to the limb and the results obtained is referred to as blood flow in the limb. Placement of the strain gauge at the maximal circumference of the limb with a distal occlusion cuff at the level of the wrist or ankle is regarded as standard in this technique. However, even if the procedure is standardized in this manner, events taking place under one strain gauge cannot be strictly duplicated in adjacent portions of the limb. Small variations in sympathetic tone and venous pressure can influence blood flow measurements with the VOP technique, even in intra-individual comparisons. This variability should be taken into account when strain-gauge plethysmography is applied for limb blood flow determination, especially in interventional studies.

III) SMALL MUSCLE INJURY - ALTERED EFFECT OF ADRENALINE

Can a small muscle injury change the balance of vasodilatory and vasoconstrictory influences of adrenaline (ADR)?

Study design: Healthy subjects (n=8), blood flow was measured by ^{133}Xe (Xenon) clearance in the gastrocnemius muscle. Two ways of ^{133}Xe administration; conventional intramuscular injection using a thin needle or through a fine tube close to an intramuscular microdialysis catheter

Intervention: ADR infusion or placebo infusion.

Expt 1 – conventional ^{133}Xe administration – ADR infusion.

Expt 2 – ^{133}Xe close to a microdialysis catheter – ADR infusion.

Expt 3 – ^{133}Xe close to a microdialysis catheter – Placebo infusion.

The microdialysis catheter-induced muscle trauma alters the balance of vasodilatory and vasoconstrictory influences of ADR in a vasoconstrictive direction. The adverse ADR effect is likely to be related to the degree of invasiveness and/or the presence of the catheter. Further studies are needed to find out if any type of invasive measuring device causing a muscle injury is able to provoke a similar reaction.

This finding in has a general physiological implication, but has also implications for the use of invasive techniques to investigate blood flow regulation in skeletal muscle.

IV) CHRONIC MUSCLE DAMAGE - ALTERED EFFECT OF ADRENALINE

Can chronic muscle damage change the balance of vasodilatory and vasoconstrictory influences of adrenaline (ADR)?

Study design: Healthy subjects (n=8), Patients suffering from tennis elbow (TE) (n=8). Blood flow was measured by ^{99m}Technetium-clearance in the main portion of the ECRB muscle.

Intervention: ADR infusion

The blood flow reaction to adrenaline was markedly different in the two study groups. Whereas the infusion did not significantly influence ^{99m}Technetium-clearance in the ECRB of controls there was a significant decrease in the patients. The altered adrenaline effect indicates a vascular dysregulation in TE, which is likely to be of clinical significance by contributing to the development and maintenance of the chronic muscle pain in this large patient group. Whether the vasoregulatory alteration, which would be expected to involve recurring relative muscle ischemia, represents the primary aetiology in TE or is a secondary effect of the muscle injury cannot be determined.

Conclusion study III-IV

In conclusion, a small muscle injury, acute or chronic, seems to alter the effect of ADR on skeletal muscle blood flow in a vasoconstrictory direction.

1 INTRODUCTION

1.1 BACKGROUND

Blood flow determinations in human skeletal muscle are not easily made. The different measuring methods available are connected with various individual problems and the "perfect" method is yet to be invented. The reasons for these individual limitations and disadvantages are heterogeneous. Venous occlusion plethysmography (VOP) is a commonly used, non invasive, method for determination of limb blood flow [1]. VOP in its modern form, measuring volume expansion rate over an isolated limb segment, is associated with problems due to the curvo-linear pressure/volume relationship in the veins and the fact that redistribution of blood can occur between individual limb segments. The inert gas clearance method [2], in which a radioactive tracer is injected into the muscle (e.g. Xenon clearance) is restricted due to the radioactivity involved and due to limitations during prolonged measurements [3]. Methods employing insertion of a measuring device into the muscle are obviously more invasive simply due to the caliber of the device and the fact that the instrument remains inside the muscle during the procedure, e.g. the microdialysis ethanol technique [4]. A concern in this instance is the trauma inflicted to the muscle by the insertion of the device which may influence the fine tuned control of the local circulation and hence possibly the accuracy of the method. In fact, recent reports have indicated that the insertion of a microdialysis catheter might change the local blood flow response to adrenaline (ADR) [5,6]. The purpose of the investigations in this thesis has been to study blood flow regulation in human skeletal muscle, with different methods, under normal conditions and after a minor muscle trauma. Special

emphasis has been put on the ADR induced blood flow reaction and if a minor muscle injury in some way alters this effect.

1.2 CONTROL OF BLOOD VESSELS

A variety of vasoregulatory systems are involved in the complex control of local blood flow. To simplify, these systems can be divided into a hierarchy of three control systems, each able to override or modify the lower one [7]. The lowest level of control is the local myogenic auto-control, generating basal tone in resistance vessels. The second level of control is maintained by local factors such as adenosine, lactate and NO etc. regulating local blood flow to match the metabolic or circulatory demands of the tissue; for example in an exercising muscle. The third level of control is the neuroendocrine system which generates central and reflex control to the vascular system in favor for the organism as a whole, e.g. blood pressure stabilization. This level is made up by sympathetic nerve fibers (noradrenaline) and hormonal control (ADR, angiotensin II and vasopressin).

1.3 ADRENALINE

The sympathetic nervous system, and the balance between its α- and β-effects, is a vital part of the blood flow control in skeletal muscle. Representing the hormonal part of the sympathetic nervous system, ADR contributes as one of our most potent regulators of blood flow. The different locations and relative abundance of the adrenergic receptors in the body is an important but complex matter to appreciate when the ADR effect on muscle blood flow is discussed.

1.3.1 Historical background

The first pure extract of ADR was produced by the Japanese chemist Jokichi Takamine in 1901 who patented the formulation under the name “Adrenalin” [8,9]. (Latin: ad = “near”, renes = “kidneys”). ADR was first synthesized by Stolz [10] and Dakin [11], independently, in 1904. After studying synthetic amines related to ADR, Barger and Dale in 1910 came close to discover noradrenalin (NADR) being the sympathomimetic nerve transmitter [9,12]. They showed that the effects of sympathetic nerve stimulation are more closely reproduced by the injection of sympathomimetic primary amines than by ADR or secondary amines [9]. However, it took until 1946 before NADR finally was identified by von Euler [13]. Subsequently Ahlquist, in 1948 [14], examined the effects of ADR, NADR and Isoprenaline (a synthetic catecholamine) and postulated the existence of two types of receptors; alpha (α) and beta (β), since the effect of these catecholamines fell into two distinct patterns (fig 1). Various α-receptor antagonists were known at that time but selective β-receptor antagonists were not developed until about ten years later [15]. By the use of these selective receptor antagonists Ahlquist’s classification could be confirmed [16-18]. Further subdivision into α_1, α_2, β_1 and β_2 receptors followed [19]. In 1974, the hypothesis of presynaptic autoreceptors modulating the sympathetic effect was described by Langer [9,20] (see paragraph 1.3.2) and was later verified [18,21]. During the following about fifteen years the subclassification of the receptors came to rely on anatomical or functional characteristics. Recently, by identification of potent and highly selective α_1- and α_2-adrenoceptor agonists and antagonists, subclassification based on pharmacological properties has been found to be more appropriate [18]. Consequently, the historical classification into two major subtypes, α- and β-receptors, has been revised into three major types: α_1-, α_2- and β-receptors based on

three lines of evidence; their affinity of selective drugs, difference in second messenger response and predicted amino acid sequences of the receptors [18]. Further subdivisions of each of the three major types of receptors have been proposed [18,22,23]. The constellation of the adrenergic receptors and their individual location are thus complex and further knowledge is constantly evolving (fig 1).

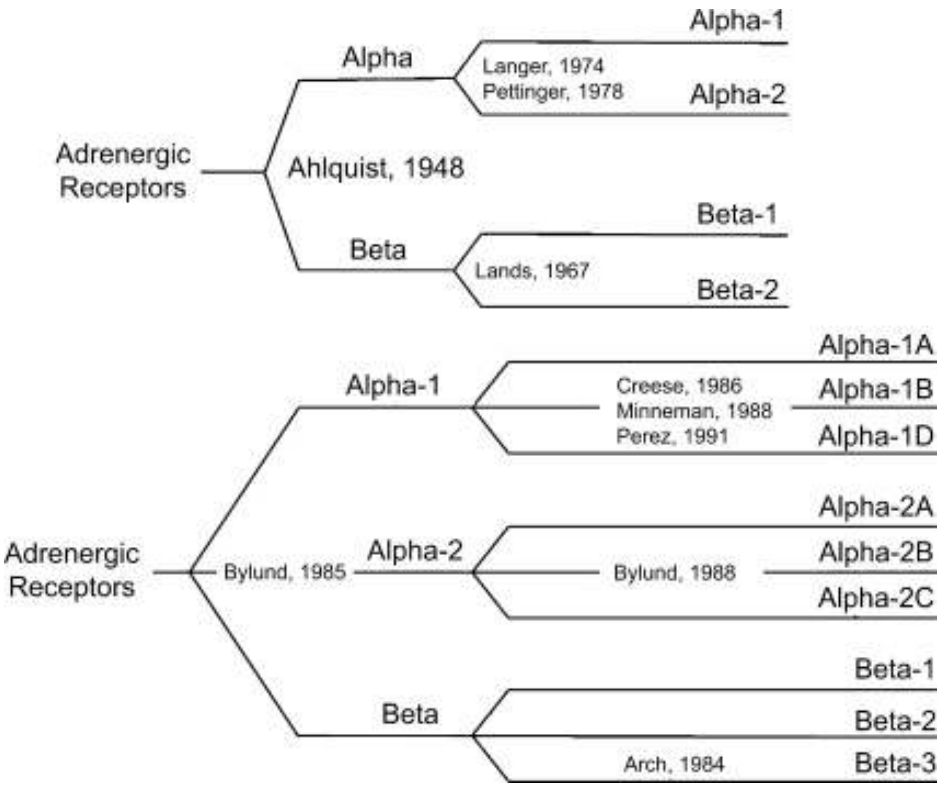

Figure 1. The original (top) and current (bottom) classifications of adrenergic receptors, emphasizing the early and prominent role of Ahlquist's 1948 paper.
Ahlquist RP. Am J Physiol 1948 [14]. **Bylund DB.** The Adrenergic Receptors in the 21st Century,: Humana, 2005 [24]. **Bylund DB.** Am Physiol Soc. Am J Physiol Endocrinol Metab , 2007[25], used with permission.

1.3.2 Adrenergic receptor location

It was originally believed that the α_1/α_2 classification corresponded directly with the post- and pre-synaptic location of the receptors [26]. Several exceptions to this are now known. In blood vessels, the postsynaptic α_1-receptor is the predominant postsynaptic receptor, mediating vasoconstriction [27]. In addition, the postsynaptic

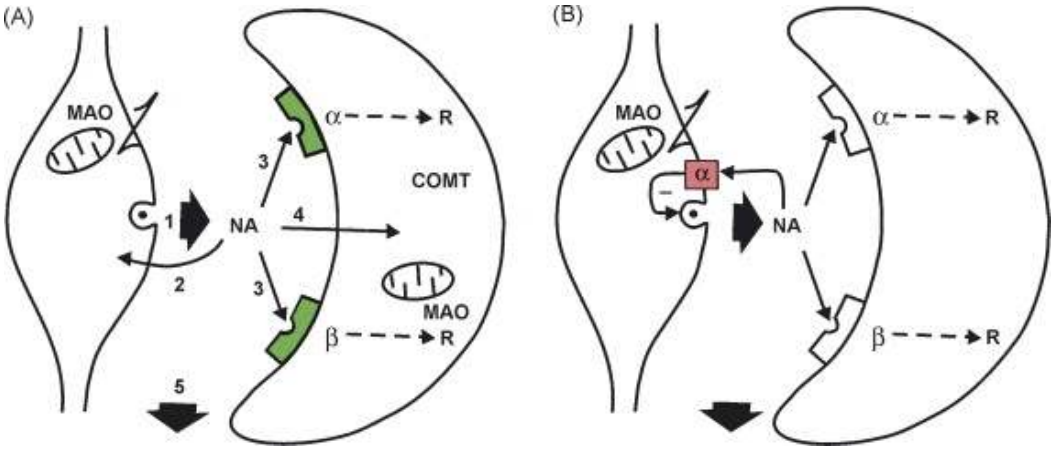

Figure 2. (A) Classical view. Classical concept of neurotransmission in the 1960s. Varicosities of noradrenergic terminals are involved in the synthesis, storage and calcium-dependent release in response to the occurrence of an action potential. Postsynaptic receptors on effector cells. The generally held opinion was that nerve endings had no receptors. (1)—exocytotic release of NA; (2)—noradrenaline transporter mediating neuronal uptake; (3)—effects on α-or β-adrenoceptors on effector cells; (4)—extraneuronal uptake; R—response; MAO—monoamine oxidase; COMT—catechol-O-methyltransferase. (B) View in 2007. In addition to the well-established postsynaptic receptors of the effector organ, terminal varicosities possess α_2-presynaptic receptors which modulate NA release through a negative-feedback mechanism which plays a physiological role in neurotransmission.

Reprinted from Neurochemistry International, 52, Salomon Z. Langer, Presynaptic autoreceptors regulating transmitter release, 26–30, Copyright (2008), with permission from Elsevier.

(http://www.sciencedirect.com/science/journal/01970186)

α_2-receptor subtype also mediates vasoconstriction [26]. This subtype, which is highly sensitive to ADR, appears to be predominantly extrasynaptic [26,28], where it may be the target of circulating cathecholamines rather than neuronally released NADR [27,28]. Furthermore, autoreceptors located presynaptically on the neuron itself regulate the sympathetic effect. Presynaptic α_2-receptors modulate NADR release through a negative feedback mechanism [27,29] (Fig. 2). In contrast, presynaptic β_2 receptors facilitate positive feedback modulation [19,30,31].

1.3.3 Cardiovascular effects of Adrenaline

All blood vessels have been found to have α- and β-receptors [32]. However, the relative abundance of each subtype varies in different tissues. In skin, renal and splanchnic circulations α- receptors predominate, but in the nutrient vessels in skeletal muscle, β_2-receptors predominate [32]. In the heart, the β_1- receptors prevail [15]. In contrast to NADR, a predominantly α- agonist, ADR stimulate both receptors, producing a mixed action [15]. The cardiovascular effects of ADR can be summarized into:

- Increased heart rate and cardiac force of contraction (β_1-effect).
- Diversion of blood from the skin, renal, and splanchnic circulations (α-effect, vasoconstriction) to skeletal muscles where vasodilatation takes place (β_2-effect).
- Expansion of the pulse pressure, as a consequence to the effects above, with an increase in systolic blood pressure and a decrease in diastolic blood pressure.
- Venoconstriction, reducing the venous capacity (α-effect).

The overall effect of ADR is to increase the output capacity of the human body.
It should be acknowledged, however, that vasoregulation is a complex issue and that ADR might influence other vasoactive mechanisms and control systems as well, such as NO and endothelin. (See section 4.3 for a more detailed discussion)

1.4 BLOOD FLOW DETERMINATIONS IN HUMAN SKELETAL MUSCLE

During a long period of time after its discovery, ADR was mainly regarded as a vasoconstrictor and hypertensor. However, as reviewed by Lundholm [33], this understanding was gradually opposed by increasing evidence that ADR actually causes vasodilatation in skeletal muscle, reducing the peripheral resistance. It has been demonstrated that ADR, when infused intravenously or intraarterially, causes an increased blood flow in skeletal muscle (cats) [33]. This vasodilatory ADR effect has been shown to be true also in humans [34,35], and different measuring devices have registered similar results. Freyshuss et al found an increase in limb blood flow (venous occlusion plethysmography) in humans in response to intravenous infusions of increasing concentrations of ADR [36]. An increase in skeletal muscle blood flow during intravenous infusion of ADR has also been found with the ^{133}Xe-clearance technique [37,38]. However, exceptions to these unanimous results have been reported with the microdialysis ethanol technique. Rosdahl et al. found that ADR, when infused locally through a microdialysis catheter, causes vasoconstriction in skeletal muscle (rats) [6]. It has been speculated that this ADR-induced vasoconstriction might be due to either the local route of administration or by a higher local ADR concentration reached during microdialysis administration [5]. Recently, Widegren et al. came up with interesting findings when human skeletal blood flow was determined with three different methods simultaneously during an intravenous ADR infusion or a Mental Stress test (Stroop Colour Word test) [5]. ^{133}Xe-clearance and VOP registered an increase in blood flow during both interventions. However, the microdialysis ethanol technique registered an increase in blood flow during mental stress test but a significant decrease in response to the intravenous ADR infusion. They concluded that ADR causes vasoconstriction in

skeletal muscle when blood flow is measured with the microdialysis ethanol technique, irrespective of the mode of administration (referring to the previous finding by Rosdahl et al.). This paradoxal ADR-induced vasoconstriction can possibly be explained by the more extensive local trauma involved in the microdialysis technique, which may in some way shift the balance of vasoconstrictor and vasodilator effects of ADR.

1.4.1 Venous occlusion plethysmography

Venous occlusion plethysmography (VOP) has been used to study blood flow in human extremities for more than a century [1]. The method is non-invasive and the underlying principle is simple; by obstructing venous outflow, but not arterial inflow, the volume of the limb distal to the occlusion cuff will increase at a rate corresponding to the blood flow entering this part [39]. This elementary principle is valid as long as one records the expansion of the whole limb to which the blood can enter and from which the venous return is occluded. The original method lived up to this principle but required large rigid water jackets in which the entire limb was enclosed [1]. The currently used method represents an adaptation of this principle and employs mercury-in-rubber strain gauges that encircle a segment of the part being examined [40]. With this modification, the swelling of the entire volume of the extremity beyond the level of venous occlusion is no longer recorded. In this case, the assumption has to be made that the volume expansion of the segments covered by the strain gauges is representative of the volume of tissue distal to the occlusion cuff.

The relationship between the transmural pressure (p) and the venous volume (v) is not linear and, furthermore, changes with an increasing sympathetic tone [41]. When the veins are in a relaxed state, the curve is S-shaped with a lower slope of the p/v

curve at high transmural pressure than at low pressure [7,42]. In contracted veins, the curve is shifted to the right (Figure 7, section 4.1), resulting in an increased venous pressure and lower venous volume. Depending on the position of the limb and whether the veins are in a relaxed or contracted state at the time of venous occlusion the obtained results will thus differ. This circumstance is a problem inherent in the strain gauge VOP method. Segments with low transmural pressure and almost collapsed veins will be able to expand more and faster than segments with contracted and/or filled veins. Furthermore, the p/v relationship is probably different in skin and muscle veins, the relative abundance of which also varies along the limb [43]. During VOP, the major portion of blood entering the limb after the venous occlusion accumulates in the veins, which act as communicating vessels. The swelling of a particular segment will, therefore, be dependent not only on the arterial inflow to it and the venous outflow from it, but also on the volume of the veins within that segment and the pressure–volume relationships of these veins in relation to adjacent communicating segments. Furthermore, the relative volume of veins within a segment is higher in segments with a high fraction of soft tissue, which varies along the limb.

VOP has experienced a renaissance during the last few decades and is frequently used in various research situations, e.g. studies of endothelial function. The previously mentioned problem with segmental volume expansion recordings has become more immediate since many studies apply the VOP technique with one strain gauge per limb, and refer the obtained results as blood flow in the whole extremity.

1.4.2 Inert gas-clearance technique

Clearance methods apply the principle that indicators diffusing freely across cell membranes can be used to measure local blood flow, since their clearance is determined by the blood flow only [2]. The diffusible tracer is mixed with saline and injected into the muscle under study. The radioactivity over the injection site is continuously monitored with an external gamma detector. After subtraction of background radiation all obtained values are converted into natural logarithms. These values are then plotted against time on a linear scale. Muscle blood flow, in ml min^{-1} 100g $tissue^{-1}$, can be calculated from this decay curve according to the equation:

$$\text{Muscle blood flow} = \lambda * k * 100$$

Where λ is the tissue to blood partition coefficient for the used isotope, and *k* is the slope of the least square linear fit. The clearance curve has an initial rapid phase followed by a fairly constant clearance rate which slowly decreases over time and finally ends up in a slow clearance rate (the tail part of the curve), see fig 3. The first about 30 minutes of the washout curve is considered to possibly be influenced by the injection trauma and therefore excluded [3]. The *inert gas clearance method* does not allow for blood flow determinations during prolonged measurement. It has previously been documented with the ^{133}Xe clearance technique that values obtained at the tail part of the clearance curve yields an underestimation of the actual blood flow by several mechanisms [3], see *Study III* for details.

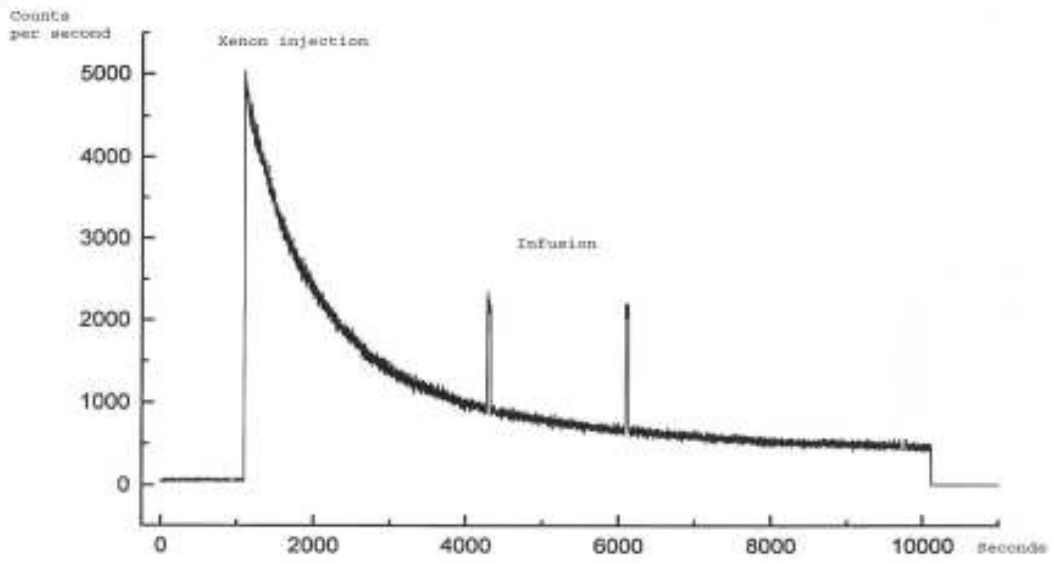

Figure 3. A typical clearance curve with an initial rapid washout phase, followed by a fairly constant clearance rate which subsequently ends up in a slow clearance rate at the tail part of the curve. The beginning and the end of the 30 minute ADR infusion is illustrated by event marks.

Several radioactive tracers have been utilized in measuring skeletal muscle blood flow with this technique; most commonly ^{133}Xe, which has been used for estimation of regional blood flow in human skeletal muscle since the sixties [2]. The ^{133}Xe clearance technique has, at least to some extent, been regarded as “golden standard” for peripheral blood flow measurements in general. The reason for this is that it has been the only available method for determination of nutritive blood flow in humans. However, ^{133}Xe has some disadvantages, as Seto et al. recently remarked [44]: Due to its lipophilic nature it is not sufficiently washed out of fat tissue which hampers precise measurement. Furthermore, it migrates easily from saline to air, thereby making it difficult to maintain the quality (radioactivity) of ^{133}Xe solution. And finally, ^{133}Xe gas-containing saline has recently become commercially more or less unavailable. We experienced difficulties to obtain ^{133}Xe solution for the forth study and were forced to find an alternative. Fortunately, Technetium has been proven to

be a satisfactory substitute. Its short-lived (half life 6 hours) gamma-emitting nuclear isomer $^{99m}TcO_4^-$ (metastable ^{99}Tc pertechnetate) has good properties which facilitate fast elimination and thus little radiation burden to the body [44,45]. Pertechnetate is an oxoanion with the chemical formula TcO_4^- which is used as a water-soluble source for carrying Tc isotopes [46], in particular the ^{99m}Tc. The disparity in the characteristics of ^{133}Xe and ^{99m}Tc has been debated since it may influence the results of blood flow measurements [44,46-48]. The uncharged ^{133}Xe (half life 5 days) can cross the endothelial membrane freely due to its lipophilic nature while the charged pertechnetate ($^{99m}Tc\ O_4^-$) most likely have other transportation pathways from the extravascular space into the capillaries due to its highly hydrophilic nature [47,48]. This difference is said to make ^{133}Xe clearance perfusion-limited while ^{99m}Tc clearance has been suggested to be diffusion-limited [47]. Diffusion-limitation increases with increasing perfusion, which may influence the recordings in absolute values during high perfusion, e.g. muscle exercise [47]. Still, the ability to detect relative changes in blood flow is probably comparable irrespective of isotope used, at least at rest. Furthermore, ^{99m}Tc is convenient to handle since it is less volatile than ^{133}Xe, and is retained more stably in the saline within the syringe [44]. Another important advantage of ^{99m}Tc is the availability since it can be obtained at most larger hospitals at low cost [46].

Technetium was discovered in 1937 with subsequent discoveries of its different isotopes [49]. ^{99m}Tc is nowadays commonly used in nuclear medicine for a wide variety of diagnostic tests and research [50]. The usage of ^{99m}Tc clearance for determination of skeletal muscle blood flow is however fairly recent [44,46], although early attempts were done in the seventies [45].

Technetium is a radioactive metal and not a radioactive gas as Xenon. The term *inert gas clearance technique* was accordingly changed into *isotope clearance technique* in order to be more correct when Technetium was used instead of Xenon (*Study IV*).

1.5 AIMS OF THE THESIS

General

The general aim of this thesis was to further study blood flow regulation in human skeletal muscle under normal conditions and after a minor muscle trauma.

Specific aims of the thesis

- To characterize the effects of environmental temperature and limb position on blood flow in human extremities as determined by VOP.
- To evaluate the effect of excluding the hand circulation during VOP on redistribution of blood in the forearm.
- To study the effect of ADR on nutritive muscle blood flow in humans.
- To determine if an inserted microdialysis catheter alters the effect of intravenously administered ADR on muscle blood flow.
- To study if ADR induces decreased blood flow in a chronic muscle pain condition such as tennis elbow.

2 SUBJECTS AND METHODS

2.1 ETHICS

Study I was approved by the Local Ethics Committee at Linköping University. *Study II-IV* was approved by the Local Ethics Committee at Karolinska Insitutet. The Isotope committee at Karolinska Hospital and Södersjukhuset approved *Study III* and *IV*, respectively. The experimental protocol in each study was explained to each subject and informed consent according to the Declaration of Helsinki was obtained from each individual prior to participation.

2.2 SUBJECTS

All subjects participating in *Study I-III* were healthy volunteers (n=6 in *Study I-II*, n=8 in *Study III*). In *Study IV* a healthy control group (n=8) was compared with a patient group suffering from tennis elbow (TE) (n=8). The subjects in the patient group were recruited at the outpatient clinic at the department of Hand Surgery, Södersjukhuset, Stockholm, Sweden. Information concerning eventual participation in *Study IV* was presented, in a strict manner, after decision regarding surgical treatment (ECRB elongation, “Garden procedure” [51]) for their distinct TE condition had been taken. No special treatment where given regardless of participation or not. All subjects, in both study groups, in *Study IV* volunteered. None of the subjects in the studies had a history of cardiovascular of peripheral vascular disease. All subjects were non-smokers.

2.3 PROCEDURES

2.3.1 Study I-II

VOP employing mercury-in-rubber strain gauges were used in *Study I-II*. Both studies were carried out in a climate chamber in warm, normal and cold temperatures and 40% relative air humidity (n=6 in both studies). Strain gauges were applied around four segments of the leg in *Study I* and three segments of the forearm in *Study II*. The varying positions of the limb in relation to the rest of the body were similar in *Study I* (leg) and *Study II* (arm) (i.e.; elevated 10˚, horizontal and lowered 15˚). A wrist cuff inflated to supra systolic pressure was used to exclude the hand circulation in half of the measurements in *Study II*. The varying limb positions were employed for affecting venous volume (gravity) and the different temperatures for altering transmural pressure (sympathetic tone).

2.3.2 Study III

Local blood flow in the gastrocnemius muscle was measured by ^{133}Xe clearance during normal conditions and during i.v. infusion of ADR with or without the influence of a small muscle injury induced by inserting a microdialysis catheter. Three experiments at different occasions where carried out in the same subjects (n=8). In experiment 1, the ^{133}Xe solution was administered conventionally by injection into the muscle via a thin intramuscular needle (diameter 0.4 mm). In the two other experiments (expt 2 and 3) a microdialysis catheter was introduced into the muscle through an insertion cannula (outer diameter 1 mm). A thin tubing was inserted along with the microdialysis catheter (see paragraph 2.4.4 for details). ^{133}Xe was injected through the thin tubing, which thereafter was withdrawn while the microdialysis catheter was fixed and remained in place during the whole experiment,

but was not perfused. Radioactivity was measured with an external scintillation detector. Blood flow was determined at rest and during an intravenous infusion of ADR (0.1 nmol · kg^{-1} · min^{-1}) (expt 1 and 2) and placebo (expt 3).

2.3.3 Study IV

Blood flow in the extensor carpi radialis brevis (ECRB) muscle was measured by local clearance of ^{99m}Tc. Patients suffering from distinct TE was compared with healthy controls (n=8 in each group). Each subject was investigated on a single occasion in experiments where blood flow in the main portion of the ECRB muscle was measured before, during and after an intravenous infusion of ADR. The ^{99m}Tc injection procedure was carried out under sterile conditions by means of an ultrasonography guided muscle puncture (see paragraph 2.4.5 for details). The ADR (0.1 nmol · kg^{-1} · min^{-1}) was infused intravenously (fossa cubiti) in the contra lateral arm during 30 minutes.

2.4 METHODS

2.4.1 Venous occlusion plethysmography, Study I-II

Mercury-in-silastic rubber strain gauges were used and the relative change of the volume of the limb segments under the strain gauges was registered on two two-channel chart recorders (Plethysmograph SP2; Medimatic, Copenhagen, Denmark). The strain gauges were calibrated at the different temperatures. The occlusion cuff was placed around the distal part of the thigh (*Study I*) and the upper arm (*Study II*) and the occlusion pressure was set at 50 mmHg. See the individual papers for specific strain gauge positioning. The strain gauges were connected to the two pletysmographs in a randomized fashion. The occluding proximal cuff was inflated for about 45 s and then deflated, the measurements being done on the linear part of

the curve during the first 30 s. This cycle was repeated six times during 10 min for each set of determinations and the mean value was used. A wrist cuff, inflated to supra-systolic pressure (220 mmHg) prior to the venous occlusion, was used in half of the measurements in *Study II* for exclusion of the hand circulation.

2.4.2 Xenon and Technetium clearance, Study III-IV

The isotope clearance method was used for determinations of muscle blood flow in the gastrocnemius muscle (*Study III*, ^{133}Xe) and the ECRB muscle (*Study IV*, ^{99m}Tc), see section 1.4.2 for methodological details. The radioactivity over the injection site was continuously monitored with an external gamma detector (see section 2.4.7). The first 30-35 minutes of the washout curve is considered to possibly be influenced by the injection trauma and was thus excluded. The following 10 min was used as basal period (the 35-45 min period in *Study III*, the 30-40 min period in *Study IV*). The basal period was followed by the 30 min ADR infusion. Placebo was infused instead of ADR in *Study III*, experiment 3. After subtraction of background radiation all obtained values were converted into natural logarithms. These values are then plotted against time on a linear scale. Muscle blood flow, in ml min^{-1} 100g tissue^{-1}, was calculated from this decay curve according to the equation:

$$\text{Muscle blood flow} = \lambda * k * 100$$

Where λ is the tissue to blood partition coefficient for the used isotope, in our case ^{133}Xe and ^{99m}Tc [44,46,52], and k is the slope of the least square linear fit.

2.4.3 Radioactive tracers, used in Study III-IV

Study III

Approximately 35 µCi (1.3 Mbq) of ^{133}Xe dissolved in 100-200 µl of isotonic saline was delivered in a glass ampoule from the Pharmacy at Karolinska hospital, Stockholm, Sweden. The ^{133}Xe solution was injected after withdrawal from the ampoule.

Study IV

^{99m}Technetium pertechnetate was extracted from a generator at the department of Nuclear medicine, Södersjukhuset, Stockholm. About 0.1 ml of the isotope solution, with an activity of approximately 10 MBq/0.1 ml, was delivered in a 1 ml syringe.

2.4.4 Catheter insertion and ^{133}Xe injection, Study III

In two of the experiments in *Study III* (expt 2 and 3) a microdialysis catheter was introduced into the muscle using the "guide tubing procedure"[53]. This involves the insertion into the muscle (parallel to the muscle fiber direction) of a steel cannula with a plastic guide tubing fitted on the outside (outer diameter 1.0 mm). This is followed by withdrawal of the steel cannula leaving the guide tubing in place in the tissue, through which the microdialysis catheter is inserted. Along with the microdialysis catheter a thin tubing (outer diameter 0.15 mm) was inserted through the guide tubing, to the level of the middle of the membrane part of the microdialysis catheter. When the microdialysis catheter plus the thin tubing had been inserted, the plastic guide tubing was removed by splicing upon retraction. ^{133}Xe was then injected via the thin tubing which thereafter was removed. The microdialysis catheter was fixed and remained in place during the whole experiment, but was not perfused.

2.4.5 Ultrasonography guided muscle injection, Study IV

An Acuson Sequoia 512 ultrasound apparatus with a transverse (linear) 8 MHz transducer was used for an ultrasonography guided ^{99m}Tc injection in the main portion of the ECRB muscle (Fig. 4-6). The subjects were placed in a comfortable sitting position with the forearm resting on a table. The injection procedure was carried out under sterile conditions. The forearm was prepared and a needle guide was clipped on to the transducer. Adjacent muscles around the ECRB was identified and avoided during the injection procedure. The extensor digitorum communis muscle was easily located by letting the subject move their fingers. The extensor carpi radialis longus muscle was visualized lying partly superficial and radial to the ECRB muscle. Subsequently, the main portion of the ECRB muscle was identified in the dorsal aspect of the proximal forearm (between the proximal and middle third of the ECRB muscle, about 8-10 cm distal to the lateral humeral epicondyle). A 25 gauge (0,5 mm) and 3½ inch (90 mm) long spinal needle (BD Medical, Franklin Lakes, NJ, USA) was advanced through the needle guide into the central part of the muscle where approximately 0.1 ml of the isotope solution (10 MBq/0.1 ml) was injected slowly. With the needle kept in place, the syringe was filled with a small amount of air (about 0.1 ml) which also was injected in order to empty the needle and inject as much of the isotope as possible. After completed injection, the needle was held in position during 1 minute and then pulled out in a stepwise fashion, in order to avoid the isotope from flowing back through the injection channel.

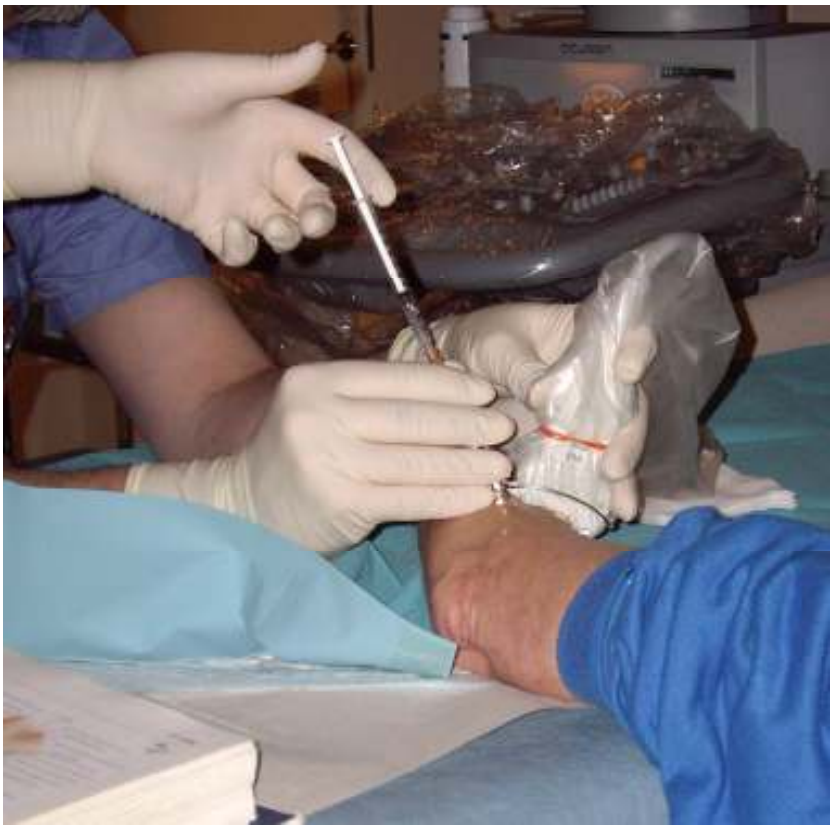

Figure 4. Ultrasosgraphy guided ^{99m}Tc injection in the ECRB muscle. The needle guide on the transducer enables a precise injection in the main portion of the muscle.

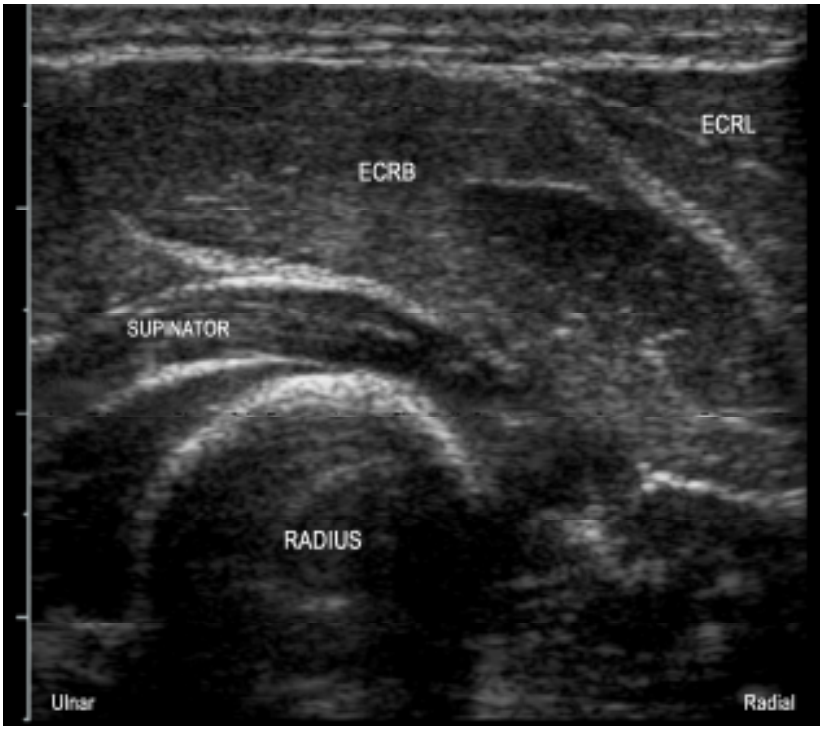

Figure 5. A cross sectional view of the dorso-radial part of the proximal forearm (ultrasonograhy, right arm). The ECRL muscle (extensor carpi radialis longus) is located partly superficial and radial to the ECRB muscle. The supinator muscle insertion to the radius is seen deep to the ECRB muscle. The major tick marks to the left represents 1 cm in between.

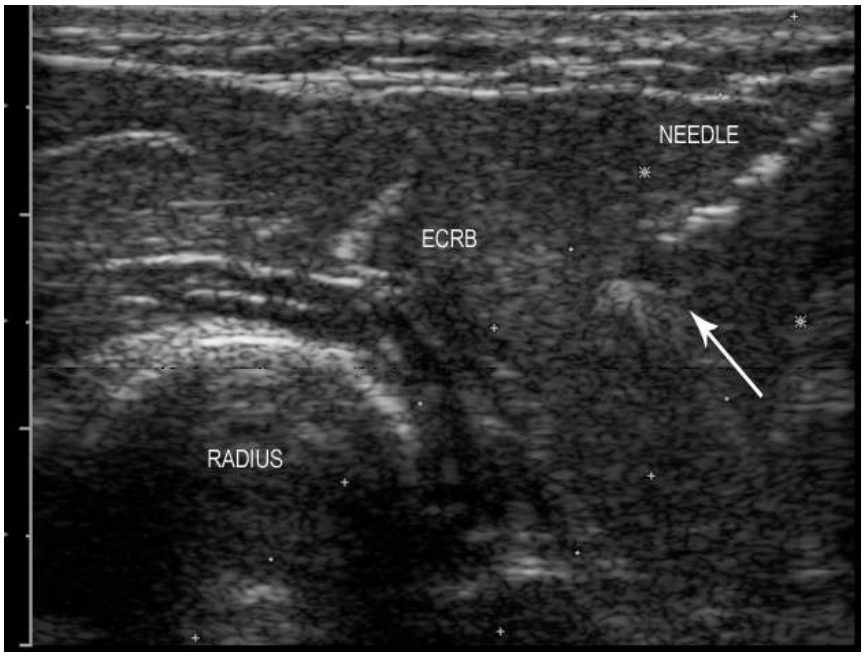

Figure 6. Immediately after the ^{99m}Tc injection in the main portion of the ECRB muscle. The white dots represent the injection "channel" into which the needle is guided. The needle tip is seen in the upper right corner. The injected ^{99m}Tc is visible as a darker area just distal to the needle tip (arrow).

2.4.6 Monitoring of heart rate and blood pressure

Study III

Heart rate was continuously monitored throughout *Study III* by telemetry using the Polar Sport Tester heart rate monitor (Polar Electro, Kempele, Finland). Heart rate data were averaged over 1 minute periods. A tourniquet was placed on the upper, non-infused, arm for intermittent blood pressure monitoring.

Study IV

Heart rate and blood pressure were measured intermittently, every third minute, during the whole procedure using a wrist cuff (Omron R3, Omron Healthcare Europe, Hoofddorp, the Netherlands) on the infused arm, distal to the intravenous infusion access.

2.4.7 Scintillation detector

Measurement of radioactivity, *Study III-IV*

The detector, a Sodium Iodide crystal (Canberra, Uppsala, Sweden) ∅ 43 mm, was located 8 cm inside the opening (∅ 34 mm) of a surrounding lead cylinder, which was placed perpendicular to and adjoining the surface of the skin. Radioactivity was measured each second period and expressed as counts min^{-1} (cpm).

3 RESULTS

3.1 STUDY I-II

VOP with multiple strain gauges were used for blood flow determinations in the upper and lower extremity at varying environmental temperature (high, low or room temperature) and limb position (elevated, horizontal or lowered). Most of the tendencies observed in *Study I* were more distinctly demonstrated in *Study II*. However, a few differences in obtained results in the two studies require some clarification.

Study I

The major finding of *Study I* was that the mutual relationship between the swelling rates of individual segments is dependent on the environmental temperature and the position of the leg.

Influence of temperature (sympathetic tone)

Environmental temperature clearly influenced the obtained values (high values at high temperature and low values at low temperature) and the influence was more distinct the more distal the strain gauge was positioned.

Influence of limb position (intravasal pressure)

With the leg in the lowered position, the values obtained were decreased, on average to 68% of that recorded at the horizontal position. There was a general trend for the magnitude of this decrease to become progressively larger the more distal the segment was situated. Elevating the leg resulted, in most cases, in an increase in calf volume expansion rate. This effect on volume expansion rate by changing leg position was accentuated at warm, and tended to be decreased at cold temperature.

Influence of strain gauge position

In all nine combinations, there was a clear tendency towards higher segmental volume expansion rate values at the maximal circumference of the calf relative to the proximal and distal calf levels, regardless of the temperature of the room and leg position.

Study II

A wrist cuff was used in half of the measurements in *Study II*, for exclusion of the hand circulation. The obtained results differed in several aspects when hand circulation was included or excluded:

The simple main effect of temperature was highly significant ($P<0.001$) in both settings (high values at high temperatures and low values at low temperature).

With excluded hand circulation (but not when included), there was a significant two factor interaction between arm position – strain gauge position ($P<0.05$), which was independent of temperature since the three factor interaction was non-significant. The highest expansion rate was found in the proximal segment when the arm was elevated, but in the distal segment when the arm was lowered. This pattern was found in all temperatures, but was not seen in *Study I* (lower limb).

With hand circulation (but not without) there was a significant two factor interaction between temperature and strain gauge position ($P<0.01$), which was independent of the positioning of the arm since the three factor interaction was non-significant. The highest expansion rate was found in the distal segment at normal and high temperatures, but in the proximal segment at low temperature. This pattern was found with all arm positions. In *Study I* (lower limb) the highest expansion rate was generally found at the maximal circumference of the calf (relative to the other calf segments).

3.2 STUDY III

Heart rate and blood pressure

In the absence of plasma adrenaline measurements, heart rate and blood pressure were recorded to document the systematic reaction to adrenaline in *Study III* and *IV*. The ADR-infusions in expt 1 and 2 generated a moderate increase in heart rate (about 3-5 beats per minute). The increase was statistically significant in expt 1, $p<0.05$. Furthermore, the pulse pressure expanded significantly in both expt 1 ($p<0.001$) and 2 ($p<0.05$), although the changes in systolic and diastolic blood pressure did not achieve statistical significance. The placebo infusion in expt 3 did not influence heart rate and blood pressure.

Blood flow

Muscle blood flow tended to increase with the adrenaline infusion in expt 1 (i.v. ADR infusion, ^{133}Xe deposited normally in the muscle, no microdialysis catheter inserted) from 1.17 ± 0.10 (basal) to 1.39 ± 0.15 ml min^{-1} 100 g tissue^{-1} (6–15 min into the infusion period), N.S. On the contrary, in expt 2, the blood flow decreased during the identical ADR infusion (i.v. adrenaline infusion, ^{133}Xe deposited close to an inserted microdialysis catheter in the muscle) from 1.39 ± 0.14 to 1.03 ± 0.14 ml min^{-1} 100 g tissue^{-1}, $P<0.001$. The blood flow change in response to the ADR infusion was significantly different in expt 1 and expt 2 ($P<0.05$). Blood flow also decreased during the placebo infusion in expt 3 (control experiment with placebo infusion, ^{133}Xe deposited close to an inserted microdialysis catheter in the muscle), from 1.15 ± 0.10 to 1.00 ± 0.09 ml min^{-1} 100 g tissue^{-1}, $P<0.01$. The blood flow decrease in response to ADR infusion in expt 2 was significantly larger than during the placebo infusion in expt

3, P<0.01. Basal blood flow values were not significantly different in the three experiments.

There was a gradual and constant decrease in the rate of ^{133}Xe-clearance over time in all three experiments, only interrupted by the adrenaline-induced effects which were most pronounced during the 6–15 min period of infusion (expts 1 and 2). A continuous decrease in clearance rate during the measurement period is expected and is a characteristic feature of the isotope clearance method; see the Discussion section 4.2 for details and references.

3.3 STUDY IV

Heart rate and blood pressure

Basal heart rate levels were similar in the two study groups, 63.4 ± 3.0 (patients) and 65.8 ± 3.5 (controls), and increased about 10 bpm in both groups during the 30 min period of intravenous adrenaline infusion (p<0.01). The heart rate returned towards basal after the adrenaline infusion. Basal levels of heart rate were obtained after about 15 min of the recovery period.

The systolic blood pressure had a tendency to increase and the diastolic blood pressure to decrease during the intravenous adrenaline infusion. This resulted in a tendency towards increased pulse pressure in both groups during the adrenaline infusion (statistically significant in the control group during the 16-30 min interval as well as during the recovery period).

Blood flow

A continuous decrease in isotope clearance rate over time is expected and a characteristic feature of the isotope clearance technique (see the Discussion section 4.2 for details and references).

In the control group, this decrease was interrupted by the adrenaline-induced vasodilatory effects which were most pronounced during the first 15 min of the 30-min infusion period. No such effect was detected in the patient group. The different pattern of blood flow change in response to the adrenaline infusion in the patient and control groups, respectively, was highly significant (statistical interaction group * effect, $p<0.002$). Mean values of ^{99m}Tc clearance rate during the 0-15 min of the adrenaline-infusion period was 55.9 ± 5.9 % of basal in the patient group and 97.4 ± 4.1 % of basal in the control group ($p<0.001$). Basal ^{99m}Tc clearance rates was similar in the two groups and corresponded to a muscle blood flow of 2.71 ± 0.39 and 2.54 ± 0.55 ml · min^{-1} · 100 g tissue^{-1} in Patients and Controls, respectively.

4 DISCUSSION

As outlined in the introduction, blood flow determinations in human skeletal muscle are not easily made. The present series of experiments highlights a number of methodological problems with VOP and the microdialysis ethanol technique. Furthermore, the experiments also emphasize that these methods are dependent on sympathetic tone, which may influence the obtained results considerably if not appreciated. In addition, by studying these problems and by applying our findings to a chronic muscle injury we have found evidence indicating a possible pathological process within the ECRB muscle of patients suffering from TE.

4.1 METHODOLOGICAL ASPECTS ON VOP (STUDY I-II)

In *Study I-II*, venous occlusion plethysmography (VOP) was used for measuring volume expansion rate in the upper and lower extremity. It should be stressed that the VOP method detects the volume expansion rate of a limb and not the actual blood flow in a particular muscle, hence making the method relatively unspecific. On the other hand, the technique is easy to use, non-invasive and fairly reproducible if handled properly. The modern form of the VOP method employs mercury-in-rubber strain gauges that encircle a segment of the part being examined. With this modification of the original method, which enclosed the whole limb, the assumption has to be made that the volume expansion of the segments covered by the strain gauge is representative of the volume of tissue distal to the occlusion cuff. The potential pitfalls inherent in the method with this assumption are clearly demonstrated in *Study I-II*, especially if only one strain gauge is applied.

The fundamental circumstance on which both of these studies were based is that the relationship between the transmural pressure (p) and the venous volume (v) is not linear and, furthermore, changes with an increasing sympathetic tone. The venous volume expansion rate is determined by the arterial inflow and the capacity for venous expansion. The latter is determined by the position on the venous pressure-volume curve at any given time and eventual redistribution of venous blood to or from a particular segment. Hence, depending on the position of the limb and whether the veins are in a relaxed or contracted state at the time of venous occlusion the obtained results will differ. When the veins are in a relaxed state, the pressure-volume curve is S-shaped with a lower slope of the curve at high transmural pressure than at low pressure. In contracted veins, the curve is shifted to the right. The results in *Study I-II* indicate that the venous blood was redistributed from segments with a high transmural pressure and, therefore, low compliance, to segments with a lower transmural pressure and, therefore, higher compliance. The changes in temperature were used to obtain low, intermediate and high sympathetic activation of the venous vasculature. At low sympathetic activation (high temperature) the positioning on the venous pressure-volume curve is far to the left (see Fig. 7). In this position the capacity for venous expansion is maximal since the veins are almost empty and the VOP consequently registers the highest values at the highest temperatures.

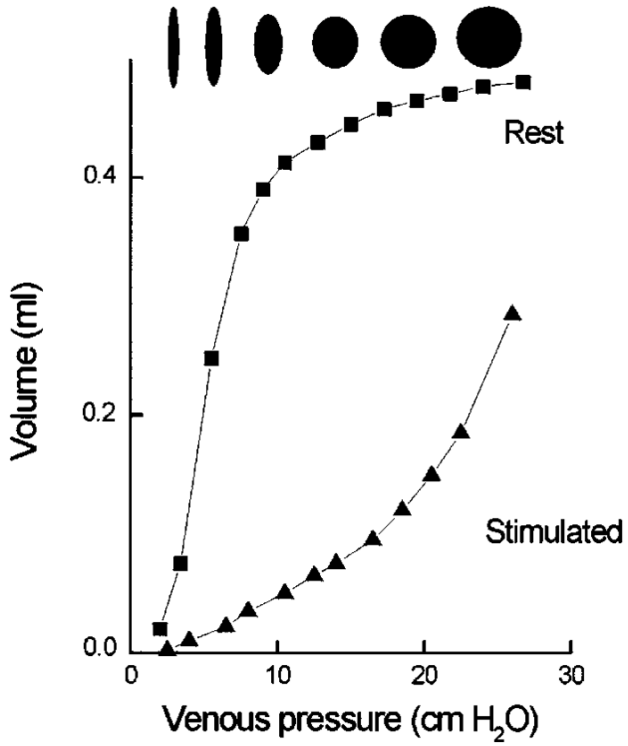

Figure 7. Schematic representation of vein distensibility in a relaxed (upper curve) and maximally contracted (lower curve) state. The change in cross-sectional profile at different pressure (relaxed state) is indicated schematically at the top of the figure.
(Modified from An Introduction to Cardiovascular Physiology by J. R. Levick, reprinted by permission of Elsevier Science Limited. Data points for isolated canine saphenous vein reprinted with kind permission from Springer Science+Business Media: Pflügers Archiv, The reactivity of isolated venous preparations to electrical stimulation, 306, 1969, 341–353, Vanhoutte P. M. and Leusen I, figure 5. Reprinted with kind permission from Dr P. M. Vanhoutte.)

Another way to obtain a position on the far left side of the venous pressure-volume curve is to keep the limb elevated. This is well illustrated by the data obtained in *Study I*; with elevated leg and with the veins in a relaxed state (warm temperature) the distal thigh showed a relatively low expansion rate, whereas the calf segments showed a high expansion rate. This is in line with a relatively lower initial transmural pressure in the distal calf segment (which is elevated) compared with that of the distal thigh segment at the start of venous occlusion. At this time, the veins of the distal calf, therefore, are empty and in an almost collapsed, state. During occlusion,

this particular segment, therefore, has the ability to increase its volume much more than the more proximal segments.

Furthermore, it is likely that venous outflow occurs to more proximal segments as well. Therefore the swelling of a segment depends not only on the arterial inflow, but also on redistribution of venous blood. The veins will act as communicating vessels, and the expansion of a segment after more proximal venous occlusion depends on the total compliance of the capacitance vessels within the segment and the time constant for translocation of venous blood to and from that segment. An example regarding the influence of limb position can be found in a report from Rojek et al. [54] who studied forearm blood flow (FBF) with VOP using a single strain gauge positioned about seven cm distal to the olecranon. FBF was measured at rest and during dynamic hand grip contractions with the arm 10 cm above and below the level of the heart. They found a significantly lower FBF reading at rest when the forearm was placed below the level of the heart than compared with above, which is in accordance with our findings. Conversely, during exercise, the FBF values were significantly higher below the level of the heart, compared with above [54].

An interesting exception to the general pattern of reaction occurred in *Study II* when the hand circulation was excluded and the arm was elevated. According to the principle that elevation of the limb empties the veins, especially in the distal part, the expected result would have been an increase in volume expansion rate in the distal segment. On the contrary, the values obtained were lower in the distal segment as compared with the more proximal segments. A possible explanation to this might be related to the venous drainage of the hand and its topographical anatomy. The venous drainage of the hand is predominantly superficial through the

subcutaneous veins on the dorsum of the hand and further proximally, still superficial, through the Cephalic and Basilic veins in the forearm [55], whereas the deep venous system is relatively more involved in the venous drainage of the forearm [56]. The cross sectional area of the distal forearm segment is dominated by less vascularized tissues (bone, tendons) while the muscle fraction predominates in the more proximal segments. The superficial venous system thus plays a relatively more important role in segmental volume expansion in the distal part of the forearm during VOP. As the wrist cuff is inflated and hand circulation is excluded the normal venous drainage to the large superficial veins in the forearm will be blocked and the filling of these veins will be dependent on communicating branches in between the deep and superficial venous systems during proximal venous occlusion (VOP measurement). One important communicating branch is the v. profundus cubitalis in the fossa cubiti [55,57,58]. A hypothetical explanation to our finding is that these communications are less sufficient in the elevated position of the forearm compared to the horizontal and lowered position. A mechanism behind this difference might be found in the competence of the venous valves. The valves are positioned to direct the blood from distal to proximal and from superficial to deep [57-59] and therefore some valvular incompetence is needed to enable the blood to flow in the opposite direction. According to the law of LaPlace, $P = T/R$, an infinite pressure would be demanded in order to increase wall tension (to enable some valvular incompetence) if the vein is fully collapsed [60]. This would explain the lower volume expansion rate at the distal segment when the arm is elevated (and the superficial veins collapsed).

Some concordance with this explanation can be found in an interesting anecdote regarding valvular incompetence in clinical practice. The distally based pedicled radial forearm flap (Chinese flap) with a reversed arterial blood flow caused astonishment in the Western world when it was introduced by Yang et al. in 1981 [61]. It challenged the prevailing principals of flap surgery at the time. The arterial supply to the flap was straightforward with blood from the ulnar artery through the superficial palmar arc and in retrograde direction into the radial artery. The controversy was the venous drainage which was a mystery since the valves are situated to prohibit blood flow in distal direction. In order to accomplish retrograde venous return through the pedicle of the flap the valves had to be incompetent. However, the venous drainage worked and the flap has survived the test of time. According to the current theory [60] several factors are needed to enable valvular incompetence, of which two are:

- A sufficient but not very high venous pressure
- The maintenance of blood flow in the veins to avoid their flattening, because when a vein flattens the pressure to fill it up is infinite according to LaPlace´s law.

We have shown that small variations in sympathetic tone and venous pressure can influence blood flow measurements, with the VOP technique, even in intra-individual comparisons. This variability should be taken into account when strain-gauge plethysmography is applied for limb blood flow determination, especially in interventional studies. As mentioned previously, VOP is frequently used with only one strain gauge attached to the limb and the results obtained are referred to as blood flow in the limb. Placement of the strain gauge at the maximal circumference of the limb with a distal occlusion cuff at the wrist or ankle is regarded as standard in

this technique [40,62,63]. However, even if the procedure is standardized in this manner, the results in *Study I-II* clearly demonstrates that events taking place under one strain gauge cannot be strictly duplicated in adjacent portions of the limb.

4.2 METHODOLOGICAL ASPECTS ON THE ISOTOPE CLEARANCE TECHNIQUE (STUDY III-IV)

Several radioactive tracers have been utilized in measuring skeletal muscle blood flow with the isotope clearance technique; most commonly ^{133}Xe, which has been used for estimation of regional blood flow in human skeletal muscle since the sixties [2]. The ^{133}Xe clearance curve has an initial rapid phase followed by a fairly constant clearance rate which slowly decreases over time and finally ends up in a slow clearance rate (the tail part of the curve), see section 1.4.2 fig 3. Calculated blood flow from the steep first part of the clearance curve, immediately after the isotope injection, has been shown to overestimate the directly measured blood flow [3]. Accordingly, the first about 30 minutes of the washout curve is considered to possibly be influenced by the injection trauma and should therefore be excluded. In addition, values obtained at the tail part of the ^{133}Xe clearance curve yields an underestimation of the actual blood flow by several mechanisms [3,64-68], see *Study III* for details. Hence, the intermediate part of the clearance curves, when fairly accurate blood flow measurement can be obtained, is relatively short (about 30-45 min). However, despite these inherent limitations the isotope clearance method is sensitive in detecting relative blood flow changes [69] following some kind of intervention, e.g. an ADR infusion. Even with a slowly decaying isotope clearance curve (with clearance rate as ordinate and time as abscissa), an upward shift represents an increased clearance rate, which in turn indicates an increase in blood flow and thus a probable vasodilatation. Consequently, a downward

shift in the isotope clearance curve reflects a decrease in blood flow and therefore most likely a vasoconstriction.

Determination of blood flow in skeletal muscle using ^{133}Xe clearance has been widely used for nearly fifty years [2] while measurement of muscle blood flow based on ^{99m}Tc clearance is still fairly new in comparison [44,46,48]. The properties of the two isotopes have some differences (discussed previously in section 1.4.2); the charged $^{99m}TcO_4^-$ (pertechnetate ion) has a hydrophilic nature while the uncharged ^{133}Xe is much more lipophilic. Even so, the obtained isotope clearance curves in *Study III* (^{133}Xe) and *Study IV* (^{99m}Tc) were similar in a general perspective with a gradual decrease in clearance rate over time, only interrupted by the events provoked by the ADR infusion.

"Muscle blood flow" (ml min^{-1} 100g $tissue^{-1}$) is widely used as the unit of measurement in isotope clearance studies [2,3,44,68,70]. In accordance with this and for ease of comparisons with previous studies we used this unit in the third study [71]. However, considering the overestimation of blood flow at the initial rapid washout phase and the underestimation at the tail part of the curve, the obtained results with the isotope clearance technique does only in a short time interval correspond directly in absolute terms to the actual muscle blood flow. This is also illustrated by the continuously decaying clearance rate over time found in *Study III-IV*. The obtained clearance values and the relative changes in clearance rate provoked by the ADR infusion should thus be looked upon as qualitative rather than quantitative. Consequently, the unit of measurement was changed from "muscle blood flow" into "isotope clearance rate" in the forth study in order to be more correct.

4.3 SMALL MUSCLE INJURY - ALTERED EFFECT OF ADRENALINE (STUDY III)

Several indications in the literature support the concept that a microdialysis catheter-induced trauma alters the balance of vasodilatory and vasoconstrictory influences of ADR, as proposed in *Study III*. Hodges et al. recently reported that the cutaneous vascular response to whole body heating was diminished by the presence of an inserted microdialysis catheter [72]. Further evidence in the same direction is a study from Rosdahl et al. who noted that an ADR infusion through the microdialysis catheter resulted in vasoconstriction [6]. Moreover, Widegren et al. found a decrease in skeletal muscle blood flow in response to intravenous ADR infusion when determined with the microdialysis ethanol technique, although simultaneous measurements with ^{133}Xenon clearance and venous occlusion plethysmography (VOP) recorded an increase in blood flow as expected [5].

In *Study III*, the ADR infusion caused a significant decrease in blood flow when the ^{133}Xe deposit was administered next to the microdialysis catheter (insertion cannula Ø = 1 mm) (expt 1) whereas the muscle blood flow instead tended to increase with the ADR infusion when the ^{133}Xe was injected conventionally with a thin intramuscular needle Ø ≈ 0.4 mm (no microdialysis catheter inserted) (expt 2). There was a small but significant decrease in muscle blood flow also during the placebo infusion (expt 3) (control experiment with placebo infusion, ^{133}Xe deposited close to an inserted microdialysis catheter in the muscle). This decrease might possibly be due to the cold placebo infusion in combination with the characteristic continuous decrease in isotope clearance over time in the inert gas clearance technique. However, the blood flow decrease in response to ADR infusion in expt 2 (microdialysis catheter inserted) was significantly larger than during the placebo

infusion in expt 3 (microdialysis catheter inserted). Basal blood flow values were not significantly different in the three experiments. Hence, the two ways of ^{133}Xe administration revealed different blood flow reactions to ADR. First of all, there is a discrepancy in the magnitude of the trauma inflicted to the muscle in the two procedures. Secondly, the microdialysis catheter (in expt 2) stayed in place during the procedure while the thin needle (expt 1) was removed after the injection. However, it is not possible to determine if the actual presence of the catheter or the muscle injury per se is the most likely reason for evoking the adverse ADR effect in Expt 2. A potential explanation might be the combination of both. Furthermore, it might be suggested that an enhanced vasoconstrictory influence around a muscle injury is an appropriate physiological response when blood is directed to the muscles during sympathetic activation with high ADR levels.

A possible mechanism behind this effect of a small muscle injury on the local tissue might be related to the complex constellation of the adrenergic receptors and their different locations [18,19,21]. Vasodilatation mediated by vascular β-adrenoceptors is the normally observed vascular response to intravenously infused ADR in human skeletal muscle [37,38]. Since the opposite reaction was seen close to a small muscular injury, in *Study III*, it is conceivable that the normal β-effect is either blunted and/or overridden by a vasoconstrictive α-effect, or by other mechanisms induced by the ADR infusion. Indirect support to the first scenario, that the catheter-induced muscle trauma in some way blunts the β_2-effect, can be found in a study by Bolli et al. who infused ADR in the brachial artery of healthy and hypertensive subjects following β-adrenergic receptor blockade and found that ADR caused vasoconstriction via activation of postsynaptic α_2-adrenoceptors [73]. An enhanced

α-effect overriding the normal β-effect is the other conceivable scenario. It is possible that the imposed trauma could increase the concentration of ADR at receptors - not normally directly exposed to humoral ADR. These receptors may be (1) prejunctional β2-receptors [19,31] which facilitate NADR discharge through a positive feedback mechanism leading to vasoconstriction via postsynaptic α-receptors and (2) postsynaptic α-receptors [27] (direct effect). Both α_1 and α_2 -receptors are present postsynaptically in blood vessels, where they mediate vasoconstriction [27]. The anatomical location and the physiological role of the postsynaptic receptors are of particular interest. It has been suggested that the predominant α_1-receptors are located primarily in the adventitial-medial border, accessible to neuronally released NADR, whereas the postsynaptic α_2-receptors are located closer to the intima, and predominantly outside the synaptic region [29,74], where it may be accessible to circulating catecholamines rather than neuronally released NADR [27,74]. Studies in various vascular preparations have subsequently shown that both postsynaptical α-receptor subtypes can be innervated by sympathetic nerves and, therefore, this classification of the α_1 and α_2 -receptors by anatomical location should no longer be utilized [75]. Still, the anatomical location of the postsynaptic α_2-receptor is interesting as a possible explanation to the finding in *Study III*. In addition, data from pharmacological studies in experimental animals indicate that α_1-receptors are located primarily on larger resistance vessels, whereas α_2-receptors are located distally on smaller arterioles [26]. Although this receptor distribution pattern has not been exactly documented in humans, recent data are to some extent consistent with this [26]. Hence, it is possible that postjunctional extrasynaptic α_2-receptor located on distal terminal arterioles becomes more exposed to ADR following a minor muscle injury. Moreover, it has been suggested in

the literature that this α_2-receptor is very sensitive to ADR [27]. Since ADR is a potent α-agonist [32,76] it could be suggested that if enhanced access is achieved to either one of the postsynaptic α -receptors, the effect of circulating ADR would be vasoconstriction instead of the expected vasodilatation mediated through the vascular β_2-receptor. In addition, enhanced stimulation of the exquisitely sensitive prejunctional β_2-receptors, which augments neuronal release of NADR [30,31], is also likely to be improved after a muscle trauma giving ADR extravascular access.

It should be acknowledged, however, that vasoregulation is a complex issue and that perturbations induced by the inserted microdialysis catheter of several other vasoactive mechanisms may also be involved in the vasoconstrictive response to ADR seen in *Study III*. A considerable factor in this instance might be the endothelium which plays a direct role in vasomotor function by integrating the controlling factors: reflexes, humoral, and local factors [77]. Endothelial cells release several substances acting directly on vascular smooth muscle cells, causing either contraction (e.g. endothelin-1) or relaxation (e.g. nitric oxide (NO) and prostacyclin). The interaction between these opposing factors is complex; e.g. the synthesis of endothelin-1 is inhibited by released NO [77], prostacyclin facilitates NO release and in turn NO potentiates prostacyclin effects in smooth muscle [77]. Another relevant factor released by the endothelium is angiotensin II (A_{II}) by means of hydrolyzing angiotensin-I through angiotensin converting enzyme (ACE). A_{II} is a potent vasoconstrictor and antagonist to NO [78]. Besides its own vasoconstrictive effect, A_{II} stimulates endothelin converting enzyme that degrades big-endothelin to endothelin-1 [78]. As can be seen, the interrelationship and balance between endothelium-derived agonists and antagonists is very complex and delicate.

However, for these mechanisms to have a role in the presently detected vasoconstrictive effect of the ADR infusion in *Study III* and *IV*, there has to be a connection between ADR (and the muscle injury) and these endothelium-derived factors. An interesting circumstance in this regard is that the β_2-mediated vasodilatation is 30-40% dependent on NO in human limbs [79]. In theory, a blunted NO effect could thus partly explain an absent β_2-effect during the ADR infusion. Moreover, the synthesis of endothelin-1 can be initiated by a variety of factors including catecholamines [77,80]. Another possible factor to consider is the inflammatory reaction induced by the catheter insertion. In accordance with this is a recent report by Mellergård et al., who found increased release of cytokines after insertion of microdialysis catheters in the brain and suggested that this might be directly related to the insertion trauma [81]. In addition, it has been suggested that ADR affects the immune system on a cellular level as well as the secretion of cytokines. Direction and nature of the effects may depend on the time and dose of exposition to the catecholamine [82].

Furthermore, there are other indications in the literature to suggest that the local site of administration, i.e. which receptor site that is stimulated, may be important for the vasoconstrictor effect to appear. Uvnäs et al [83] conducted interesting studies where they used rezerpin to deplete sympathetic nerves from their NADR. They subsequently reloaded the neurons with intravenously injected ADR and upon stimulation of the nerves a vasoconstriction occurred. These findings suggested that ADR released by sympathetic nerve stimulation hits vascular receptors different from the receptors stimulated by ADR given intravenously. Several authors [76,84-86] have shown that infused ADR can be converted into a cotransmitter by neuronal uptake

and on subsequent release augment the simultaneous discharge of NADR. It has also been demonstrated that local ADR administration (in a wide range of concentrations) on the outside of isolated blood vessels causes vasoconstriction, even if intravenous injection of ADR causes the same vessels to dilate [87]. Altogether, these indications may suggest that receptor sites not normally exposed to circulating ADR may be responsible for the adverse ADR effect seen in *Study III*.

The microdialysis technique is used for a variety of different purposes in both research and clinical practice, e.g. neurosurgery, plastic and reconstructive surgery etc. Whether the purpose is blood flow measurements or sampling of extracellular substances, it is apparently important that the tissue in which the catheter is introduced is as unaffected as possible by the insertion procedure per se. If the insertion of a measuring-device changes the natural state of the tissue and, as demonstrated in *Study III* - the blood flow response to ADR, the obtained results might be compromised. Depending on the plasma ADR concentration at any given moment, which varies endogenously (se section 4.4 for details), the blood flow around the catheter may fluctuate.

It is reasonable to believe that the adverse ADR effect is related to the degree of invasiveness. In this case, it could be expected that any type of invasive measuring device causing a muscle injury would possibly be able to provoke a similar reaction. The finding in *Study III* has a general physiological implication, but has also implications for the use of invasive techniques to investigate blood flow regulation in skeletal muscle. Although the microdialysis technique in previous studies has been able to adequately detect blood flow changes of various origin in resting skeletal

muscle and adipose tissue [4,88-91], the results in *Study III* indicate that caution is warranted when this technique is used in studies of blood flow regulation.

4.4 CHRONIC MUSCLE DAMAGE - ALTERED EFFECT OF ADRENALINE (STUDY IV)

The aim of the fourth study was to determine if a diffuse and widespread chronic "muscle injury" can change the balance of vasodilatory and vasoconstrictory influences of ADR, similar to the findings after an acute small muscle injury in *Study III*. Our hypothesis was based on several reports about impaired muscle blood flow and morphological signs of muscle damage in chronic muscle pain syndromes in general and in the tennis elbow (TE) condition in particular.

TE, often referred to as lateral epicondylitis, is one of the most common causes of chronic muscle pain in the upper extremity. The suffix "itis" in the term epicondylitis indicate a disorder of inflammatory origin, but recent comprehensive reviews have stressed that the condition is an overuse syndrome with a failed reparative process rather than active inflammation [92-94]. The term epicondylitis is thus a misnomer and several other names have been proposed and used for the disorder e.g. lateral epicondylagia [95], angiofibroblastic tendinosis [96] and lateral elbow tendinopathy [97-99], of which the latter is the latest contribution. The term tennis elbow, which originate from a report by Major in 1883 [100] and still persists as a colloquial eponym for this condition [101], is used as designation for the condition in this thesis.

Although the aetiology is commonly related to repetitive overuse [102], the patophysiology of TE is still unclear [93]. The overall consensus is that the extensor

carpi radialis brevis (ECRB) muscle-tendon unit has a central role in the condition [51,92,93,102-104]. Previous studies of the disorder have mainly focused on the painful extensor origin at the lateral epicondyle and histological changes within the ECRB tendon [96,105-107]. The vascularity of the proximal ECRB tendon has been studied in detail. Schneeberger and Masquelet found that the undersurface of the tendon seemed macroscopically avascular [108]. Bales et al. delineated the microvascular blood supply further and reported 2 hypovascular zones: one at the lateral epicondyle and the other 2-3 cm proximal to the ECRB origin, still in the tendon [109]. Several studies report degenerative changes in the ECRB tendon with dense populations of immature fibroblasts and signs of non-functional vascular hyperplasia [93,109]. These changes have been suggested to evolve due to insufficient blood supply resulting in failed attempts to heal an injury after a repetitive microtrauma [109]. The term angiofibroblastic hyperplasia/tendinosis originate from these histological findings [96,110]. Furthermore, Ljung et al studied the autonomic innervation of the blood vessels of the ECRB tendon and reported an imbalance between vasoconstrictor and vasodilator innervation at the arteriolar level which may predispose to hypoxic degeneration of the tendon [111,112]. However, during the last decade there has been an increase in reports about morphological and physiological abnormalities more distally, at muscle level. In ECRB muscle biopsies taken 5 and 10 cm distal to the lateral epicondyle in TE patients, Ljung et al. found moth-eaten muscle fibres (muscle fibres with an uneven distribution of mitochondrial enzyme activity), fibre necrosis and signs of de- and regeneration with a conversion of muscle fibres into more oxidative forms [113]. Hence, tissue damage and degeneration seems to be part of the TE condition also at muscle level. In support of an impaired blood supply at muscle level, Oskarsson et al. measured blood flow in

the ECRB muscle bilaterally in patients with unilateral tennis elbow and reported significantly lower blood flow levels on the affected side [114]. In a subsequent study they treated the same subjects with botulinum toxin to induce muscle relaxation and reported significant relief of pain at 3 and 12 months follow-up accompanied by improved intramuscular blood flow in the ECRB muscle [115]. Although these studies do not indicate whether the blood flow impairment in TE is primary or secondary, they support the concept that a change in the balance between vasoconstriction and vasodilatation in the ECRB muscle may be central in the TE condition. The shift towards vasoconstriction with ensuing hypoxia may thus explain why TE often becomes chronic and difficult to treat. In further support of a vascular disturbance in TE, a slow reperfusion response in the ECRB muscle after release of an upper arm tourniquet has been noticed on the painful side in patients with unilateral tennis elbow (Ljung et al, unpublished data, Laser Doppler single fibre technique with on line registration of blood flow). In addition, Smith et al. found a local dysfunction of the sympathetic blood flow control in the skin overlying the affected enthesis and suggested that it may be associated with the pathogenesis in TE [116]. Taken together, the whole ECRB muscle-tendon unit is involved in the TE condition and the recent findings at muscle level emphasize the complexity of the disorder. This is also underlined by the multitude of operative and non-operative treatment options and their response (and non-response) which indicate that our understanding of the disorder currently is incomplete [93,101].

The vascular reaction in response to the ADR infusion in *Study IV* was distinctly different in the two study groups, illustrated by the highly significant interaction effect ($p<0.002$). Whereas the ADR infusion did not significantly influence the blood

flow in the ECRB of controls, there was a significant decrease in ^{99m}Technetium clearance in the patient group. The result is in agreement with our hypothesis that the muscle damage in TE might shift the balance of vasoactive influences in the ECRB muscle in a vasoconstrictory direction. The result is also in accordance with the altered ADR effect seen in *Study III* after an acutely inflicted small muscle injury. The exact mechanism behind the vascular effect of a muscle injury cannot be determined and several different vasoactive systems may be involved as discussed in Section 4.3. The important finding is that this mechanism is present also in the TE condition. Evidently, it seems to be a distinct association between a small muscle injury, acute or chronic, and an abnormal blood flow reaction to adrenaline.

A moderate increase in muscle blood flow is normally reported following intravenous ADR infusion in a dose equivalent to what we used in *Study III-IV*. This was also found in a previous study from our group [5], but could not be reproduced in *Study III-IV* even though a tendency towards an increased blood flow during the first 15 min of ADR infusion was seen in the control group in *Study IV* (fig 2, *Study IV*) and when conventional ^{133}Xe administration was used in *Study III* (fig 1 (expt 1), *Study III*).

It is possible that the muscle damage in the TE condition in some way blunts the vasodilatation mediated by β-adrenoceptors (or by other mediators) and therefore makes the tissue more reactive towards vasoconstrictive influence, such as α-adrenergic stimulation. Other possible mechanisms behind the adverse ADR effect in the ECRB muscle of TE might be similar to the alternatives discussed in section 4.3 regarding an acute small muscle injury. As suggested previously, an enhanced

vasoconstrictory influence around a muscle injury might possibly be an appropriate physiological response in situations with high ADR levels in plasma.

In *Study IV* we used an ultrasonography guided muscle puncture in order to be absolutely certain that the ^{99m}Tc was administrated in the central portion of the ECRB muscle. The cross sectional depth and width of the ECRB muscle at this level is approximately 1.5 and 4 cm, respectively (see figure 5, section 2.4.5). The muscle is located ulnar and partly deep to the ECRL muscle and superficial to the Supinator muscle. Hence, ultrasonography guidance is vital in order to be exact with the muscle puncture. In addition, with the findings of *Study III* in mind, special precautions were taken to minimize the injection trauma and avoid the injection injury per se to alter the ADR effect. The injection was performed in a single puncture procedure by an experienced radiologist (MW) with a thin needle (0.5 mm in diameter) and only 0.1 ml of ^{99m}Tc solution was injected.

The gradual decrease in isotope clearance rate over time could give the impression that the blood flow reaction in the TE group during the adrenaline infusion was an absence of vasodilatation only. An additional control group with TE patients infused with placebo would have been necessary to confirm that a vasoconstriction had indeed occurred in the TE group. Such a confirmation of the vasoconstrictive effect of the muscle injury was obtained in *Study III* [71].

The altered ADR effect, seen in *Study IV*, indicates a vascular dysregulation in TE, which is likely to be of clinical significance by contributing to the development and maintenance of the chronic muscle pain in this large patient group. Ischemia is well

known to cause pain and the human skeletal muscle is vulnerable for hypoxia. Since ADR is an endogenous stress hormone it is tempting to believe that the ECRB muscle experiences recurrent hypoxia or even ischemia on daily basis in patients with an established tennis elbow disorder. However, the obtained results cannot be directly extrapolated to a defined physiological situation, such as psychosocial stress. In the latter situation, the predominant physiological response is an activation of the cardiovascular system by the sympathetic nervous system leading to a profound increase in heart rate and blood pressure, but where the contribution of the limited (two-fold or less) increase in the plasma ADR concentration is relatively small [117]. With the presently used ADR infusion, the concentration of ADR in arterial plasma will increase by 8-10-fold [118], but with a much smaller increase in heart rate than during mental stress, and with small or non-existent increases in systolic blood pressure and perceived stress [117,118]. Irrespective of these differences, there is evidence that the increase in the plasma ADR concentration that occurs during mental stress is large enough to influence the vascular resistance and blood flow in skeletal muscle [119]. Another situation in which the plasma ADR concentration will influence blood flow in skeletal muscles is physical exercise, where the ADR level in plasma may increase by 6-7-fold [120]. Therefore, the altered blood flow response to ADR in the ECRB muscle in tennis elbow, as shown in *Study IV*, may still be of direct importance in everyday situations.

The present results may furthermore be extrapolated to other conditions involving widespread and diffuse muscle damage with signs of disturbed mitochondrial function and increased reliance of non-oxidative metabolism [113], such as myalgia of the shoulder (m. trapezius) and neck. Also in these conditions, there is evidence

that microcirculatory impairment is of central importance for the occurrence of the symptoms [121-123]. In light of such data, and in accordance with the present findings, it has been proposed that impaired muscle blood flow and its consequences on the cellular level might have a general implication on chronic muscle pain in humans [124].

Most patients with TE recover spontaneously within a year [93] and conservative treatment is the cornerstone in dealing with this large patient group [102]. However, in a small fraction of these patients the condition becomes chronic and surgery might be indicated. Several of the operative procedures to treat TE involves some kind of release or lengthening of the ECRB tendon [93], such as the Z-lenghtening of the distal ECRB tendon introduced by Garden 1961 [51]. The latter procedure has been shown to produce a significant sarcomere shortening [125] and is considered to relieve the mechanical stress on the painful muscle origin. However, based on the present results it could be suggested that an important effect of the procedure is related to an increased blood flow in the ECRB muscle, which is facilitated by the relative muscle relaxation after the tendon lengthening.

The TE condition is generally looked upon as an overuse syndrome with degenerative changes in the proximal ECRB tendon. *Study IV* underlines that the ECRB muscle is also involved in the disorder and that TE is associated with a vascular dysregulation at muscle level. New ways of thinking about the condition may be required and even pharmacological treatment might be an option to improve the blood supply and turn a possible vicious circle of pain-muscle damage-impaired blood flow-increased pain etc, which may be an important feature of the condition.

4.5 CLINICAL IMPLICATIONS

- VOP is frequently used with only one strain gauge attached to the limb and the results obtained is referred to as blood flow in the limb. Placement of the strain gauge at the maximal circumference of the limb with a distal occlusion cuff at the level of the wrist or ankle is regarded as standard in this technique. However, even if the procedure is standardized in this manner, events taking place under one strain gauge cannot be strictly duplicated in adjacent portions of the limb.

- Small variations in sympathetic tone and venous pressure can influence blood flow measurements with the VOP technique, even in intra-individual comparisons. This variability should be taken into account when strain-gauge plethysmography is applied for limb blood flow determination, especially in interventional studies.

- Enhanced vasoconstrictory influences around a muscle injury may be an appropriate physiological response when blood is directed to the muscles during sympatho-adrenal activation.

- Both an acute small muscle injury (*Study III*) and a widespread and diffuse muscle damage, such as in the ECRB muscle of TE patients (*Study IV*), causes an abnormal blood flow reaction to ADR.

- The adverse ADR effect caused by the inserted microdialysis catheter in *Study III* may be related to the degree of invasiveness. In this case, it would be expected that any type of invasive measuring device causing a small muscle injury would possibly be able to provoke a similar reaction.

- Whether the vasoregulatory alteration observed in *Study IV* represents the primary aetiology in TE or is a secondary effect of the muscle injury cannot be determined, but it is likely to contribute to the development and maintenance of the chronic muscle pain in this large patient group.

4.6 FUTURE PERSPECTIVES

- VOP employing only one strain gauge attached to the limb will most likely be one of the most common methods for determination of limb blood flow also in the future. This is due to the relatively simple and non-invasive nature of the procedure. However, it is important to be meticulous in the placement of the strain gauge, the positioning of the limb and to avoid situations that alter the sympathetic tonus.

- The ^{133}Xe clearance technique for blood flow determinations in skeletal muscle is likely to be less common in the future as the isotope is becoming commercially unavailable. Meanwhile, the use of ^{99m}Tc clearance probably will increase since it is easy to obtain and have several other advantages compared to ^{133}Xe.

- Microdialysis ethanol technique will still be a necessary technique in order to determine blood flow fluctuation during microdialysis experiments and furthermore be an alternative for determination of local blood flow in skeletal muscle, although caution is warranted in interventional studies especially if ADR is involved in the procedure.

- After completion of *Study IV* all patients in the TE group underwent Z-lenghtening of the ECRB tendon at forearm level (Garden procedure) [51]. This technique has been used as standard surgical treatment for chronic TE cases after failed conservative treatment during the last 10-15 years at the Hand Surgery department in Stockholm. The surgery, performed by the author (TV), was successful and all subjects had a complete recovery. The obvious follow up study would be to reinvestigate the TE group to find out if the ADR effect also is normalized.

- After Z-lengthening of the ECRB tendon most patients recover and become more or less pain free within about three months postoperative. An even more interesting study would thus be prospective, investigating blood flow and ADR effect in the ECRB muscle in a new group of TE patients – preoperative and at several occasions during the recovery period, eventually being able to demonstrate a parallel improvement with normalization of the ADR effect and a simultaneous remission of the pain.

- If the altered ADR effect on muscle blood flow is normalized after a Garden procedure it may have profound impact on the understanding of the patophysiology behind the TE condition and may also be extended into other chronic muscle pain conditions as myalgia of the shoulder and neck.

5 CONCLUSIONS

5.1 STUDY I

The venous pressure-volume curve is S-shaped with a lower slope of the curve at high transmural pressure than at low pressure. In contracted veins, the curve is shifted to the right. Hence, depending on the position of the limb and whether the veins are in a relaxed or contracted state at the time of venous occlusion the obtained results will differ. The venous blood will be redistributed from segments with a high transmural pressure and, therefore, low compliance, to segments with a lower transmural pressure and, therefore, higher compliance.

5.2 STUDY II

The usage or non-usage of a distal wrist cuff during VOP for exclusion of hand circulation influences the results in several aspects. *With included hand circulation* the highest expansion rate was found in the distal segment at normal and high temperatures, but in the proximal segment at low temperature. This pattern was found with all arm positions. *With excluded hand circulation*, there was a significant two factor interaction between arm position – strain gauge position, which was independent of temperature. The highest expansion rate was found in the proximal segment when the arm was elevated, but in the distal segment when the arm was lowered.

5.3 STUDY III

The microdialysis catheter-induced muscle trauma alters the balance of vasodilatory and vasoconstrictory influences of ADR in a vasoconstrictive direction. The adverse ADR effect is likely to be related to the degree of invasiveness and/or the presence of the catheter. Further studies are needed to find out if any type of invasive measuring device causing a muscle injury is able to provoke a similar reaction.

This finding has a general physiological implication, but has also implications for the use of invasive techniques to investigate blood flow regulation in skeletal muscle.

5.4 STUDY IV

The altered ADR effect, seen in *Study IV*, indicates a vascular dysregulation in TE, which is likely to be of clinical significance by contributing to the development and maintenance of the chronic muscle pain in this large patient group. Whether the vasoregulatory alteration, which would be expected to involve recurring relative muscle ischemia, represents the primary aetiology in TE or is a secondary effect of the muscle injury cannot be determined. New ways of thinking about the condition may be required and even pharmacological treatment might be an option to improve the blood supply and avoid further damage to the affected ECRB muscle-tendon unit.

Evidently, it seems to be a distinct association between a small muscle injury, acute or chronic, and an abnormal blood flow reaction to adrenaline.

6 ACKNOWLEDGEMENTS

Many people have been involved in various ways in the completion of this thesis, thanks to you all. In particular, I want to express my sincere appreciation to the following.

Two grand men, *my captain* and *my navigator*, deserve a special gratitude.
Professor **Jan Henriksson,** my chief supervisor, for sharing his immense scientific knowledge and for teaching me the art of research. His patience and belief in me combined with his warm generosity and friendly spirit have been a most gratifying experience.
Professor **Lennart Jorfeldt**, my co-supervisor, for his brilliant mind and scientific excellence. His guidance through this process has been truly inspiring and educational, seasoned with enlightening anecdotes about “fresh fish” and “pink elephants”.

Björn-Ove Ljung, my co-supervisor and former chief of staff, for providing me with time and means to fulfill this project but also for the suggestion to study blood flow in the ECRB muscle of patients suffering from tennis elbow.

Marianne Arner, chief of staff at the department of Hand Surgery, Södersjukhuset, Stockholm, for her more than generous support and understanding.

Michael Werner, my co-author, for fruitful discussions and his remarkable skills in the art of ultrasonography, making the ECRB muscle injection in the forth study look easy.

Malin Pers, my dear colleague and former chief of staff, for her wonderful way as a senior surgeon to make me feel confident and important, since day one.

Sören Nylén, my external mentor, for his support and good sense of humour

Fredrik Roos, my roommate at work, for friendly and trustful conversations about anything and for being such a nice person.

My **colleagues and co-workers** at the department of Hand Surgery, Södersjukhuset, for their support and friendship.

Present and past Doctoral students at the department of Physiology and Pharmacology, Karolinska Institutet, especially **Björn Alkner, Kerstin Hamrin, Ulrika Widegren** and **Charlott Wretman** for valuable collaboration and discussions.

All **subjects** who generously participated in the studies.

The Mojlanen Masters gang for extraordinary friendship and indispensable yearly gatherings somewhere in the world with golf, laughter and happiness.

Team Stian "il porci ballante" for all the good times over the years. Briegel is back…

Relatives and **friends** for encouragement and support, especially **Jonas Vedung** for valuable language help in the forth study.

My parents, **Henry** and **Ingrid Vedung**, to whom I am truly grateful. Their love and support together with a fair amount of trust have allowed me to explore and make my own decisions in life.

Finally, **Kristin**, the love of my life, you and our kids **Johannes** and **Vera** make my life complete.

Abstract in Swedish

Flera olika kontrollsystem är involverade i den komplexa regleringen av blodflöde i human skelettmuskel. Interaktionen mellan dessa där ett system kan dominera eller modifiera ett annat komplicerar ytterligare. Mätning av muskelblodflöde kan därför vara utmanande särskilt om man betänker att tillgängliga mätmetoder är behäftade med olika tillkortakommanden och begränsningar. Denna doktorsavhandling har två olika inriktningar inom detta område varav båda relaterar till effekten av sympatiskt tonus.

De två inledande studierna belyser problemen med att använda venös ocklusions-pletysmografi med endast en strain gauge-slynga runt extremiteten. Denna modifiering av den ursprungliga metoden har uppenbara risker. *Studie I-II* påvisar att små variationer i sympatisk tonus och venöst tryck påverkar mätresultaten avsevärt. Den underliggande orsaken till detta är att förhållandet mellan tryck och volym i venerna inte är linjärt och det faktum att blod kan redistribueras mellan olika extremitetssegment. Resultaten demonstrerar tydligt att volymsexpansion i ett segment inte kan dupliceras och sägas gälla även intilliggande segment.

De avslutande två studierna fokuserar kring möjligheten att en mindre muskelskada kan framkalla ett förändrat blodflödessvar på adrenalin. Flera rapporter indikerar att detta kan stämma. Vi undersökte om en akut tillfogad muskelskada (*Studie III*) och en kroniskt skadad muskel (*Studie IV*) förändrar adrenalinets effekt på muskelblodflödet. I enlighet med hypotesen gav den akuta muskelskadan orsakad av en inlagd mikrodialyskateter ett signifikant minskat blodflöde under intravenös adrenalininfusion (^{133}Xenon clearance intill en inneliggande men oanvänd kateter). Om ^{133}Xe injektionen administrerades konventionellt utan en kateter i muskeln, påvisades ingen signifikant blodflödesförändring. Den avvikande adrenalineffekten på blodflödet är troligen relaterad till graden av invasivitet. Alla typer av invasiva mätinstrument som orsakar en muskelskada borde därför kunna framkalla en liknande reaktion. Detta fynd har en generell fysiologisk betydelse men påverkar också användandet av invasiva mätmetoder vid studier av blodflödesreglering i skelettmuskel.

Tidigare studier av tennis armbåge har visat att den involverade muskeln, extensor carpi radialis brevis (ECRB), uppvisar tecken till en diffus och utbredd muskelskada och ett minskat blodflöde. Vi genomförde en fall-kontroll studie (*Studie IV*) med hypotesen att muskelskadan i ECRB, hos patienter med tennisarmbåge, orsakar ett förändrat blodflödessvar på adrenalin. Muskelblodflöde mättes med ^{99m}Technetium clearance lokalt i ECRB muskeln i samband med en intravenös adrenalininfusion. I enlighet med hypotesen var blodflödessvaret distinkt olika i de två undersökningsgrupperna (statistiskt signifikant interaktion, grupp * effect). Adrenalinet gav inte någon förändring av ^{99m}Technetium clearance i kontrollgruppen men framkallade en signifikant minskning i tennisarmbågsgruppen. Den förändrade adrenalineffekten indikerar att det föreligger en störd kärlreglering i ECRB muskeln vid tennisarmbåge. Troligen kan detta ha klinisk betydelse i utvecklandet och underhållandet av den kroniska muskelsmärtan i denna stora patientgrupp. Om den förändrade kärlregleringen, som troligen kan orsaka återkommande relativ syrebrist i muskeln, representerar primärpatologin vid tennisarmbåge eller om den är sekundär till muskelskadan kan dock inte avgöras.

Sammanfattningsvis, en mindre muskelskada, akut eller kronisk, förefaller kunna förändra blodflödessvaret på adrenalin i kärlsammandragande riktning.

7 REFERENCES

1 Joyner, M.J., Dietz, N.M., Shepherd, J.T. (2001) From Belfast to Mayo and beyond: The use and future of plethysmography to study blood flow in human limbs. J Appl Physiol. **91**, 2431-2441.

2 Lassen, N.A., Lindbjerg, J., Munck, O. (1964) Measurement of blood-flow through skeletal muscle by intramuscular injection of Xenon-133. Lancet. **15**, 686-689.

3 Tønnesen, K.H., Sejrsen, P. (1970) Washout of ^{133}Xenon after intramuscular injection and direct measurement of blood flow in skeletal muscle. Scand J Clin Lab Invest. **25**, 71-81.

4 Hickner, R.C., Rosdahl, H., Borg, I., Ungerstedt, U., Jorfeldt, L., Henriksson, J. (1992) The ethanol technique of monitoring local blood flow changes in rat skeletal muscle: Implications for microdialysis. Acta Physiol Scand. **146**, 87-97.

5 Widegren, U., Hickner, R.C., Jorfeldt, L., Henriksson, J. (2010) Muscle blood flow response to mental stress and adrenaline infusion in man: Microdialysis ethanol technique compared to ^{133}Xe clearance and Venous occlusion plethysmography. Clin Physiol Funct Imaging. **30**, 152-161.

6 Rosdahl, H., Samuelsson, A.C., Ungerstedt, U., Henriksson, J. (1998) Influence of adrenergic agonists on the release of amino acids from rat skeletal muscle studied by microdialysis. Acta Physiol Scand. **163**, 349-360.

7 Levick, J.R. (1995) An introduction to cardiovascular physiology, Butterworth-Heinemann, Oxford

8 Takamine, J. (1902) The isolation of the active principle of the suprarenal gland. J Physiology. **27**, xxix-xxx.

9 Bennett, M.R. (1999) One hundred years of adrenaline: The discovery of autoreceptors. Clin Auton Res. **9**, 145-159.

10 Stolz, F. (1904) Uber adrenalin und alkylaminoacetobrenzcatechin. Ber Dtsch Chem Ges. **37**, 4149-4154.

11 Dakin, H.D. (1905) The synthesis of a substance allied to noradrenaline. Proc Roy Soc Lond Series B. **LXXVI**, 491-497.

12 Barger, G., Dale, H.H. (1910) Chemical structure and sympathomimetic action of amines. J Physiology. **41**, 19-59.

13 Euler, U.S.V. (1946) A specific sympathomimetic Ergone in Adrenergic nerve fibres (Sympathin) and its relations to Adrenaline and Noradrenaline. Acta Physiol Scand. **12**, 73-97.

14 Ahlquist, R.P. (1948) A study of the adrenotropic receptors. Am J Physiol. **153**, 586-600.

15 Rang, H.P., Dale, H.H. (1991) Pharmacology, 2nd ed., Churchill Livingstone, London

16 Moran, N.C., Perkins, M.E. (1958) Adrenergic blockade of the mammalian heart by a dichloro analogue of isoproterenol. J Pharmacol Exp Ther. **124**, 223-237.

17 Powell, C.E., Slater, I.H. (1958) Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. J Pharmacol Exp Ther. **122**, 480-488.

18 Bylund, D.B., Eikenberg, D.C., Hieble, J.P., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Molinoff, P.B., Ruffolo, R.R., Jr., Trendelenburg, U. (1994) International union of pharmacology nomenclature of adrenoceptors. Pharmacol Rev. **46**, 121-136.

19 Langer, S.Z. (2008) Presynaptic autoreceptors regulating transmitter release. Neurochem Int. **52**, 26-30.

20 Langer, S.Z. (1974) Presynaptic regulation of catecholamine release. Biochem Pharmacol. **23**, 1793-1800.

21 Starke, K. (2001) Presynaptic autoreceptors in the third decade: Focus on alpha2-adrenoceptors. J Neurochem. **78**, 685-693.

22 Hieble, J.P., Bylund, D.B., Clarke, D.E., Eikenburg, D.C., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Ruffolo, R.R., Jr. (1995) International union of pharmacology. X. Recommendation for nomenclature of alpha 1-adrenoceptors: Consensus update. Pharmacol Rev. **47**, 267-270.

23 Civantos Calzada, B., Aleixandre de Artinano, A. (2001) Alpha-adrenoceptor subtypes. Pharmacol Res. **44**, 195-208.

24 Bylund, D.B. (2005) Adrenergic receptors: Historical perspectives from the 20th century. in The adrenergic receptors in the 21st century,(ed) Perez, D.M., pp 3-21,Totowa, Humana

25 Bylund, D.B. (2007) Alpha- and beta-adrenergic receptors: Ahlquist's landmark hypothesis of a single mediator with two receptors. Am J Physiol Endocrinol Metab. **293**, E1479-1481.

26 Dinenno, F.A., Joyner, M.J. (2006) Alpha-adrenergic control of skeletal muscle circulation at rest and during exercise in aging humans. Microcirculation. **13**, 329-341.

27 Langer, S.Z., Hicks, P.E. (1984) Alpha-adrenoreceptor subtypes in blood vessels: Physiology and pharmacology. J Cardiovasc Pharmacol. **6 Suppl 4**, S547-558.

28 Guimaraes, S., Moura, D. (2001) Vascular adrenoceptors: An update. Pharmacol Rev. **53**, 319-356.

29 Gentili, F., Pigini, M., Piergentili, A., Giannella, M. (2007) Agonists and antagonists targeting the different alpha2-adrenoceptor subtypes. Curr Top Med Chem. **7**, 163-186.

30 Stjärne, L., Brundin, J. (1975) Dual adrenoceptor-mediated control of noradrenaline secretion from human vasoconstrictor nerves: Facilitation by beta-receptors and inhibition by alpha-receptors. Acta Physiol Scand. **94**, 139-141.

31 Stjärne, L., Brundin, J. (1976) Beta2-adrenoceptors facilitating noradrenaline secretion from human vasoconstrictor nerves. Acta Physiol Scand. **97**, 88-93.

32 Ahlquist, R.P. (1976) Present state of alpha- and beta-adrenergic drugs I. The adrenergic receptor. Am Heart J. **92**, 661-664.

33 Lundholm, L. (1956) The mechanism of the vasodilator effect of adrenaline. I. Effect on skeletal muscle vessels. Acta Physiol Scand. **39**, 1-52.

34 Barcroft, H., Swan, H.J.C. (1953) Sympathetic control of human blood vessels, Edward Arnold & Co., London

35 Duff, R.S., Swan, H.J. (1951) Further observations on the effect of adrenaline on the blood flow through human skeletal muscle. J Physiol. **114**, 41-55.

36 Freyschuss, U., Hjemdahl, P., Juhlin-Dannfelt, A., Linde, B. (1986) Cardiovascular and metabolic responses to low dose adrenaline infusion: An invasive study in humans. Clin. Sci. **70**, 199-206.

37 Alpert, J.S., Coffman, J.D. (1969) Effect of intravenous epinephrine on skeletal muscle, skin, and subcutaneous blood flow. Am J Physiol. **216**, 156-160.

38 Barcroft, H., Briggs, M., Gimlette, T.M.D., Nasrallah, A. (1967) Validity of the ^{133}Xenon method for determination of muscle blood flow in man as evaluated by simultaneous Venous occlusion plethysmography during intravenous infusion of adrenaline. Cardiovascular Res. **1**, 229-232.

39 Greenfield, A.D. (1960) Venous occlusion plethysmography. Methods Med Res. **8**, 293-301.

40 Whitney, R.J. (1953) The measurement of volume changes in human limbs. J Physiol. **121**, 1-27.

41 Sumner, D.S. (1985) Volume plethysmography in vascular disease: An overview. in Noninvasive diagnostic techniques in vascular disease,(ed) Bernstein, E.F., pp 97-118,St Louis, Mosby CV

42 Bevegard, B.S., Shepherd, J.T. (1965) Effect of local exercise of forearm muscles on forearm capacitance vessels. J Appl Physiol. **20**, 968-974.

43 Clarke, R.S., Hellon, R.F. (1957) Venous collection in forearm and hand measured by the strain-gauge and volume plethysmograph. Clin Sci (Lond). **16**, 103-117.

44 Seto, M., Bunko, H., Shuke, N., Takahashi, K., Sakaibori, Y., Terada, H., Imabayashi, E., Kuji, I., Matsuda, H., Yokoyama, S. (2008) Quantitative regional blood flow measurements in exercising leg skeletal muscle based on ^{99m}Tc-pertechnetate clearance. Nucl Med Commun. **29**, 770-774.

45 Cutajar, C.L., Brown, N.J., Marston, A. (1971) Muscle blood-flow studies by the technetium (^{99m}Tc) clearance technique in normal subjects and in patients with intermittent claudication. Br J Surg. **58**, 532-537.

46 Brown, S.L., Hunt, J.W., Hill, R.P. (1988) A comparison of the rate of clearance of xenon (^{133}Xe) and pertechnetate ion ($^{99m}TcO_4^-$) in murine tumors and normal leg muscles. Int J Rad Appl Instrum B. **15**, 381-390.

47 Peters, M.A. (2009) Letters to the editor: Quantitative regional blood flow measurements in excercising leg skeletal muscle based on ^{99m}Tc-pertechnetate clearance. Nucl Med Commun. **30**, 651-653.

48 Seto, M., Bunko, H., Shuke, N., Takahashi, K., Sakaibori, Y., Terada, H., Imabayashi, E., Kuji, I., Matsuda, H., Yokoyama, S. (2009) Reply (letters to the editor). Nucl Med Commun. **30**, 320-321.

49 de Jonge, F.A., Pauwels, E.K. (1996) Technetium, the missing element. Eur J Nucl Med. **23**, 336-344.

50 Eckelman, W.C. (2009) Unparalleled contribution of Technetium-99m to medicine over 5 decades. JACC Cardiovasc Imaging. **2**, 364-368.

51 Garden, R. (1961) Tennis elbow. J Bone Joint Surg Am. **43B**, 100-106.

52 Conn, H.L., Jr. (1961) Equilibrium distribution of radioxenon in tissue: Xenon-hemoglobin association curve. J Appl Physiol. **16**, 1065-1070.

53 Rosdahl, H.: Microdialysis sampling from skeletal muscle and adipose tissue with special reference to the effects of insulin on tissue blood flow and glucose metabolism: Department of Physiology and Pharmacology. Stockholm, Karolinska Institutet, 1998, PhD, pp 10-11.

54 Rojek, A.M., Wood, R.E., Stewart, I.B. (2007) The effect of changing limb position on the validity of venous occlusion plethysmography. Physiol Meas. **28**, 861-867.

55 Strauch, B., Yu, H.-L. (2006) Atlas of microvascular surgery, 2nd, Thieme, New York

56 Wahren, J. (1966) Quantitative aspects of blood flow and oxygen uptake in the human forearm during rhythmic exercise. Acta Physiol Scand Suppl. **269**, 1-93.

57 Timmons, M.J. (1986) The vascular basis of the radial forearm flap. Plast Reconstr Surg. **77**, 80-92.

58 Valentino, J., Funk, G.F., Hoffman, H.T., McCulloch, T.J. (1996) The communicating vein and its use in the radial forearm free flap. Laryngoscope. **106**, 648-651.

59 Imanishi, N., Nakajima, H., Aiso, S. (2000) Anatomic study of the venous drainage architecture of the forearm skin and subcutaneous tissue. Plast Reconstr Surg. **106**, 1287-1294.

60 Mascuelet, A.C., Gilbert, A. (2005) An atlas of flaps of the musculoskeletal system, 3rd, Martin Dunitz, Oxon

61 Yang, G.F., Chen, P.J., Gao, Y.Z., Liu, X.Y., Li, J., Jiang, S.X., He, S.P. (1997) Forearm free skin flap transplantation: A report of 56 cases. 1981. Br J Plast Surg. **50**, 162-165.

62 Benjamin, N., Calver, A., Collier, J., Robinson, B., Vallance, P., Webb, D. (1995) Measuring forearm blood flow and interpreting the responses to drugs and mediators. Hypertension. **25**, 918-923.

63 Wilkinson, I.B., Webb, D.J. (2001) Venous occlusion plethysmography in cardiovascular research: Methodology and clinical applications. Br J Clin Pharmacol. **52**, 631-646.

64 Novotny, J.A., Mayers, D.L., Parsons, Y.F., Survanshi, S.S., Weathersby, P.K., Homer, L.D. (1990) Xenon kinetics in muscle are not explained by a model of parallel perfusion-limited compartments. J Appl Physiol. **68**, 876-890.

65 Homer, L.D., Weathersby, P.K. (1986) How well mixed is inert gas in tissues? J Appl Physiol. **60**, 2079-2088.

66 Bonde Petersen, F., Siggaard-Andersen, J. (1967) Blood flow in skin and muscle, evaluated by simultaneous Venous occlusion plethysmography and ^{133}Xe clearance. Scand J Clin Lab Invest. 113-119.

67 Sejrsen, P., Tønnesen, K.H. (1972) Shunting by diffusion of inert gas in skeletal muscle. Acta Physiol Scand. **86**, 82-91.

68 Sparks, H.V., Mohrman, D.E. (1977) Heterogeneity of flow as an explanation of the multi-exponential washout of inert gas from skeletal muscle. Microvasc Res. **13**, 181-184.

69 Radegran, G. (1999) Limb and skeletal muscle blood flow measurements at rest and during exercise in human subjects. Proc Nutr Soc. **58**, 887-898.

70 Larsen, O.A., Lassen, N.A., Quaade, F. (1966) Blood flow through human adipose tissue determined with radioactive Xenon. Acta Physiol Scand. **66**, 337-345.

71 Vedung, T., Jorfeldt, L., Henriksson, J. (2010) Intravenous adrenaline infusion causes vasoconstriction close to an intramuscular microdialysis catheter in humans. Clin Physiol Funct Imaging. **30,** 399-405.

72 Hodges, G.J., Chiu, C., Kosiba, W.A., Zhao, K., Johnson, J.M. (2009) The effect of microdialysis needle trauma on cutaneous vascular responses in humans. J Appl Physiol. **106**, 1112-1118.

73 Bolli, P., Erne, P., Ji, B.H., Block, L.H., Kiowski, W., Buhler, F.R. (1984) Adrenaline induces vasoconstriction through post-junctional alpha 2 adrenoceptors and this response is enhanced in patients with essential hypertension. J Hypertens Suppl. **2**, S115-118.

74 Ariens, E.J., Simonis, A.M. (1983) Physiological and pharmacological aspects of adrenergic receptor classification. Biochem Pharmacol. **32**, 1539-1545.

75 Piascik, M.T., Soltis, E.E., Piascik, M.M., Macmillan, L.B. (1996) Alpha-adrenoceptors and vascular regulation: Molecular, pharmacologic and clinical correlates. Pharmacol Ther. **72**, 215-241.

76 Floras, J.S., Aylward, P.E., Victor, R.G., Mark, A.L., Abboud, F.M. (1988) Epinephrine facilitates neurogenic vasoconstriction in humans. J Clin Invest. **81**, 1265-1274.

77 Stankevicius, E., Kevelaitis, E., Vainorius, E., Simonsen, U. (2003) Role of nitric oxide and other endothelium-derived factors. Medicina (Kaunas). **39**, 333-341.

78 Esper, R.J., Nordaby, R.A., Vilarino, J.O., Paragano, A., Cacharron, J.L., Machado, R.A. (2006) Endothelial dysfunction: A comprehensive appraisal. Cardiovasc Diabetol. **5**, 4.

79 Joyner, M.J., Dietz, N.M. (2003) Sympathetic vasodilation in human muscle. Acta Physiol Scand. **177**, 329-336.

80 Hynynen, M.M., Khalil, R.A. (2006) The vascular endothelin system in hypertension--recent patents and discoveries. Recent Pat Cardiovasc Drug Discov. **1**, 95-108.

81 Mellergard, P., Aneman, O., Sjogren, F., Pettersson, P., Hillman, J. (2008) Changes in extracellular concentrations of some cytokines, chemokines, and neurotrophic factors after insertion of intracerebral microdialysis catheters in neurosurgical patients. Neurosurgery. **62**, 151-157; discussion 157-158.

82 Jakob, S.M., Ensinger, H., Takala, J. (2001) Metabolic changes after cardiac surgery. Curr Opin Clin Nutr Metab Care. **4**, 149-155.

83 Uvnäs, B. (1984) From Physiologist to Pharmacologist--promotion or degradation? Fifty years in retrospect. Annu Rev Pharmacol Toxicol. **24**, 1-18.

84 Berecek, K.H., Brody, M.J. (1982) Evidence for a neurotransmitter role for epinephrine derived from the adrenal medulla. Am J Physiol. **242**, H593-601.

85 Floras, J.S., Aylward, P.E., Mark, A.L., Abboud, F.M. (1990) Adrenaline facilitates neurogenic vasoconstriction in borderline hypertensive subjects. J Hypertens. **8**, 443-448.

86 Floras, J.S. (1992) Epinephrine and the genesis of hypertension. Hypertension. **19**, 1-18.

87 Grant, R.T. (1964) Direct observation of skeletal muscle blood vessels (rat cremaster). J Physiol. **172**, 123-137.

88 Hickner, R.C., Ekelund, U., Mellander, S., Ungerstedt, U., Henriksson, J. (1995) Muscle blood flow in cats: Comparison of microdialysis ethanol technique with direct measurement. J Appl Physiol. **79**, 638-647.

89 Fuchi, T., Rosdahl, H., Hickner, R.C., Ungerstedt, U., Henriksson, J. (1994) Microdialysis of rat skeletal muscle and adipose tissue: Dynamics of the interstitial glucose pool. Acta Physiol Scand. **151**, 249-260.

90 Hickner, R.C., Ungerstedt, U., Henriksson, J. (1994) Regulation of skeletal muscle blood flow during acute insulin-induced hypoglycemia in the rat. Diabetes. **43**, 1340-1344.

91 Rosdahl, H., Ungerstedt, U., Jorfeldt, L., Henriksson, J. (1993) Interstitial glucose and lactate balance in human skeletal muscle and adipose tissue studied by microdialysis. J. Physiol. **471**, 637-657.

92 Buchbinder, R., Green, S.E., Struijs, P.A. (2008) Tennis elbow. Clin Evid (Online). **2008**,

93 Faro, F., Wolf, J.M. (2007) Lateral epicondylitis: Review and current concepts. J Hand Surg Am. **32**, 1271-1279.

94 Karkhanis, S., Frost, A., Maffulli, N. (2008) Operative management of tennis elbow: A quantitative review. Br Med Bull. **88**, 171-188.

95 Vicenzino, B. (2003) Lateral epicondylalgia: A musculoskeletal physiotherapy perspective. Man Ther. **8**, 66-79.

96 Nirschl, R.P., Ashman, E.S. (2003) Elbow tendinopathy: Tennis elbow. Clin Sports Med. **22**, 813-836.

97 Ali, M., Lehman, T.A. (2009) Lateral elbow tendinopathy: A better term than lateral epicondylitis or tennis elbow. J Hand Surg Am. **34**, 1575; author reply 1576.

98 Rayan, G.M., Coray, S.A. (2001) V-Y slide of the common extensor origin for lateral elbow tendonopathy. J Hand Surg Am. **26**, 1138-1145.

99 Stasinopoulos, D., Johnson, M.I. (2006) 'lateral elbow tendinopathy' is the most appropriate diagnostic term for the condition commonly referred-to as lateral epicondylitis. Med Hypotheses. **67**, 1400-1402.

100 Major, H.P. (1883) Lawn-tennis elbow. Bmj. **2**, 557.

101 Boyer, M.I., Hastings, H., 2nd (1999) Lateral tennis elbow: "Is there any science out there?" J Shoulder Elbow Surg. **8**, 481-491.

102 Flatt, A.E. (2008) Tennis elbow. Proc (Baylor University Medical Center). **21**, 400-402.

103 Coonrad, R.W., Hooper, W.R. (1973) Tennis elbow: Its course, natural history, conservative and surgical management. J Bone Joint Surg Am. **55**, 1177-1182.

104 Cyriax, J.H. (1936) The pathology and treatment of tennis elbow. J Bone Joint Surg Am. **18**, 921-940.

105 Doran, A., Gresham, G.A., Rushton, N., Watson, C. (1990) Tennis elbow. A clinicopathologic study of 22 cases followed for 2 years. Acta Orthop Scand. **61**, 535-538.

106 Galliani, I., Burattini, S., Mariani, A.R., Riccio, M., Cassiani, G., Falcieri, E. (2002) Morpho-functional changes in human tendon tissue. Eur J Histochem. **46**, 3-12.

107 Regan, W., Wold, L.E., Coonrad, R., Morrey, B.F. (1992) Microscopic histopathology of chronic refractory lateral epicondylitis. Am J Sports Med. **20**, 746-749.

108 Schneeberger, A.G., Masquelet, A.C. (2002) Arterial vascularization of the proximal extensor carpi radialis brevis tendon. Clin Orthop Relat Res. 239-244.

109 Bales, C.P., Placzek, J.D., Malone, K.J., Vaupel, Z., Arnoczky, S.P. (2007) Microvascular supply of the lateral epicondyle and common extensor origin. J Shoulder Elbow Surg. **16**, 497-501.

110 Nirschl, R.P. (1992) Elbow tendinosis/tennis elbow. Clin Sports Med. **11**, 851-870.

111 Ljung, B.O.: Wrist extensor muscle mechanics: With special reference to the pathophysiology of tennis elbow: Department of Orthopaedics, Institute of Surgical Sciences. Gothenburg, Gothenburg University, 1998, PhD

112 Ljung, B.O., Forsgren, S., Friden, J. (1999) Sympathetic and sensory innervations are heterogeneously distributed in relation to the blood vessels at the extensor carpi radialis brevis muscle origin of man. Cells Tissues Organs. **165**, 45-54.

113 Ljung, B.O., Lieber, R.L., Friden, J. (1999) Wrist extensor muscle pathology in lateral epicondylitis. J Hand Surg Br. **24**, 177-183.

114 Oskarsson, E., Gustafsson, B.E., Pettersson, K., Aulin, K.P. (2007) Decreased intramuscular blood flow in patients with lateral epicondylitis. Scand J Med Sci Sports. **17**, 211-215.

115 Oskarsson, E., Piehl Aulin, K., Gustafsson, B.E., Pettersson, K. (2008) Improved intramuscular blood flow and normalized metabolism in lateral epicondylitis after botulinum toxin treatment. Scand J Med Sci Sports.

116 Smith, R.W., Papadopolous, E., Mani, R., Cawley, M.I. (1994) Abnormal microvascular responses in a lateral epicondylitis. Br J Rheumatol. **33**, 1166-1168.

117 Lindqvist, M., Kahan, T., Melcher, A., Bie, P., Hjemdahl, P. (1996) Forearm vasodilator mechanisms during mental stress: Possible roles for epinephrine and anp. Am J Physiol. **270**, E393-399.

118 Freyschuss, U., Hjemdahl, P., Juhlin-Dannfelt, A., Linde, B. (1986) Cardiovascular and metabolic responses to low dose adrenaline infusion: An invasive study in humans. Clin Sci (Lond). **70**, 199-206.

119 Freyschuss, U., Hjemdahl, P., Juhlin-Dannfelt, A., Linde, B. (1988) Cardiovascular and sympathoadrenal responses to mental stress: Influence of beta-blockade. Am J Physiol. **255**, H1443-1451.

120 Galbo, H., Holst, J.J., Christensen, N.J. (1975) Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. J Appl Physiol. **38**, 70-76.

121 Larsson, R., Cai, H., Zhang, Q., Oberg, P.A., Larsson, S.E. (1998) Visualization of chronic neck-shoulder pain: Impaired microcirculation in the upper trapezius muscle in chronic cervico-brachial pain. Occup Med (Lond). **48**, 189-194.

122 Larsson, R., Oberg, P.A., Larsson, S.E. (1999) Changes of trapezius muscle blood flow and electromyography in chronic neck pain due to trapezius myalgia. Pain. **79**, 45-50.

123 Larsson, S.E., Bodegard, L., Henriksson, K.G., Oberg, P.A. (1990) Chronic trapezius myalgia. Morphology and blood flow studied in 17 patients. Acta Orthop Scand. **61**, 394-398.

124 Raymer, G.H., Green, H.J., Ranney, D.A., Marsh, G.D., Thompson, R.T. (2009) Muscle metabolism and acid-base status during exercise in forearm work-related myalgia measured with 31p-mrs. J Appl Physiol. **106**, 1198-1206.

125 Friden, J., Lieber, R.L. (1994) Physiologic consequences of surgical lengthening of extensor carpi radialis brevis muscle-tendon junction for tennis elbow. J Hand Surg Am. **19**, 269-274.