Non-invasive measurement of skeletal muscle blood flow using photoplethysmography

-Experimental studies on the human leg-

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Göteborg, Sweden, 2002

NON-INVASIVE MEASUREMENT OF SKELETAL MUSCLE BLOOD FLOW USING PHOTOPLETHYSMOGRAPHY

Experimental studies on the human leg

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I. **Zhang Q, Lindberg LG, Kadefors R, Styf J.** A non-invasive measure of changes in blood flow of the human anterior tibial muscle. European Journal of Applied Physiology 2001; 84(5): 448-452.

II. **Zhang Q, Styf J, Lindberg LG.** Effects of limb elevation and increased intramuscular pressure on human tibialis anterior muscle blood flow. European Journal of Applied Physiology 2001; 85(6): 567-571.

III. **Zhang Q, Styf J.** Abnormally elevated intramuscular pressure impairs muscle blood flow at rest after exercise. Submitted, 2002.

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V. **Zhang Q, Andersson G, Lindberg LG, Styf J.** Muscle blood flow in response to concentric muscular activity versus leg activation with venous foot pump. Submitted, 2002.

ABSTRACT

Qiuxia Zhang, **Non-invasive measurement of skeletal muscle blood flow using photoplethysmography. Experimental studies on the human leg.** Department of Orthopaedics, Institute of Surgical Sciences, Göteborg University, Göteborg, Sweden.

The purpose of this thesis was to validate a new non-invasive photoplethysmography (PPG) method for measuring relative changes in local muscle blood flow (MBF) in the human leg. Experimental conditions expected to impede or increase local MBF were used to test responsiveness of the PPG.

The PPG method for monitoring blood flow in muscle following arterial occlusion and isometric and concentric contractions was validated (Study I). Invasive single-fibre laser-Doppler flowmetry was used as a reference method. MBF increased significantly after arterial occlusion and following isometric and concentric contractions, as measured with both methods. Skin blood flow increased significantly after arterial occlusion but did not change significantly after muscle contractions. The effects of limb elevation and increased intramuscular pressure (IMP) on MBF and leg neuromuscular function were investigated (Study II). MBF decreased significantly, by 50%, and perfusion pressure was reduced from 65 mmHg to 17 mmHg when IMP was elevated to 40 mmHg by venous obstruction (elevated casted leg). Subjects experienced sensory dysfunction and muscular weakness only in the elevated leg. Study III examined whether or not PPG could detect MBF changes in response to exercise during elevated IMP and under conditions of normal IMP. MBF at rest after exercise was significantly lower when IMP was elevated compared to normal IMP. Study IV evaluated the ability of PPG to measure MBF during foot-transmitted vibration exposure. The filtering technique applied produced a marked reduction of the vibration-induced artefacts on the PPG signal, thus making it possible to measure MBF during vibration exposure. In Study V, changes in MBF following concentric muscular activity or leg activation with a venous foot pump were compared. MBF increased significantly following concentric muscular activity, but not following leg activation by the venous foot pump. MBF was significantly greater in the exercised leg compared with the leg exposed to the venous foot pump during venous obstruction.

In conclusion, photoplethysmography, with the aid of a custom-designed probe, enables non-invasive, continuous assessment of relative changes in local MBF in response to, for example, arterial occlusion, muscle contractions, and increased intramuscular pressure. The PPG technique is a promising non-invasive diagnostic tool for direct measurement of local MBF under clinical conditions with and without abnormally elevated IMP. MBF can be measured by PPG during vibration exposure using a filtering technique, thus extending the possibilities of applying this technique to different physiological and ergonomic conditions.

Keywords: photoplethysmography, skeletal muscle blood flow, perfusion pressure, intramuscular pressure, venous obstruction, muscle exercise, non-invasive, limb elevation, vibration exposure, hyperaemia, foot pump.

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ABBREVIATIONS

IMP	Intramuscular pressure
LDF	Laser-Doppler flowmetry
MAP	Mean arterial blood pressure
MBF	Muscle blood flow
MCP	Muscle contraction pressure
PP	Perfusion pressure
PPG	Photoplethysmography

INTRODUCTION

1. Background

Muscle function depends on blood flow at the microvascular level since all exchange of oxygen, nutrients, fluids and waste products occurs at this level. Blood flow is one of the important factors allowing for continued contraction of skeletal muscle during exercise (Kalliokoski et al. 2000). Blood flow changes in skeletal muscle tissue are of great interest in both health and disease. Therefore, measurements of local muscle blood flow are of particular importance, because they provide valuable information about the function and regulation of the circulatory system. Until recently, measurements of local muscle blood flow have been limited, owing to the absence of suitable methods (Jensen et al. 1995; Hughson & Tschakovsky 1999).

Blood flow (Q) depends on the pressure difference between arterial pressure (P_A) and venous pressure (P_V), and resistance (R) to the blood flow, $Q = (P_A-P_V)/R$. This formula states that the blood flow is directly proportional to the pressure difference, but inversely proportional to the resistance (Feigl 1974a). Therefore, measurements of blood flow can reflect changes in the pressure difference in the circulatory system. When tissue pressure rises, the pressure in the local veins must also rise (Kjellmer 1964). The increased venous pressure reduces the local arteriovenous gradient, and thereby local blood flow (Burton 1954; Matsen et al. 1980).

2. Anatomy of the anterior compartment of the leg

The anterior compartment contains the extensor muscles. These are the tibialis anterior, extensor digitorum longus, extensor hallucis longus, and the fibularis (peroneus) tertius. These muscles dorsiflex the ankle, invert the foot, and extend the toes. The anterior tibial artery is the major source of blood supply to the anterior compartment. The deep peroneal nerve supplies the muscles in the anterior compartment. This nerve terminates between the big toe and second toe and can be tested at this point.

The muscles of the anterior compartment are enclosed in a relatively unyielding osteo-facial space (Fig. 1), which means that if the muscles in the compartment swell, the fascial may not be capable of sufficient expansion to accommodate these changes.

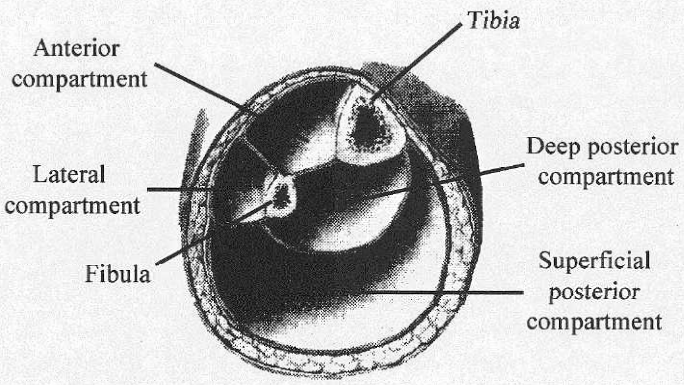

Figure 1. Cross-section of the leg.

3. Muscle blood flow

Central control mechanisms

These consist of both humoral and neural control systems, responsible for regulating cardiac activity and regional vascular tone throughout the body during exercise. Investigators utilising pharmacological blockade of cholinergic muscarinic receptors and sympathectomy have concluded that neither sympathetic cholinergic nor adrenergic neural mechanisms are involved in the initial hyperaemia (Delp 1999). The exercise-induced increase in blood flow to skeletal muscle is primarily the result of local vascular control systems within the muscle tissue (Delp & Laughlin 1998).

Metabolic or chemical mechanisms

Blood flow is very low in resting skeletal muscle, in part because the muscles are inactive (Delp 1999). Peak muscle blood flow during exercise is estimated to be up to between 300 and 400 ml/min/100g. This represents a 20- to 100- fold increase relative to that at rest (Rowell 1988). There is normally a close relationship between skeletal muscle metabolic rate and muscle perfusion. When the metabolic rate of the tissue increases or oxygen delivery to the tissue decreases, more vasodilator substance is formed and blood flow increases (Berne & Levy 1996). The increased blood flow and oxygen extraction that result from metabolic vasodilatation increase oxygen supply to a level compatible with tissue oxygen demand (Delp & Laughlin 1998).

Myogenic mechanisms

The myogenic theory of local blood flow control is based on the assumption that vascular resistance is determined in part by transmural pressure at the arteriolar level because of the effect of stretch on vascular smooth muscle tone (Meininger & Davis 1992). According to this theory, the smooth muscle is thought to be sensitive to transmural pressure. This mechanism seems particularly well suited for responding to skeletal muscle contraction. The increase in intramuscular pressure would trigger vasodilatation in response to the corresponding fall in transmural pressure (Segal & Kurjiaka 1995).

Muscle pump mechanism

The mechanism of rhythmic muscular contractions, repeatedly emptying the veins and facilitating perfusion of skeletal muscle, is often referred to as "the muscle pump" (Folkow et al. 1970; Lash 1996; Laughlin & Schrage 1999). Immediately on release of a contraction, the pressure in the veins is probably close to zero. This implies that the pressure difference across all the capillaries in the muscle has increased (Delp & Laughlin 1998; Delp 1999; Hughson & Tschakovsky 1999). It has been proposed that this occurs through two mechanisms associated with the pumping action of muscle (Laughlin 1987). First, the muscle pump imparts kinetic energy to the blood. Muscular contraction produces compression of the veins causing blood to be transported out of compressed segments. The refilling of the "muscle pump" occurs during muscle relaxation (Stick et al. 1992; Delp 1999). The second mechanism is that the muscle pump increases the pressure difference across the capillaries by lowering pressure in the venules and deep small veins during the relaxation phase of the contraction cycle (Laughlin 1987). The decreased pressure in small veins produced by their expansion in the muscle during relaxation is a key to the muscle pump (Laughlin & Schrage 1999). The muscle pump is thought to play a key role in the immediate rise in blood flow. It can augment flow before any vasodilatation seen with the onset of contractions (Sheriff et al. 1993; Tschakovsky et al. 1996).

Autoregulation

Autoregulation means that blood flow to an organ remains constant over a wide range of perfusion pressures (Feigl 1974b). Autoregulation of blood flow has also been observed in skeletal muscle. When perfusion pressure (P_A-P_V) initially decreases, blood flow falls because of the relationship among pressure, flow and resistance [$Q = (P_A\text{-}P_V)/R$]. When blood flow falls, metabolic or myogenic mechanisms cause arteriolar vasodilatation and a fall in resistance (R). As resistance decreases, blood flow increases, despite the presence of reduced perfusion pressure.

Active hyperaemia

Active hyperaemia is the increase in organ blood flow associated with increased metabolic activity of an organ or tissue. With increased metabolic activity, vascular resistance decreases due to both vasodilatation and vascular recruitment (Guyton 1991). Active hyperaemia is also termed "post-exercise hyperaemia" or "functional hyperaemia". Post-exercise hyperaemia, reflecting vasodilatation following isometric contractions, is assumed to be attributable to an insufficient blood flow during the preceding contraction (Byström & Kilbom 1990). The magnitude of the active hyperaemia is closely related to the increase in metabolic activity. Immediately after both static and dynamic exercise, blood flow to the exercised muscles increases markedly. The increased blood flow is maintained throughout the period of increased metabolic activity. It subsides after normal metabolism is restored (Laughlin 1987; Walloe & Wesche 1988; Bangsbo & Hellsten 1998). In general, four basic types of mechanisms might contribute to exercise hyperaemia (Joyner & Proctor 1999). First, there are mechanical interactions between the contracting and relaxing muscles and the blood vessels (Laughlin 1987; Sheriff et al. 1993). These interactions empty the venous system to create a muscle pump mechanism that can augment flow independently of vasodilatation by increasing effective perfusion pressure in the active muscles (Sheriff et al. 1993). Second, skeletal muscle vasodilatation during exercise might be caused primarily by substances released from the active skeletal muscles (Sheriff et al. 1993; Tschakovsky et al. 1996). Third, vasodilating substances might also be transported by blood to the active muscles (Jia et al. 1996). Fourth, vasodilating substances might be released by nerves in close proximity to the contracting muscle fibres (Segal & Kurjiaka 1995).

Reactive hyperaemia

Reactive hyperaemia is the transient increase in organ blood flow that occurs following a brief period of ischemia (e.g., arterial occlusion). Upon sudden occlusion of the artery supplying the studied muscle region, blood flow stops completely, after which, upon release of the arterial occlusion, blood flow quickly increases to a peak value clearly above the normal flow level for a short time. This phenomenon is called reactive hyperaemia. The longer the period of occlusion, the greater the increase in blood flow above pre-occlusion levels. Such a linear relation seems reasonable from a physiological point of view, since blood flow might be the crucial parameter. It re-establishes a balance between metabolic tissue demand and nutritional flow. The various mechanisms thought to play a significant role in reactive hyperaemia can be subdivided into two types: 1) metabolic or chemical mechanisms and 2) myogenic mechanisms (Burton & Johnson 1972).

4. Techniques for measuring local muscle blood flow

It has, until now, been difficult to measure local muscle blood flow directly in humans. The magnitude of muscle blood flow has been estimated from measurements of total limb flow. It has been demonstrated that skeletal muscle accounts for the majority of limb blood flow, and that the traction of resting muscle blood flow increases as a function of limb blood flow

(Raitakari et al. 1996). However, Elia and Kurpad investigated whether forearm and calf plethysmography largely reflect local muscle blood flow as measured by ^{133}Xe (Elia & Kurpad 1993). They found that less than half of the total blood flow was directed towards muscle tissue. Thus these investigators suggested that non-muscular blood flow is greater than muscle blood flow, but provided no information on the relationship between total limb and local muscle blood flow.

When choosing a method to measure local muscle blood flow, it should be borne in mind that non-invasive methods for measuring local muscle blood flow are required, so as to exert minimal influence on the measurement object. However, most techniques available for measuring local blood flow in muscle today are invasive ones.

4.1. Regional blood flow measurements

Invasive methods

Invasive methods include the use of electromagnetic flow meters, indo-cyanine green dye dilution, and thermodilution.

Electromagnetic flow meters are based on induction of voltage changes proportional to the flow of the blood (Djordjevich & Sadove 1981). The electromagnetic flow probe is mounted intraoperatively directly on the vessel where the flow is to be measured (Hall 1969). Blood flow is calculated from the product of the cross-sectional area of the vessel and the mean flow velocity (Rådegran 1999). The method is invasive and is not suitable for measuring blood flow in healthy human subjects.

Ido-cyanine green dye dilution is a method based on the infusion of Indocyanine Green into the femoral artery, and on the assumption that indicator concentration in blood is flow-dependent (Jorfeldt & Wahren 1971). Blood may be measured by infusion of dye of a known concentration at a specific rate, at a site upstream in a vessel, after which the dye concentration is determined in blood samples drawn at a point downstream after thorough mixing. Continuous indo-cyanine green infusion gives reliable measurements at rest when performed for an extended period of time, i.e. approximately 3 minutes (Jorfeldt & Wahren 1971). It also gives accurate intermittent steady-state measurements during incremental exercise up to peak effort when infused for approximately 80 seconds (Jorfeldt & Wahren 1971). However, the technique is invasive.

Thermodilution method allows accurate measurement of average flow in a vessel. Blood flow is determined by measuring the temperature change in a vessel during infusion of cold saline. The temperature deflection is proportional to the temperature and rate of infusion of the saline solution, as well as the flow rate of the blood (Rådegran 1999). A thermistor and a catheter are inserted into a peripheral vessel in the proximal and either proximal or distal direction, respectively. Using continuous saline infusion, measurements can be performed during exercise, as it is unaffected by movement artefacts. However, the technique is invasive in nature, requiring either arterial or venous cannulation (McCully & Posner 1995). Furthermore, there has to be strict control of the volume and frequency of saline infusion to avoid tissue cooling, haemodilution and a too large saline load (Rådegran 1999).

Non-invasive methods

Non-invasive methods include plethysmography, ultrasound Doppler, and Magnetic resonance velocity imaging.

Venous occlusion plethysmography is based on determining the volume increase in a limb segment when venous outflow is temporarily arrested but arterial inflow is intact. The general venous occlusion plethysmography procedure involves applying a pressure cuff to the limb. The cuff is inflated above venous pressure but below diastolic pressure so that blood can flow through the arteries but not through the veins (McCully & Posner 1995). Since the veins

are drained, any increase in limb volume due to the accumulation of blood is attributed to arterial inflow. The rate of increased volume of the limb during occlusion is used to assess the rate of arterial inflow. The strain-gauge plethysmography utilises a strain-gauge sensor placed around the widest part of the limb, to measure the volume of the limb. The blood flow measured using this technique represents total flow to various tissues, so the technique cannot measure local muscle blood flow. The ability to evaluate how abnormally increased intramuscular pressure affects local muscle blood flow is clinically important for further study of the physiopathology of compartment syndrome. However, it is impossible to investigate the relationship between blood flow and tissue pressure from the same muscle using this technique. Furthermore, sealing of the limb without inducing tissue constriction is also problematic (Rådegran 1999).

Blood flow over the limb can also be determined using the *ultrasound Doppler*, which measures arterial inflow to a human limb during intermittent static contraction (Rådegran 1997). The velocity of blood flow through a large vessel can be calculated from the "Doppler-shift" of the ultrasound waves, i. e. the signal changes with changes in red blood cell velocity (Gill 1985). The ultrasound Doppler is currently the most sensitive technique for blood flow measurement at rest, at onset of and during submaximal exercise, as well as post-exercise. However, to avoid insonation failures, the exercise mode must be such that the limb and the artery are in a fixed position. The technique is expensive, technically demanding and has a significant learning curve (Rådegran 1999).

Magnetic resonance velocity imaging flow measurements are non-invasive and do not require intravascular injections or the use of ionising radiation. They are based on the principle that the hydrogen nuclei in blood moving through a magnetic field gradient accumulate a phase shift proportional to their velocity (Hundley et al. 1996). However, this technique is sensitive to movement artefacts and is thus best used in hyperaemia-type measurements of blood flow (McCully & Posner 1995). The equipment and running costs are very high. The apparatus and imaging procedures are also cumbersome, limiting the type and intensity of exercise that can be studied (Rådegran 1999).

4.2. Local blood flow measurements

Invasive methods

One direct method for assessing local blood flow in skeletal muscle is the isotope clearance technique. Several new methods including microdialysis ethanol outflow/inflow technique, near-infrared spectroscopy, and laser Doppler flowmetry have been developed to measure local muscle blood flow. However, all these methods are invasive.

The local *^{133}Xenon washout technique* introduced by Lassen and co-workers has been the most often used for determining local blood flow in skeletal muscle (Lassen et al. 1964). This method is based on the clearance of a radioactive tracer of ^{133}Xenon injected directly into the muscle. The radioactivity in the area is continuously monitored with a gamma detector placed over the skin at the injection site. With external observation of its clearance rate depicted as a washout curve (Lassen et al. 1964), a measure of capillary flow is obtained. The technique offers the possibility of studying the pathophysiology of chronic compartment syndrome during exercise and at rest after exercise (Styf et al. 1987). It is possible to record the relative changes in blood flow continuously in the tibialis anterior muscle during graded bicycle exercise (Sörensen et al. 2000). However, the local ^{133}Xenon washout technique is invasive, and the local trauma at the site of injection can complicate the flow measurements (McCully & Posner 1995). In addition to the requirement for injection of a radioactive molecule, the method was found to be non-linear and to underestimate blood flow (Clausen & Lassen 1971; Cerretelli et al. 1984). However, estimates of relative changes in blood flow may be of value (Tönnesen & Sejrsen 1970).

Microdialysis ethanol outflow/inflow technique is based on measurement of the transport of molecules across the semi-permeable membrane of a microdialysis fibre inserted into the muscle. By perfusing the fibre with an infusate containing ethanol (i.e. a tracer suggested to be metabolically insert in the muscle), and measuring the ethanol concentration in the outflowing dialysate, the loss of ethanol is suggested to be proportional to the local muscle blood flow surrounding the fibre (Hickner et al. 1991). However, there was no investigation of whether the changes in ethanol removal during muscular contractions only represented changes in muscle blood flow, or whether they reflected other factors causing alterations in the molecular transport across the membrane (Rådegran 1999).

Using *near-infrared spectroscopy (NIRS)* and a light-absorbing tracer, it is possible to measure blood flow. A photo-densitometer is used to detect the dye concentration. The method is based on detecting the light attenuation induced in the muscle during infusion of a tracer. The rate of accumulation of a tracer in a given tissue is equal to its rate of inflow minus its rate of outflow (Boushel et al. 2000). The technique is unique, as it allows estimation of local muscle oxygenation in relation to local perfusion. Although the NIRS technique is non-invasive, the method requires invasive indo-cyanine green infusion and arterial blood sampling (Rådegran 1999).

Recent developments in *laser Doppler flowmetry (LDF)* now allow for the assessment of blood flow in deeper tissues by means of a single-fibre probe. With this technique, the laser light is continuously guided via an optical fibre inserted through the subcutaneous tissue into the muscle (Salerud & Öberg 1987). The light strikes moving blood cells in the microcirculation causing a change in wavelength of the emitted light (a Doppler shift). The light back-scattered from the blood cells is collected through the same fibre. The laser Doppler signal responds to the Doppler shifted light, which, in turn, reflects the average velocity of red blood cells times the number of cells (Nilsson et al. 1980). Single-fibre laser-Doppler flowmetry combined with an optic fibre probe allows the circulation to be measured continuously at microvascular level (Salerud & Öberg 1987). This technique has also been applied to human muscle for measuring continuous changes in local muscle blood flow in relation to current muscle work (Kvernebo et al. 1990; Hoffmann et al. 1995; Jensen et al. 1995; Larsson et al. 1995). However, tissue trauma caused by insertion of the single-fibre probe into muscle may disturb local blood flow, even when using an optical fibre with a diameter of 0.5 mm. Furthermore, the optical fibre is influenced by motion (Öberg 1990) and the blood flow may be overestimated using this method.

Non-invasive method

Photoplethysmography (PPG) is a non-invasive optical technique for measuring tissue blood perfusion (Lindberg & Öberg 1991). Hertzman and Spealman illustrated changes in blood volume produced by exercise, cold and the Valsalva manoeuvre in 1937. They contributed greatly to the understanding of the PPG technique in their earlier work (Hertzman & Spealman 1937). Hertzman called his apparatus a "photoelectric plethysmography" in 1938 (Hertzman 1938). Since then numerous photoplethysmographic measuring devices have been developed for various applications. PPG is a common non-invasive optical technique for measuring changes in blood flow and has been used mainly for monitoring blood perfusion in skin (Challoner 1979; Kamal et al. 1989).

The PPG technique requires a light source and a photodetector, placed adjacent to each other or a light source applied on one side and a photodetector on the opposite side of the measuring volume. The principle on which PPG is based is simple, although the underlying detailed optical mechanisms remain unknown (Kamal et al. 1989). A beam of light is directed toward the part of tissue in which blood flow (or volume) is to be measured. The emitted light is reflected, absorbed and scattered in the vascular bed, with only a small fraction being

received by the photodetector (Tahmoush et al. 1976). Changes in the intensity of the reflected and scattered light recorded by the photodetector are related to blood flow changes in the underlying tissue (Lindberg & Öberg 1991; Tahmoush et al. 1976). Light penetration into the tissue increases primarily with increasing wavelength (Anderson & Parrish 1981). The light penetration depth also depends on the optical geometry of the PPG probe. A increasing distance between the light source and the photodetector enable to image vascular bed at different depth in human tissue (Fridolin et al. 2000; Fridolin & Lindberg 2000).

According to above, determinations of actual skeletal muscle blood flow are not easily made in humans. Different methods measure different levels in the circulatory system, such as limb blood flow, whole muscle blood flow, and local microcirculation. However, there is no published method in which local blood flow has been measured non-invasively in human skeletal muscle.

5. Intramuscular pressure

Intramuscular pressure (IMP) has primarily been used in experimental and clinical studies of acute (Matsen et al. 1976; Hargens et al. 1977) and chronic compartment syndromes (Styf & Körner 1986a). The most rigid compartment is the anterior compartment.

Guyton described the total tissue pressure as a combination of interstitial fluid pressure and solid tissue pressure (Guyton 1971). The fluid pressure can be divided into hydrostatic and osmotic pressure. The physiological intramuscular pressure in a non-contracting muscle with no external compression varies between 4 and 10 mmHg (0.5 and 1.3 kPa[1]). During contraction, the intramuscular pressure reaches 150-250 mmHg in leg muscles (Styf & Körner 1986b). Tissue pressure in a compartment may be increased either by a decrease in the size of the compartment or by an increase in the volume of its contents (Matsen 1975).

Different techniques have been developed for direct measurement of tissue pressure within a muscle. The different techniques for pressure recordings used in the clinical situation may be classified as: (a) injection techniques; (b) infusion techniques; (c) non-infusion techniques; and (d) transducer-tipped techniques (Styf 1989). Most of the methods have been thoroughly evaluated and are generally accepted for pressure recordings at rest. The microcapillary infusion technique has been evaluated and found suitable for recording pressure during exercise and at rest after exercise (Styf & Körner 1986b). The recorded pressure depends on the method used, the position of the leg (Weiner et al. 1994) and the depth of the catheter in the muscle (Nakhostine et al. 1993). Pressures exceeding 30 to 35 mmHg at rest after exercise and a time period exceeding between 6 and 15 minutes to normalise pressure have been used as a criteria for diagnosis of the compartment syndrome (Rorabeck et al. 1983; Styf & Körner 1987). Intramuscular pressure is a valuable parameter in estimating local perfusion pressure.

6. Pathophysiology of abnormally elevated IMP

The local muscle blood flow is regulated by the pressure difference along the vessels (local mean arterial blood pressure – local venous blood pressure) and the local vascular resistance (Feigl 1974a). A common factor of significance for the venous blood pressure and the local vascular resistance is the intramuscular pressure. Many investigators have demonstrated that increased intramuscular pressure reduces blood flow (Ashton 1975; Rorabeck & Macnab 1975; Rorabeck & Clarke 1978; Matsen et al. 1979a; Styf et al. 1987; Jensen et al. 1995).

Increased tissue pressure may affect local blood flow in two ways. The increased pressure exerted on the outside of a collapsible vessel wall may affect the flow through collapsible vessels by compressing vessels directly (Matsen 1975). Increased tissue pressure

[1] 1 mmHg = 0.133 kPa

may also reduce the local arteriovenous pressure difference and hence local blood flow, as local venous pressure increases to the same extent as to the rise in the surrounding tissue pressure (Nielsen 1991; Jensen et al. 1995). Parazynski and co-workers made direct measurements of normal muscle capillary pressure (Parazynski et al. 1993). They found that muscle capillary pressure varies between 20 and 30 mmHg. It has been reported that muscle relaxation pressure exceeding 35 mm Hg correlates to decreased muscle blood flow (Styf et al. 1987). When increased tissue pressure compromises local circulation to the point where the metabolic demands are no longer met, functional abnormalities occur.

7. Abnormally elevated intramuscular pressure in an experimental model

In 1975, Ashton studied effects of external compression by inflatable plastic splints on limb blood flow in human subjects (Ashton 1975). She demonstrated a significant reduction in blood flow with application of 40 mmHg pressure and elevation of the limb 9 to 35 cm above heart level. However, external compression of a muscle compartment yields compressive forces in the tissue, which deform the tissue and increase interstitial hydrostatic pressure.

By applying venous stasis to a casted leg, abnormally increased pressure in the anterior compartment of the human leg was induced (Styf et al. 1998; Wiger et al. 1998; Wiger et al. 2000). The authors suggested a human model of venous obstruction of a casted leg to increase IMP to 40 mmHg. By elevating the limb, perfusion pressure decreased to about 25 mmHg and neuromuscular dysfunction evolved. They suggested that the neuromuscular dysfunction is due to circulatory compromise and not to a direct effect of increased pressure on nerve or muscle.

Styf and Wiger compared the effects of abnormally increased pressure in the anterior compartment induced by applying venous obstruction of a casted leg and external compression by a cylindrical air splint (Styf & Wiger 1998). They indicated that both models induced the range of intramuscular pressure seen in patients with imminent compartment syndromes. However, subjects experienced impaired sensation, tingles, and numbness as well as muscular weakness only in the casted obstructed elevated leg. Therefore, venous obstruction of a casted leg for 30 minutes appears to be a suitable and safe model for studying the relationship among muscle blood flow, abnormally increased IMP, decreased perfusion pressure, and muscle and nerve function in graded hypotension in human legs.

8. Perfusion pressure and limb elevation

Matsen and co-workers reported that elevation of a limb lowers the local arterial pressure by an amount approximately equal to the resulting hydrostatic column (i.e., the amount of elevation in centimetres divided by 1.3 cm of blood per mmHg) (Matsen et al. 1977). Local perfusion pressure is calculated as mean arterial pressure (MAP) minus IMP (Heppenstall et al. 1988). Elevation of a limb reduces the local perfusion pressure. Elevation of a limb with significantly increased tissue pressure creates a more serious reduction in local perfusion pressure (Wiger & Styf 1998; Wiger et al. 2000). Furthermore, decreased tolerance for increased intracompartmental pressure in an elevated extremity has been shown to be directly related to the reduced perfusion pressure.

It is common clinical practice to elevate traumatised and post-surgical limbs to reduce swelling. Limb elevation has also been recommended as treatment for patients with post-traumatic venous hypertension (Mullins et al., 1980; Shah et al., 1989). It is clinically important to know whether or not the limb should be elevated to reduce swelling, or whether it should be maintained at heart level to better serve the local tissue perfusion. Limb elevation during prolonged surgery may also provoke acute compartment syndrome (Khalil 1987; Bergqvist et al. 1990; Slater et al. 1994). To better understand the effects of limb elevation

and increased tissue pressure on local circulation, measurements of muscle blood flow in the leg are required.

9. Vibration exposure

Vibration exposure occurs in many occupations where a worker is in contact with vibrating machinery or equipment. Adverse health effects can result from contact with almost any vibration source if the vibrations are sufficiently intense and within the frequency range of 4 to 5000 Hz for some period of time (Pelmear & Taylor 1994). Vibration is usually categorised into hand-arm vibration exposure and whole-body vibration exposure. Hand-arm vibration is usually associated with the use of vibrating hand tools, whereas whole-body vibration is experienced when the operator sits on or in a vibrating machine or vehicle. The risk of injury depends on the intensity and frequency of the vibration, the duration of exposure and the part of the body which receives the vibration energy (Taylor 1993).

Vibration-induced white fingers, originating from vibrating hand tools, affecting blood circulation (vascular effects) and nerves (neurological effects) have been described (Taylor 1993). Whole-body vibration is associated with an increased risk of low back disorders (Bovenzi & Hulshof 1999). Thus, it is clinically important to be able to evaluate physiological responses during vibration exposure in order to better understand vibration-induced disorders.

10. Muscular activity and venous foot pump

The mechanism of rhythmic muscular contractions, repeated emptying of the veins and facilitating perfusion of skeletal muscle, is often referred to as "the muscle pump" (Folkow et al. 1970; Lash 1996; Laughlin & Schrage 1999). Following the discovery in 1983 of a previously unrecognised venous pumping mechanism in the sole of the foot by Gardner and Fox, a new possibility of maintaining the circulation in immobilised patients arose. They reported a foot pump mechanism in the sole of the human foot that is capable of returning venous blood from the foot up into the abdomen with no assistance from muscular action. Passive activation of the venous plexus of the foot using a venous foot pump has been reported to reduce post-traumatic pain, swelling and compartment pressures in the leg, and to increase blood flow in the limb due to widespread diminished vascular resistance (Gardner et al. 1990)

The effects of active foot movement or leg activation with the venous foot pump on the venous return of the leg have previously been reported (Gardner & Fox 1983; McNally et al. 1997; Sochart & Hardinge 1999). However, there are no studies that have compared the effects on muscle blood flow following concentric muscular activity and leg activation with the foot pump. It is clinically important to know which mode provides better microcirculation of the muscle in the early treatment of post-operative and post-traumatic oedema of the leg.

AIMS OF THE STUDY

The aims of the present study were:

1. To validate a new non-invasive photoplethysmography (PPG) method for monitoring blood flow in the human anterior tibial muscle during arterial occlusion and following isometric and concentric contractions (*Study I*).

2. To evaluate the effects of increased intramuscular pressure and limb elevation on local muscle blood flow in a model of increased IMP in the human leg (*Study II*).

3. To evaluate PPG for detecting MBF changes in response to exercise under normal and elevated IMP conditions and to investigate if abnormally elevated IMP affects MBF at rest after exercise (*Study III*).

4. To evaluate whether the PPG method can be used to measure MBF during vibration exposure (*Study IV*).

5. To evaluate changes in local MBF following concentric muscular activity or leg activation with the venous foot pump. To compare the effects of these two types of interventions on MBF (*Study V*).

MATERIALS AND METHODS

1. Subjects

Twelve healthy subjects (five men and seven women) with a mean age of 25 (range 20-30) years participated in study I. Eight healthy subjects (four men and four women) with a mean age of 31 (range 25-39) years participated in study II. In study III, eight healthy subjects (five men and three women) with a mean age of 27 (range 19-42) years participated. Six healthy subjects (three men and three women) with a mean age of 29 (range 19-42) years participated in study IV. In study V, experiments were carried out on 48 legs in 24 healthy subjects (ten men and fourteen women) with a mean age of 32 (range 18-51) years.

A total of 58 subjects and 98 legs were investigated in the experimental studies. All subjects were active in some kind of sports and were symptom free. They were also free of any cardiac problems and metabolic diseases. No subject had been occupationally or otherwise regularly exposed to vibrations. The study was approved by the Research Ethics Committee of Göteborg University. All subjects provided their informed written consent.

2. Methods for recording muscle blood flow

2.1. Photoplethysmography

A modified PPG instrument with a custom-designed PPG probe (Department of Biomedical Engineering, Linköping University, Sweden) was developed for the purpose of measuring muscle blood flow. Using this method, blood flow changes in the anterior tibial muscles were determined (Sandberg & Lindberg 2000; Sandberg et al. 2002a; Sandberg et al. 2002b).

The PPG instrument was used to record blood flow changes in the skin and the anterior tibial muscle. The PPG probe (Zhang et al. 2001a) used in *Study I* consisted of three photodetectors and six light sources (light emitting diodes LEDs). Four LEDs emitted light of a wavelength of 560 nm (green light) and two LEDs emitted light in the near-infrared region of 880 nm (Fig. 2). All components were placed in a special pattern and embedded in black-coloured silicone. The centre to centre distance between the LEDs and the photodetectors was 3.5 mm and 20 mm for wavelengths 560 and 880 nm, respectively. The two wavelengths and the distance between each LED and the photodetectors were chosen to cover approximately 1-2 mm depth of penetration of skin using 560 nm and a part of the muscle belly regarding depth of penetration using 880 nm. The PPG probe (Zhang et al. 2001b) used in *Studies II-V* consisted of seven photodetectors and four light emitting diodes (LEDs) in the near-infrared region of 880 nm. The center-to-center distance between the LEDs and the photodetectors was 20 mm (Fig. 3).

To evaluate the depth of light penetration using 880 nm, an experiment was performed (Sandberg et al. 2002). The depth was determined in three subjects in the following way: an optic fibre was inserted into the anterior tibial muscle and connected to an Optical Power Meter for recording the radiant power of radiation in the muscle. The tip of the fibre was positioned approximately in the middle of the muscle. Exact location of the fibre tip was determined using ultrasound Doppler, by which the distance between the muscle fascia and the sensor tip was measured. The PPG sensor was placed over the tibial muscle above the fibre tip (Fig 4). The radiant power measured in the muscle tissue indicates that the near-infrared light penetrates at least down to a vascular depth of 13.0 mm from the skin surface. The distance between the skin surface and the muscle fascia was also measured in forty subjects. The mean value was 6.5 mm (range 2.1-13.8mm) with a standard deviation of 2.9 mm (Sandberg et al. 2002c).

The PPG signal consists of an AC component synchronous with the heart rate that reflects pulsatile blood flow, and a slowly varying DC component that reflects total blood

volume of the examined tissue area (Challoner 1979; Fairs et al. 1985; Kamal et al. 1989). A reduction in PPGdc signal corresponded to an increase in blood volume.

The PPG probe was placed over the muscle belly 1 cm lateral to the anterior margin of the tibia. It was connected to the designed three-channel PPG-instrument. The signals passed an amplifier, and were high-pass filtered at 0.1 Hz and low-pass filtered at 28 Hz. The output of PPG signal was converted into digital form in an A/D converter (DAQCard-700, National Instruments, USA) and recorded into a PC at a sampling frequency of 60 Hz, using a LabWindows program.

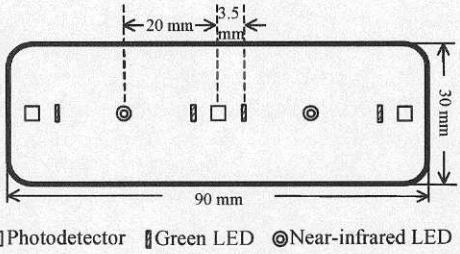

Figure 2. Configuration of the photoplethysmography (PPG) probe used in Study I showing the position of the optical components. (*LED* Light-emitting diode)

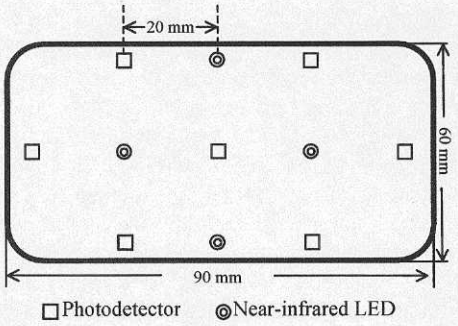

Figure 3. Configuration of the photoplethysmography probe used in Study II-V showing the position of the optical components. (*LED* Light-emitting diode)

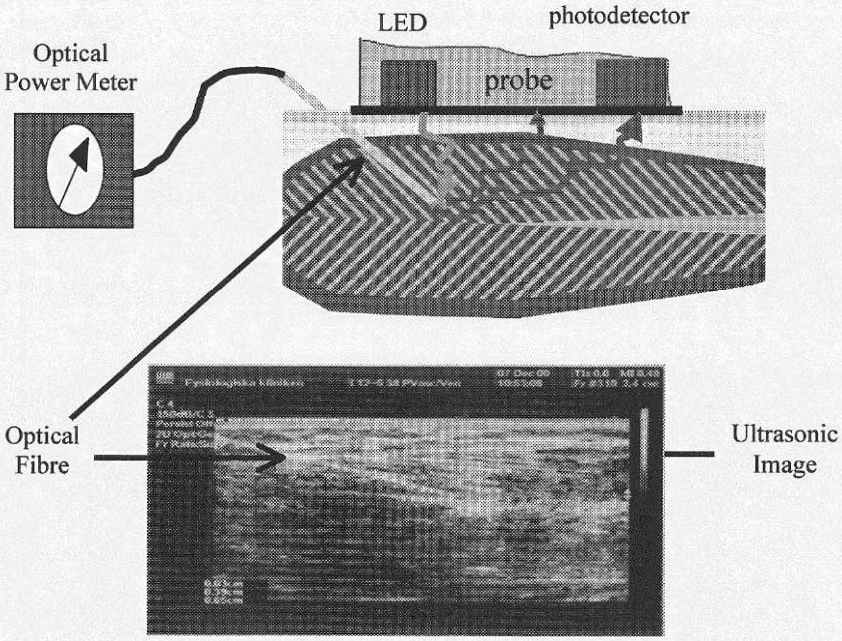

Fig. 4 The depth of light penetration from 880 nm was determined by following experimental setup.

2.2. Single-fibre laser-Doppler flowmetry

Blood flow in the anterior tibial muscle was also measured in Study I, using a three-channel, single-fibre LDF (Multiflow 3, Linköping, Sweden; time constant 0.2 s and gain 10). The instrument utilises a semiconductor laser, which emits a monochromatic light at a wavelength of 632 nm and at an intensity of 3 mW. Laser light was continuously guided through an optical fibre, which had a diameter of 0.5 mm and back-scattered light was collected through the same fibre.

A local anaesthetic 1 to 2 ml 1% lidocaine) was injected into the skin (about 7 cm below the knee joint and 2 cm lateral to the anterior margin of the tibia. Then a catheter (1.2 × 32 mm) was inserted in the distal direction into the anterior tibial muscle at an angle to the skin of ~ 30°, and the optical fibre was inserted.

The output of LDF was converted into digital form in an A/D converter (DAQCard-700, National Instruments, USA) and recorded into a PC at a sampling frequency of 60 Hz, using a LabWindows program. Mean values of the LDF signals were then calculated.

3. Method for recording intramuscular pressure

Pressure in the anterior tibial muscle was monitored with the intermittent microcapillary infusion technique in all volunteers (*Studies II and III*). The subjects were investigated when they lay supine with no external compression of the legs.

The skin was anaesthetised 2 cm lateral to the tibial tuberosity with 2 ml of 1% lidocaine. Under sterile conditions, a cannula with an outer diameter of 1.7 mm was then inserted into the anterior tibial muscle fascia in the distal direction at an angle of 30° from the plane of the skin, while the subject kept the ankle joint dorsiflexed (Styf & Körner 1986b). The tip of the needle was kept retracted within the plastic sheath of the introducer, and the set was bluntly advanced parallel to the fibres of the relaxed muscle. The needle was then withdrawn. A catheter (Myopress, Atos Medical, Hörby, Sweden) for recording of IMP was inserted 5 cm into the plastic sheath. The plastic sheath of the introducer was then removed. The catheter had four side holes at its tip and was filled with 0.9% saline. The infusion rate was 1ml/h with a pressure of 100 mmHg (*Study I*) and 1.5ml/h with a pressure of 150 mmHg (*Study III*) over the microcapillary. The catheter position was checked by observing the response to external compression and to active muscle contraction. The catheter was connected to an SC 9000 transducer and an R 50 recorder (Siemens-Elema, Mölndal, Sweden). The pressure recording system was calibrated before and after each experiment.

4. Blood pressure

Blood pressure was measured in the left forearm using a NAIS manometer (Matsushita, electronic Works, Ltd., Japan). Mean arterial pressure (MAP) was obtained by adding the diastolic blood pressure to one-third of the pulse pressure (pulse pressure = systolic minus diastolic). For the anterior compartment, perfusion pressure was estimated to be MAP minus IMP minus height of limb elevation in centimetres above heart level divided by 1.3 (Matsen et al. 1979b; Heppenstall et al. 1988), where 1.3 cm represents the conversion factor for blood to millimetres of mercury.

5. Vascular resistance

Vascular resistance was calculated from the obtained relative muscle blood flow and estimated perfusion pressure. Vascular resistance in arbitrary units was defined as PP_{mmHg} / $MBF_{\%}$.

6. Elevation of IMP in an experimental model

Pressure in the tibialis anterior muscle was elevated by venous obstruction of a casted leg (Styf & Wiger, 1998; Wiger & Styf, 1998).

A 14.5 cm wide pneumatic thigh tourniquet, inflated to between 60 and 65 mmHg, was used to obstruct venous return from the limb, with different durations in Studies II, III, and V. The pressure within the tibialis anterior muscle was then elevated to the desired level.

A four-layer plaster cast, 20 cm wide and applied over two layers of cotton padding, was extended from 10 cm above the malleoli to the proximal part of the leg (Studies II and III). To avoid external compression on the anterior compartment, the anterior part of the cast rested on the tibial tuberosity. The posterior part of the cast was adjusted to allow for 30° of knee flexion.

7. Evaluation of neuromuscular function

Sensory and motor testing was performed at fifteen-minute intervals during the period of stasis and at one- to two-minute intervals after the release of stasis (Study II). The subject estimated the sensory function of the feet on a 10 cm visual analogue scale (VAS). The ends of the scale were "normal sensibility" (10 cm) and "no sensibility" (0 cm). The subject's motor function was evaluated using a neurograph with standard surface electrodes (Keypoint, Dantec Medical A/S, Denmark). The peroneal nerve was stimulated over the fibular head, using 0.1 ms electrical pulses with an intensity sufficient to elicit maximal amplitude of the compound muscle action potential. The active surface electrode was positioned over the motor point of the extensor digitorum brevis muscles. The reference electrode was located on the fifth metatarsophalangeal joint and the ground electrode positioned on the calf. A biphasic compound muscle action potential was recorded. The stimulation site was marked on the skin with indelible ink.

8. Suppressing vibration-induced artefacts in a PPG signal

The PPG signal recorded from a limb exposed to stationary random vibration can be masked by vibration-induced artefacts. To extract the desired information, the disturbed PPG signal has to be filtered.

The PPG signal can, roughly, be considered as a distorted triangulate wave. Therefore, a PPG signal power spectrum will have its power concentrated to the few first odd multiples of its fundamental frequency. Measurements showed that its third component was 20 dB less than the fundamental component. As the heart rate is about 1 Hz and the main acceleration power of the applied vibration is between 5 and 2000 Hz, a cut off frequency of 3.1 Hz was chosen for the filter function. A fourth order Butterworth low pass-filter with a cut-off frequency of 3.50 Hz was found suitable as a base for the final filter function. To avoid phase distortion the signal was filtered twice, changing the time direction of the signal at the second filtration. This was achieved with a standard algorithm "filtfilt.m" in the Matlab® program package. The resulting filter was of eighth order and had a cut off frequency of 3.1 Hz.

9. Statistical analyses

Results are given as a mean value and one standard deviation (SD) or median and range. The Wilcoxon signed-rank non-parametric test was used for differences between paired observations. The correlation analysis between the methods was performed according to Kirkwood (1988). The level of statistical significance was set as $p < 0.05$. Linear regression was performed to estimate relative changes of muscle blood flow as a function of time.

RESULTS

1. Muscle blood flow

Blood flow in the anterior tibial muscle (*Studies I and V*) and the gastrocnemius muscle (*Study V*) was measured by PPG.

1.1. Post-occlusive reactive hyperaemia (*Study I*)

Figure 5 shows the PPG and LDF signals recorded during arterial occlusion from one subject. Figure 6 shows a close up PPG_{AC} signal. During arterial occlusion, the PPG_{AC} and LDF values approached zero. The PPG_{DC} varied according to changes in blood volume.

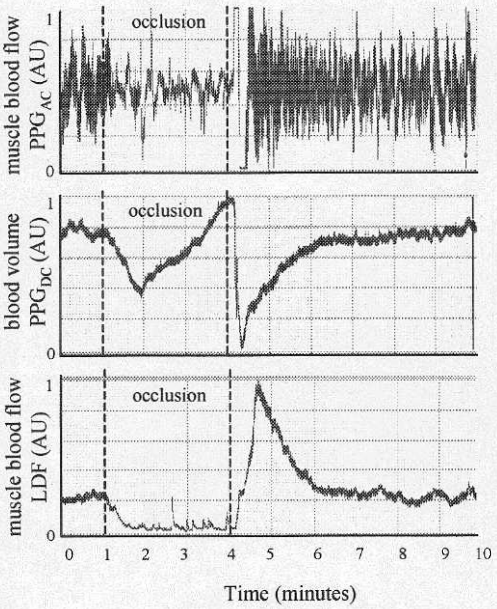

Figure 5. Simultaneous recordings of the AC component of the PPG signal (PPG_{AC}; upper curve), the DC component of the PPG signal (PPG_{DC}; middle curve) and the signal from the laser-Doppler flowmeter (LDF; lower curve) which were obtained from the anterior tibial muscle. Here PPG_{AC} and LDF indicate muscle blood flow. PPG_{DC} indicates blood volume. (*AU* Arbitrary units).

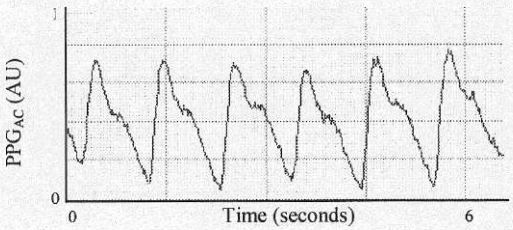

Figure 6. Expansion of the PPG_{AC}. signal shown in Fig. 2. AU = arbitrary units.

After arterial occlusion the post-occlusive reactive hyperaemia in the muscle was 150% (SD=31, p=0.003) by PPG (880 nm) and 182% (SD=66, p=0.012) by LDF (compared with baseline=100%). Skin blood flow increased to 135% (SD=57, p=0.026) by PPG (560 nm). Data obtained as a result of the post-occlusive reactive hyperaemia are shown in Fig. 7.

1.2. Exercise hyperaemia (*Study I*)

After 1 min of maximal static contraction, the blood flow increased to 150% (SD=51, p=0.003) by PPG (880 nm), and to 169% (SD=43, p=0.005) by LDF. After 1 min of maximal concentric contractions, muscle blood flow increased to 158% (SD=59, p=0.003) by PPG (880 nm) and to 170% (SD=99, p=0.008) by LDF. Skin blood flow by PPG (560 nm) showed no significant change either after 1 min of maximal isometric contraction or after 1 min of concentric contractions. Data obtained as a result of the exercise hyperaemia are shown in Fig. 7.

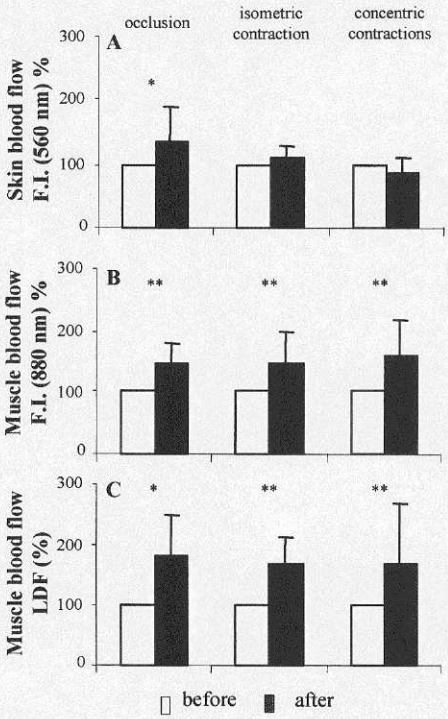

Figure 7. Comparison of blood flow in skin [**A**; *FI (560 nm)*] and muscle [**B** and **C**; *FI (880 nm)* and LDF, respectively] before (white bars) and after (black bars) arterial occlusion and isometric and concentric contractions. Values are given as a percentage of the initial resting value (100%). (*F.I.* Blood flow index). *Significant difference at $p<0.05$; **Significant difference at $p<0.01$

1.3. MBF responses to increased IMP (*Study II*)

MBF decreased significantly, by 50%, in the elevated leg when the IMP was held at 40 mmHg and perfusion pressure reduced to 17 mmHg. It decreased significantly, by 42%, in the non-elevated leg when the IMP was held at 40 mmHg and perfusion pressure reduced to 43

mmHg. These data are summarised in Table 1. The individual variations in MBF in the elevated leg during the increased IMP and reduced perfusion pressure for the eight healthy subjects are shown in Fig 8.

1.4. Vascular resistance (*Studies II & III*)

Vascular resistance calculated from the obtained relative muscle blood flow and estimated perfusion pressure is shown in Tables 1 and 2. MBF in the leg remaining at heart level decreased by 42%, corresponding to an increase in vascular resistance of 26% at 30 min of venous obstruction. In contrast, MBF in the elevated leg decreased by 50%, corresponding to a decrease in vascular resistance of 12% at 30 min of the venous obstruction (Table 1).

Vascular resistance before and after exercise in both control and test legs under different conditions are shown in Table 2. MBF increased by 179% in the control leg and by 84% in the test leg (with venous obstruction of 40 mmHg), corresponding to a decrease in vascular resistance of 60% and 62%, respectively. MBF increased by 203% in the control leg and by 56% in the test leg (with venous obstruction of 65 mmHg), corresponding to a decrease in vascular resistance of 66% and 68%, respectively. No significant differences were found between the resistance values in the control leg and the values in the test legs.

Table 1. The AC component of the photoplethysmography (PPG) signal (PPGac; assumed to reflect pulsatile muscle blood flow), the DC component of the PPG signal (PPGdc; assumed to reflect the total blood volume of the tissue area examined, the reduction in the PPGdc signal which corresponds to an increase in blood volume), intramuscular pressure (*IMP*), muscle contraction pressure (*MCP*), perfusion pressure (*PP*), vascular resistance, the amplitude (*AMPL*) and area (*AREA*) of compound muscle action potential of extensor digitorum brevis, and sensibility of the foot in the casted leg measured before, during, and after 30 minutes of venous stasis in eight healthy subjects.

Variable	Control		Venous stasis				Recovery	
			15 min		30 min			
	mean	SD	mean	SD	mean	SD	mean	SD
Non-elevated limb								
PPGac (%)	100	0	73*	24	58*	20	88	40
PPGdc (%)	100	0	91	8	91	6	101	5
IMP (mm Hg)	17.4	5.9	38.8	1.8	39.9	1.6	13.4	5.1
MCP (mm Hg)	181	84			205	76	189	90
PP (mm Hg)	65.4	9.9	46.5*	9.0	42.6*	8.4	72.9	8.8
Vascular resistance (AU)	0.65	0.10	0.71	0.32	0.82	0.36	0.93*	0.31
AMPL (mV)	5.5	2.6	4.9	3.2	4.7*	3.1	5.5	3.2
AREA (ms·mV)	20.2	7.1	17.3	11.6	15.5*	11.0	19.6	9.5
Sensibility [cm:median (range)]	10 (10-10)		9.3* (7.7-10)		8.9*(7.6-9.6)		10 (8.8-10)	
Elevated limb								
PPGac (%)	100	0	61*	25	50*	17	105	35
PPGdc (%)	100	0	94	12	94	10	105	6
IMP (mm Hg)	16.3	4.4	39.5	2.4	40.1	1.8	14.3	4.4
MCP (mm Hg)	199	116			69*	22	153	104
PP (mm Hg)	42.0	5.8	20.9*	6.4	17.3*	6.4	47.5	5.9
Vascular resistance (AU)	0.42	0.06	0.42	0.27	0.37	0.16	0.50	0.17
AMPL (mV)	6.4	2.0	4.7*	2.2	3.4*	2.4	5.6*	2.2
AREA (ms·mV)	23.3	6.2	17.4*	8.4	13.4*	10.1	21.9	8.5
Sensibility [cm:median (range)]	10 (10-10)		4.3* (1.7-8.5)		0.5* (0-8.5)		7.7* (5-10)	

*Significant difference when data were compared with their control values (before stasis), $P<0.05$

Table 2. Results (mean ± standard deviation) of muscle blood flow (MBF), intramuscular pressure (IMP), perfusion pressure (PP) and vascular resistance (VR) in the control leg and the test leg measured before and after exercise in the anterior tibial muscle of eight healthy subjects (16 legs). All data are presented as mean ± SD.

Variable	Pre-exercise		Post-exercise (0-5 min)		Post-exercise (0-5 min)	
	Control leg	Test leg	Control leg	Test leg (venous obstruction of 40 mmHg + cast)	Control leg	Test leg (venous obstruction of 65 mmHg + cast)
MBF (%)	100 ± 0	100 ± 0	279 ± 108.3	184 ± 52.6***	303 ± 95.3	156 ± 58.2***
IMP (mmHg)	11.4 ± 2.1	9.4 ± 1.3	17.8 ± 5.3	39.4 ± 7.9***	17.0 ± 5.5	58.3 ± 8.4***
PP (mmHg)	70.9 ± 6.1	72.9 ± 7.0	68.4 ± 8.2	46.8 ± 5.9***	66.8 ± 8.1	28.0 ± 7.9***
VR (AU)	0.71 ± 0.1	0.73 ± 0.1	0.28 ± 0.10	0.28 ± 0.07	0.24 ± 0.07	0.23 ± 0.15

***Significant difference between the control and test leg, P<0.001.

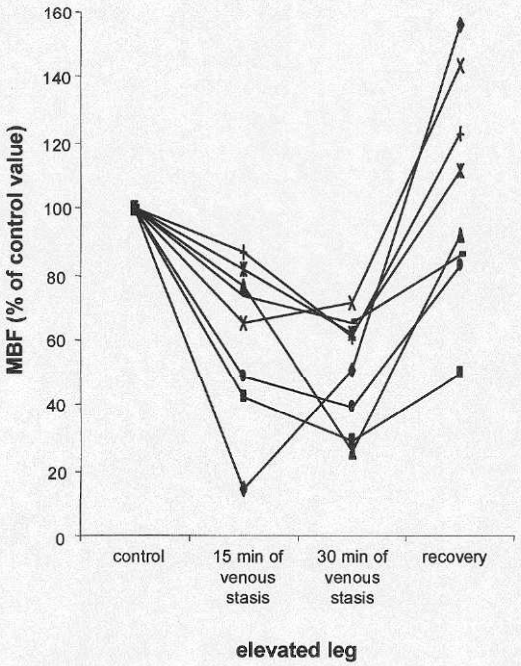

Figure 8. The effects of limb elevation and increased intramuscular pressure on blood flow in the tibialis anterior muscle in eight healthy subjects. Muscle blood flow (MBF) decreased during venous stasis (60-65 mmHg).

1.5. MBF at rest after exercise under normal and elevated IMP conditions (*Study III*)

Increased MBF was found both in the control and the test leg at rest after exercise. MBF at rest after exercise was significantly lower in the leg exposed to elevated IMP than in the leg with normal IMP. Results are summarised in Table 2.

The relationship between MBF and IMP at rest after exercise is shown in Fig. 9. MBF increased to 156% in the test leg when IMP was 58 mmHg and to 303% in the control leg

when IMP was 17 mmHg at rest after exercise. This means that the increased IMP caused a decrease in MBF at rest after exercise.

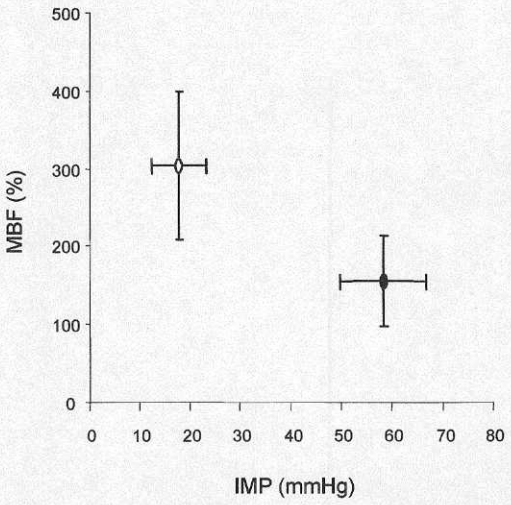

Figure 9. The effects of increased intramuscular pressure (IMP) on muscle blood flow (MBF) at rest after exercise in the test leg with venous obstruction of 65 mmHg and a plaster cast (filled circle), and in the control leg (open circle). Data are presented as mean ± SD.

The relationship between MBF and perfusion pressure at rest after exercise is shown in Fig. 10. MBF increased to 156% in the test leg when perfusion pressure was 28 mmHg and to 303% in the control leg when PP was 67 mmHg. This means that reduced local perfusion pressure caused a decrease in MBF at rest after exercise.

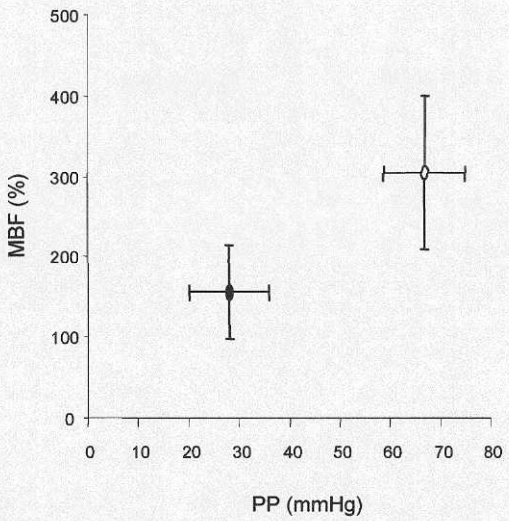

Figure 10. The effects of reduced local perfusion pressure (PP) on muscle blood flow (MBF) at rest after exercise in the test leg with venous obstruction of 65 mmHg and a plaster cast (filled circle) and in the control leg (open circle). Data are presented as mean ± SD.

1.6. MBF following concentric muscular activity and leg activation with venous foot pump (*Study V*)

Blood flow measured in the anterior tibial muscle (n=15)

MBF increased following concentric muscular activity (Fig. 11a), but not following activation with the foot pump (Fig. 11b). MBF was significantly greater in the exercised leg than in the leg exposed to activation of the foot pump (Fig. 11c), and the difference of MBF between two legs was significant ($p<0.05$).

MBF decreased in both legs when venous obstruction of 60 mmHg was applied. However, it was significantly higher in the exercised leg than in the contralateral leg (Fig. 12a). There was no significant difference in MBF between the leg activation with the foot pump and the contralateral leg (Fig. 12b). MBF decreased by 28% in the exercised leg and by 47% in the leg which was activated by the venous foot pump at the end of venous obstruction. The difference of MBF between the two legs was significant ($p<0.05$) (Fig. 12c).

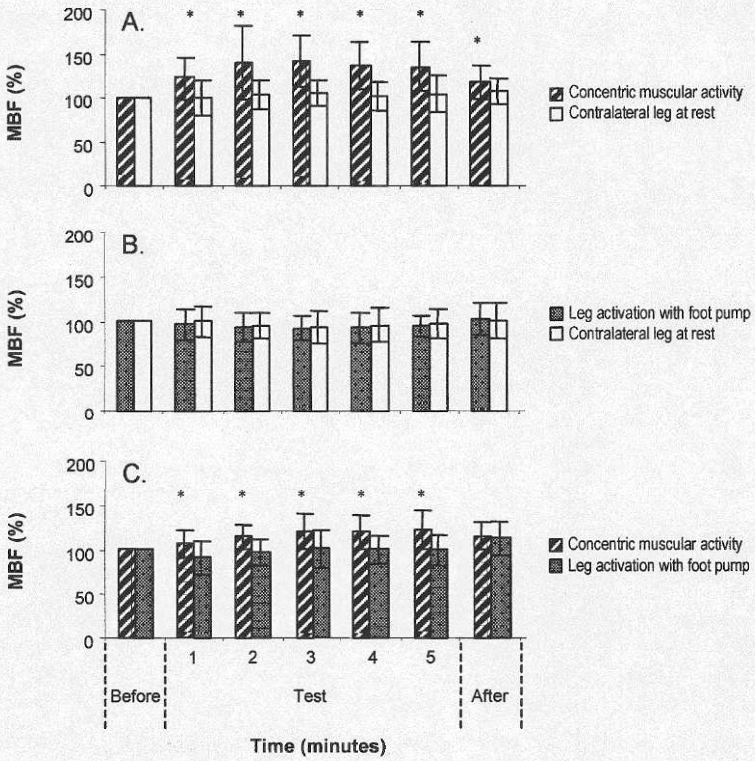

Figure 11. The effects of concentric muscular activity and leg activation with venous foot pump on muscle blood flow (MBF) in the tibialis anterior muscle (n=15). MBF values are given as a percentage of the initial resting value (100%). Each column contains the mean ± SD. Before – initial resting value, Test – concentric muscular activity or leg activation with a venous foot pump or both (1, 2, 3, 4, & 5 min), After – recovery. *Significant difference at $p<0.05$.

Blood flow measured in the gastrocnemius muscle (n=9)

When venous obstruction (60 mmHg) was applied, MBF decreased by 39% in the exercised leg and by 48% in the leg which was activated using the venous foot pump at the end of venous obstruction. The difference between the two legs was significant ($p<0.05$) (Fig. 13).

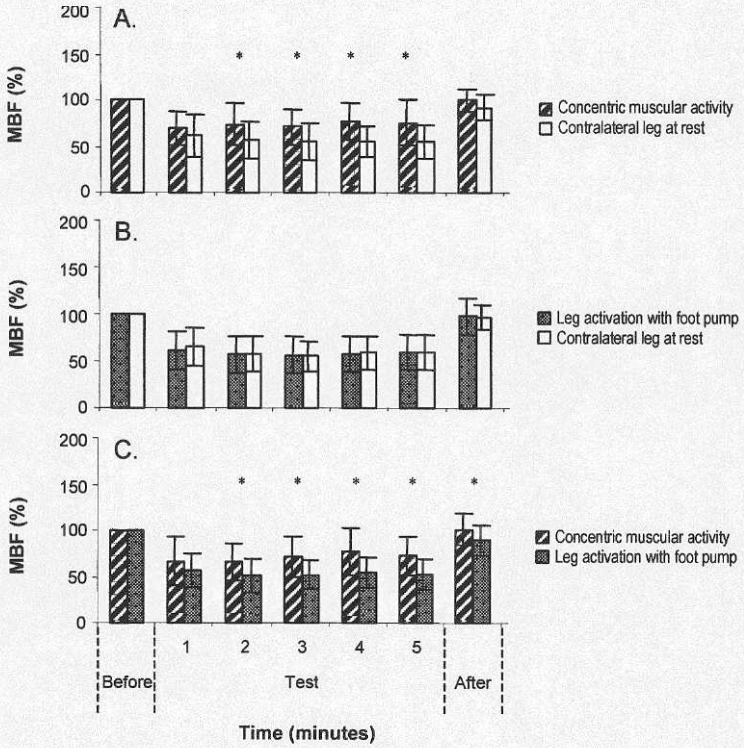

Figure 12. The effects of concentric muscular activity and leg activation with venous foot pump on muscle blood flow (MBF) in the tibialis anterior muscle (n=15) during 5 min of venous obstruction (60 mmHg). MBF values are given as a percentage of the initial resting value (100%). Each column contains the mean ± SD. Before – initial resting value, Test – concentric muscular activity or leg activation with a venous foot pump or both (1, 2, 3, 4, & 5 min), After – recovery. *Significant difference at $p<0.05$.

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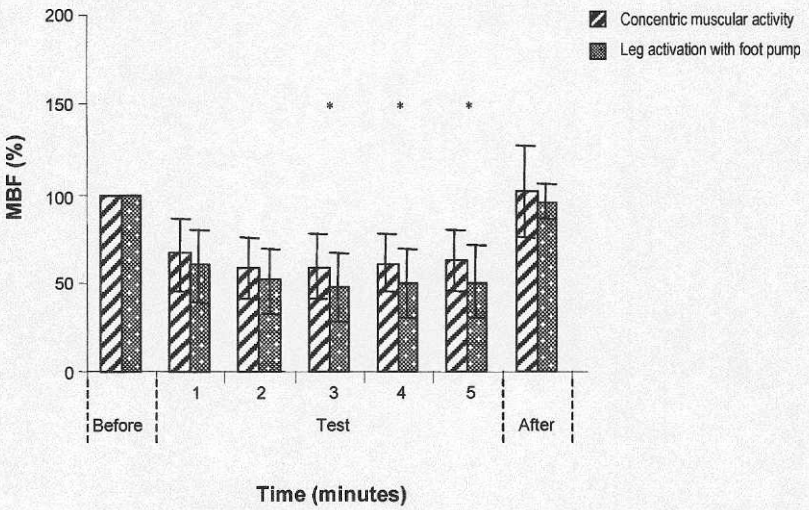

Figure 13. The effects of concentric muscular activity and leg activation with the venous foot pump on muscle blood flow (MBF) in the gastrocnemius muscle (n=9) during 5 min of venous obstruction (60 mmHg). MBF values are given as a percentage of the initial resting value (100%). Each column contains the mean ± SD. Before – initial resting value, Test – concentric muscular activity and leg activation with the venous foot pump (1, 2, 3, 4, & 5 min), After – recovery. *Significant difference at $p<0.05$.

2. Intramuscular pressure

2.1. IMP at rest

With both limbs at heart level, IMP was 7.1 (SD 2.3) mmHg in the left leg and 9.0 (SD 5.4) mmHg in the right leg (*Study II*). When the plaster cast was applied, IMP was 16.3 (SD 4.4) mmHg and 17.4 (SD 5.9) mmHg in the left and right legs, respectively. During venous stasis, IMP was about 40 mmHg in both legs.

IMP at rest before exercise was 11.4 (SD 2.1) mmHg in the control leg and 9.4 (1.3) mmHg in the test leg. No significant difference was found between the two legs (*Study III*).

2.2. IMP at rest with venous obstruction of a casted leg (*Study II*)

IMP was elevated bilaterally to 40 mmHg by venous obstruction (60-65 mmHg) lasting 30 min in the casted legs.

2.3. IMP at rest after exercise with venous obstruction of a casted leg (*Study III*).

IMP increased to 39 mmHg when the cuff pressure was 40 mmHg at rest after exercise. It increased to 58 mmHg at rest after exercise when the cuff pressure was 65 mmHg.

3. Neuromuscular function

The amplitude and area of the compound muscle action potentials showed a progressive loss in both legs during venous stasis (*Study II*). They diminished more during elevation of the limb. Subjects experienced sensory dysfunction and muscular weakness after 15 min of venous stasis only in the elevated casted leg (Table 1). Neuromuscular function returned to normal within 10 min after release of the venous stasis in all subjects.

4. Reduction of vibration-induced artefacts in PPG signal (*Study IV*)

An example of the effect of filtering a PPG signal that was masked with vibration-induced artefact arising from the stationary bandlimited acceleration used in the experiments is shown in Fig. 14. The diagrams indicate a dramatic reduction of the vibration-induced artefact.

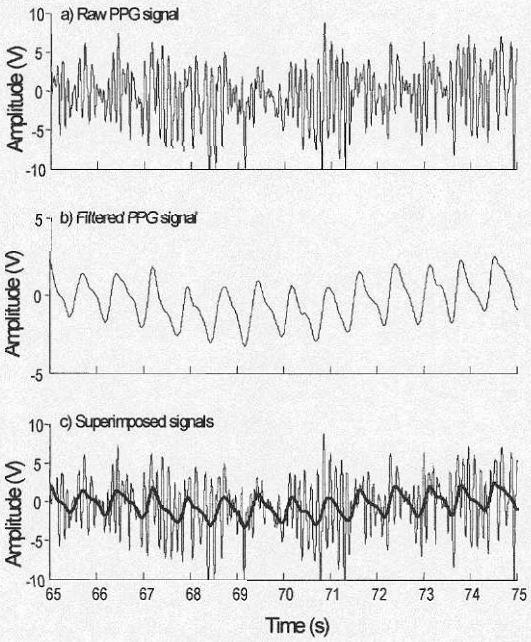

Figure 14. Figure a) shows the PPG signal affected by vibration, b) shows PPG signal after artifact suppressing filter and c) shows the two signals superimposed in the same plot.

However, filtering the PPG signal induces some distortion. This filter distortion of the PPG signal is illustrated (Fig. 15). The PPG signal originating from a measurement with no vibration exposure was applied. The filtered signal has, naturally, lost some of its high frequency content. However, some high frequency measurement noise was also suppressed.

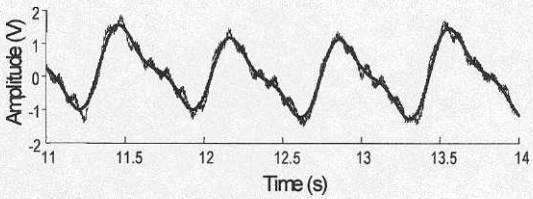

Figure 15. Illustration of the filter distortion in the PPG signal. The thin curve is a PPG signal filtered with the conventional blood flow measurement system. The bold curve illustrates the same signal filtered with the artefact suppressing filter.

GENERAL DISCUSSION

1. Subjects

Fifty-eight subjects and 98 legs were studied in this thesis. No consideration was taken of gender difference in subjects. Both sexes were included in all studies. It was not studied whether the effect of adipose tissue thickness on PPG measurements could be different for male and female subjects. It could be expected that the different amounts of subcutaneous adipose tissue might influence the measurement by PPG. Gender was not taken into account when the reactions to increased intramuscular pressure were studied.

2. Methodological considerations

2.1. Recording of muscle blood flow

Photoplethysmography

The major advantage of PPG technique is that it is a non-invasive technique, which allows the microcirculation to be continuously monitored at rest. The PPG method differs from strain-gauge plethysmography in that it measures local muscle blood flow selectively. In contrast, strain-gauge plethysmography determines total limb flow in various tissues and cannot measure local muscle blood flow. PPG is suited to situations which require measurements to be made over long periods. It can be easily applied to superficial skeletal muscle tissue in human studies. PPG is particularly attractive because of its relative low cost, portability, and ease of use, as well as for reasons of patient acceptability. With further development, it may be the most suitable technique for measuring MBF in the clinical situation.

To investigate the relationship between MBF and increased IMP, both parameters should be measured in the same muscle. PPG offers the possibility of measuring changes in local muscle blood flow in relation to increased tissue pressure in the same muscle, and so it is a suitable method to choose. The ability to measure how abnormally elevated IMP affects local muscle blood flow is of clinical interest. A non-invasive tool for monitoring muscle blood flow before and after exercise is helpful in studying the pathophysiology of exercise-induced pain in the leg and impaired skeletal muscle perfusion, such as compartment syndrome. The method may also provide information about MBF with regard to muscle performance during defined occupational demands and functional assessment of patients with work-related musculoskeletal disorders.

In the present study, PPG was compared with single-fibre laser-Doppler flowmetry. Both methods determine local muscle blood flow. Therefore, local blood flow was measured simultaneously in the same muscle using these two methods, and the results measured are comparable. The findings of post-occlusion and post-exercise hyperaemia measured using both methods were similar. There were no significant differences in muscle blood flow changes after arterial occlusion and muscle contractions between the methods. The correlation coefficient of the paired measurements between PPG and LDF was 0.76 for muscle blood flow after arterial occlusion. However, there was a poor correlation between PPG and LDF during the post-exercise hyperaemia. In comparing results of PPG and LDF it must be stressed that blood flow measured using the two methods is measured on the basis of two different physical principles. In addition, PPG and LDF reflect the blood flow over different tissue volumes. Changes in muscle blood flow recorded by LDF covers a tissue volume of approximately 1 mm^3. The PPG covers a much larger volume of the muscle belly, as it utilises several LEDs and photodetectors and due to multiple scattering, which also enhances the volume exposed to the probe. In addition, the LDF probe may be influenced by tissue movements during muscle contractions, which in turn may change the exact position of the probe. This may result in a "false" change in blood flow relative to the reference, which may explain, at least in part, why there was a poor correlation between the data provided by PPG and that provided by LDF during post-exercise hyperaemia.

Limitations of the PPG method, such as possible interference of high adipose tissue thickness, influence of superficial blood flow on MBF and influence of motion artefacts must be taken into consideration. Light penetration depends strictly upon the source-detector distance and the subcutaneous adipose tissue thickness. Since light is highly scattered in tissue, a detector positioned on the surface of the skin can measure reflections from a range of depths. Those reflections are variously absorbed, depending on whether the light encounters weakly or highly absorbing tissue. A thick skin or adipose tissue prevents some light passing through the muscle tissue. In the absence of any blood volume changes, the signal level will be determined by the muscle type, skin type, probe positioning, local temperature, surrounding light and of course the geometry and sensitivity of the sensor itself. Most of these baseline factors cannot be determined by independent measurement and will vary greatly between operators and subjects. In addition, the amplitude of the PPG signal cannot be calibrated. Therefore, only relative changes in blood flow in an individual can be measured. Furthermore, one of the difficulties with the clinical use of a PPG device is motion artefacts that have a deteriorating effect on the measurements and restrict the study design. The method cannot measure MBF during exercise.

The application of the filter technique showed a dramatic reduction in the vibration-induced artefacts in the PPG signal during vibration exposure (*Study IV*). This finding extends the possibilities of measuring changes in muscle blood flow by PPG under different physiological and ergonomic conditions. However, the transient artefacts in the PPG signal, i.e., the subject's movement, are not easily suppressed.

Further development of the PPG technique for measurement of MBF is needed. Future studies of the PPG technique for measurement of MBF should include comparisons between the PPG and the ^{133}Xenon clearance method. The ^{133}Xenon clearance method determines muscle blood flow in humans in a volume of tissue less than 1 cm^3 (Hickner et al., 1994). This may be similar to the volume measured using PPG. Strain-gauge plethysmography has been extensively used to study circulation in human extremities. However, the validity of the PPG method for determination of muscle blood flow as evaluated by simultaneous strain-gauge plethysmography is not satisfactory. Differences in measurement volume, where PPG measures local muscle blood flow while strain-gauge plethysmography determines total limb flow to various tissues, can make such comparison of limited value. Moreover changes of MBF in one leg compartment cannot be studied with the strain-gauge plethysmography.

Single-fibre laser-Doppler flowmetry

Local muscle blood flow can be measured using a modified LDF technique with only one single optical fibre. This technique provides a minimally invasive, continuous, real-time assessment of microvascular perfusion (Salerud & Öberg 1987). Tahmoush and co-workers studied blood flow in rat muscles and compared the laser-Doppler readings with radioactive microspheres (Tahmoush et al. 1983). The authors suggested that laser-Doppler monitor provides a linear index of skeletal muscle blood flow. Monteiro and co-workers also compared LDF with ^{133}Xe in assessment of blood flow changes in human masseter muscle (Monteiro et al 1989). They suggested that LDF can be used to assess changes in blood flow in the masseter; it registers a greater increase in flow during isometric contraction than does ^{133}Xe clearance. Salerud and Öberg studied the relationship between femoral artery blood flow and local muscle blood flow with a modified technique using only one single optical fibre introduced into the gastrocnemius muscle of pigs (Salerud & Öberg 1987). Femoral blood flow was recorded with an electromagnetic flowmeter. Linear regression analysis gave a correlation coefficient between femoral flow and local gastrocnemius blood flow of 0.88. It has been reported that single-fibre LDF is a reliable method which can be used for continuous

monitoring of human local muscle blood flow (Kvernebo et al. 1990; Hoffmann et al. 1995; Jensen et al. 1995; Larsson et al. 1995).

The optical fibre is sensitive to motion, giving rise to motion artefacts signals (Öberg 1990). We noticed motion artefacts in the LDF recordings at the moment when the occlusion was released or the subject relaxed the muscle after muscle contractions, which may be transferred to the optical fibre. However, after the initial phase no disturbances of the signal were observed.

The tissue trauma caused by the insertion of the optical fibre may influence muscle blood flow. In skin, this traumatic response is an eight-fold increase in flow for ten minutes. Even 70 min after the insertion, a hyperaemic trauma response was maintained (Staxrud et al. 1991). It is not known whether the trauma caused by insertion of the fibre causes a similar reaction in muscle tissue.

2.2. Recording of intramuscular pressure

Many factors contribute to changes in IMP during the measurements. It has been shown that IMP in the anterior compartment varies with depth and the position of the ankle joint (Gershuni et al. 1984; Nakhostine et al. 1993). It is important to place the catheter tip at a constant depth in the muscle and to keep the subject in a standard position while recording pressure from individual muscles. In the present study, the catheter was located into the muscle at a depth of 2.5 cm from the skin surface. The tip of the catheter was inserted parallel to the muscle fibres to reduce the risk of catheter bending and creating partial occlusion. The catheter position was checked by observing the response to external compression and to active muscle contraction. The Myopress catheter with four side holes gives good dynamic properties but the fluid filled recording system with extra corporal transducer has the disadvantage of possible hydrostatic artefacts. In the present study, infusion rate was below 1.5 ml/h. Styf and Körner reported that a microcapillary infusion of less than 1.5 ml/h does not increase the risk of recording high IMP induced by oedema formation of the infused saline (Styf & Körner 1986b).

2.3. Experimental model for generating elevated IMP

An experimental human model was used to improve understanding of the effects of increased IMP on MBF and neuromuscular function in Studies II and III. The combined effects of increased leg volume from venous obstruction and restricted swelling from plaster cast increased compartmental pressure. A thigh-shaped tourniquet was used in order to prevent the tourniquet from affecting the nerve directly during the experiments by spreading the pressure of the tourniquet over a larger area of the thigh. Pedowitz and co-workers concluded that wide cuffs and curved cuffs (designed to fit conically shaped limbs) with the minimum necessary cuff inflation give less nerve and muscle dysfunction (Pedowitz et al. 1993). Garfin and co-workers simulated increasing intracompartmental pressure in the hind limbs of dogs immobilised in plaster casts (Garfin et al. 1981). The cast was found to restrict expansion of the compartment volume by approximately 40%.

The experimental model was applied for thirty minutes, as this has been shown to be a safe way of inducing both temporary leg swelling and abnormally elevated IMP in human subjects (Wiger & Styf 1998).

3. Muscle blood flow

3.1. Post-occlusion and post-exercise hyperaemia (*Study I*)

After arterial occlusion lasting three minutes, reactive hyperaemia was observed in muscle tissue using both PPG and single-fibre LDF (Fig. 5). Post-occlusion reactive hyperaemia of the anterior tibial muscle has been measured using single-fibre LDF (Kvernebo et al. 1990;

Hoffmann et al. 1995; Larsson et al. 1995). Hoffmann and co-workers presented a mean blood flow in the anterior tibial muscle of 21 arbitrary units (AU) at rest and 46 AU after 3 min of arterial occlusion (Hoffmann et al. 1995). Compared with the resting value (100%), blood flow was seen to increase to 220% in that study, and in our study to 182% (LDF) and 150% (PPG).

Blood flow increases dramatically in active muscles after both static and dynamic exercise (Laughlin 1987; Walloe & Wesche 1988; Bangsbo & Hellsten 1998). This is consistent with our finding that after 1 min of maximal isometric and concentric contractions, a post-contraction hyperaemia was recorded using both methods in the anterior tibial muscle.

In response to arterial occlusion and muscle contraction, a low relative increase in blood flow was observed in our study compared with the findings of other studies (Hoffmann et al. 1995; Larsson et al. 1995). One reason for the difference could be the relatively short time period between the insertion of the optical fibre into the muscle and the ignition of measurements. The tissue trauma caused by the insertion of the optical fibre may influence muscle blood flow by inducing artificial vasodilatation. In skin this traumatic response is an eight-fold increase in flow for 10 min. Even 70 min after the insertion, a hyperaemic trauma response was maintained (Staxrud et al. 1991). Whether the trauma caused by insertion of the fibre causes a similar reaction in muscle tissue is not known. Measurements were started ~ 10 min after the insertion of the fibre, and this time period might be too short to allow for completion of the hyperaemic reaction to local trauma and recovery of normal flow (Monteiro et al. 1989). Thus, blood flow measured at rest may be overestimated. Another reason for the low relative increase found in the present study could be that we may have missed the maximum hyperaemia peak value. To compare MBF measured using PPG and the LDF methods, MBF should be recorded simultaneously. In some cases, there were motion artefacts in the LDF recordings at the moment when the occlusion was released or the subject relaxed the muscle after contraction, which may be transferred to the optical fibre. In order to reduce the influence of motion artefacts, the signals were analysed 60 s after occlusion. The mean time between tourniquet deflation and peak hyperaemia in the muscle has been reported to be 4 s (Larsson et al. 1995), 12-17 s (Kvernebo et al. 1990) and 19 s (Hoffmann et al. 1995). The maximum hyperaemia response, which may occur in the early recovery period, was therefore not covered in this study. Post-exercise hyperaemic muscle blood flow was measured immediately after isometric dorsiflexion of the ankle joint at maximal contraction for 1 min and full range-of-motion dorsiflexion and plantar flexion of the ankle joint for 1 min. Subject' blood pressure did not change during exercise. Therefore, the hyperaemic muscle blood flow that we obtained during post-exercise may be only attributable to local metabolic and muscle pump mechanisms.

3.2. Effects of limb elevation and increased IMP on MBF (*Study II*)

During the experiment, muscle blood flow decreased by 50% in the elevated leg and by 42% in the leg at heart level when the IMP was maintained at 40 mmHg. Perfusion pressure of the anterior compartment decreased from 42 to 17 mmHg (60%) in the elevated leg and from 65 to 43 mmHg (34%) in the leg kept at heart level.

Blood flow is determined by differences in arterial and venous pressures divided by local vascular resistance (Feigl 1974a). Increased intramuscular pressure results in an increase in local venous pressure, as local venous pressure increases to the same extent as the rise in the surrounding tissue pressure, which produces a diminished local arteriovenous pressure difference and hence blood flow. Limb elevation above heart level further reduces perfusion pressure. The fact that increased tissue pressure impairs local blood flow has been demonstrated by the plethysmographic studies of Ashton (Ashton 1975), washout rates of Xenon (Rorabeck & Clarke 1978; Styf et al. 1987), the clearance rates of Tc99 (Rorabeck &

Macnab 1975), and the argon washout technique (Matsen et al. 1979a). The findings in the present study are also in agreement with results of others, which show that IMP values between 30 and 50 mmHg and perfusion pressure below 30 mmHg may impair normal function (Ashton 1975; Hargens et al. 1979; Matsen et al. 1979b; Heppenstall et al. 1988; Styf & Wiger 1998; Wiger & Styf 1998; Wiger et al., 2000).

Autoregulation can compensate for some reduction in arteriovenous gradient by changes in local vascular resistance (Feigl 1974b) and maintain local blood flow over a range of arteriorvenous gradients. This may explain why MBF in the elevated leg was not significantly lower as compared with the non-elevated leg. Heppenstall and co-workers indicated that skeletal muscle has the ability to maintain adequate mitochondrial oxygen delivery despite marked reductions in its arteriovenous perfusion pressure gradient (Heppenstall et al. 1988). When increased tissue pressure and limb elevation reduced arteriovenous perfusion pressure, autoregulation could initially compensate for this through a reduction in peripheral vascular resistance. However, when the local blood flow was reduced to the point where it no longer met the metabolic demands of the tissue, functional abnormalities of muscle and nerve tissue occurred.

3.3. Vascular resistance

In Study II, muscle blood flow in the non-elevated leg was 58% of the control flow when perfusion pressure was kept at 43 mmHg. In contrast, MBF in the elevated leg remained at 50% of the control flow although perfusion pressure was decreased to 17 mmHg. MBF in the elevated and non-elevated legs showed no significant difference during venous obstruction. However, we noted that vascular resistance increased by 25% in the leg kept at heart level, while it decreased by 12% in the elevated leg. As resistance decreased in the elevated leg, MBF remained constant despite the presence of reduced perfusion pressure. This observation could be explained by autoregulation. This finding is in agreement with a previous study by Järhult and Mellander (Järhult & Mellander 1974). They showed that most vascular beds, including that of skeletal muscle, exhibit autoregulation of blood flow over a wide range of perfusion pressures.

The vascular resistance decreased by approximately 35% at rest after exercise compared with at rest before exercise in both control and test legs (*Study III*). Vascular resistance showed no difference between the control and test legs. However, MBF was significantly higher in the control leg with normal intramuscular pressure than in the test leg with abnormally elevated intramuscular pressure. This is because perfusion pressure in the control leg was significantly higher than in the test leg. This suggests that the local muscle blood flow, during post-exercise hyperaemia, increases with increasing perfusion pressure and with decreasing vascular resistance, which may caused by vasodilatation due to local metabolic factors.

3.4. Abnormally elevated IMP impairs MBF at rest after exercise (*Study III*)

The present study has shown that MBF was significantly lower in the leg exposed to elevated IMP than in the control leg exposed to normal IMP at rest after exercise. The lower increase in muscle blood flow at intramuscular pressures of 39.4 mmHg and 58.3 mmHg at rest after exercise compared to conditions of normal intramuscular pressure may be explained by the arteriovenous pressure difference. In addition, increased intramuscular pressure may compress the vessels at the capillary level, leading to enhanced hyperaemia after exercise. Styf and Körner reported that muscle blood flow decreased significantly during exercise when muscle relaxation pressure increased to 42.6 mmHg, in patients who had chronic compartment syndrome (Styf & Körner 1987). Delayed post-exercise hyperaemia was found, when IMP was 74.1 mmHg, in chronic compartment syndrome patients one minute after exercise

(Abraham et al. 1998). These findings are consistent with our results. They may suggest that muscle blood flow can be limited by the effect of increased intramuscular pressure at rest after exercise. The findings of the present study are also consistent with previous studies in which increased intramuscular pressure impairs muscle blood flow at rest (Clayton et al. 1977; Matsen et al. 1979a), and a significant decrease or even cessation of blood flow when tissue pressure exceeded 30 to 60 mmHg and perfusion pressure fell below 30 mmHg (Ashton 1975; Sheridan & Matsen 1975; Clayton et al. 1977; Zhang et al. 2001b).

Mohler and co-workers measured tissue oxygenation of exercised skeletal muscle with near-infrared spectroscopy in patients who had chronic compartment syndrome of the leg (Mohler et al. 1997). They reported greater relative deoxgenation during exercise as well as delayed reoxygenation after exercise. These findings are probably a consequence of impaired muscle blood flow due to elevated IMP, and support our findings that abnormally elevated intramuscular pressure impedes muscle blood flow at rest after exercise.

3.5. Effects of concentric muscular activity or leg activation with venous foot pump on MBF (*Study V*)

Blood flow in the tibialis anterior and gastrocnemius muscles produced by concentric muscular activity was significantly greater than that which resulted from leg activation with the venous foot pump during 5 minutes of exposure to venous obstruction. Activation of the leg with the venous foot pump was not followed by any significant change in leg muscle blood flow. The duration and frequency of exercise in this study was designed to facilitate the comparison of effects.

The increase in blood flow after exercise observed in this study is in agreement with observations from other physiological experiments (Laughlin 1987; Walloe & Wesche 1988; Bangsbo & Hellsten 1998). Immediately upon release of the contraction, pressure in the veins is probably close to zero, resulting in an increased pressure gradient across all capillaries in the muscle (Delp & Laughlin 1998; Delp 1999; Hughson & Tschakovsky 1999). Saltin and co-workers reported that the elevated pressure gradient between mean arterial pressure and the venous end of the capillary after the contraction is most likely sufficient to explain the very early (1-3 s) elevation in blood flow velocity without postulating any concomitant vasodilatation (Saltin et al. 1998). There is a considerable increase in the perfusion pressure of the muscles after contractions, and this may selectively increase the blood flow in muscles and thus be recognised as a highly effective venous pump. In the present study, the findings of an immediate marked elevation in blood flow following muscle contractions support the muscle pump mechanism.

The blood flow did not change significantly in the anterior tibial and gastrocnemius muscles during leg activation with the venous foot pump in the present study. It has been shown that compression on the sole of the human foot by the venous foot pump creates oscillations in intramuscular and subcutaneous interstitial fluid hydrostatic pressure in the leg (Styf 1990). The mechanism for this was explained in terms of intermittent passive muscle stretch. Furthermore, in that study, tissue pressure in the anterior tibial muscle decreased following active muscle contractions during venous obstruction, but not after leg activation with the venous foot pump. This may partly explain why muscle blood flow was lower following leg activation with the venous foot pump when compared to concentric muscular activity in the present study.

4. Intramuscular pressure

IMP at rest immediately after exercise was 17 mmHg (*Study III*). It is between 10-25 mmHg in healthy legs (Styf & Körner 1986b; Rorabeck et al. 1988; Pedowitz et al. 1990). IMP increased to 39 mmHg with 40 mmHg venous obstruction and to 58 mmHg with 65 mmHg

venous obstruction at rest after exercise. These pressure levels were very similar to those reported by Mohler and co-workers (Mohler et al. 1997). In their study, pressure of 55.4 mmHg was found one minute after cessation of exercise in patients who had chronic compartment syndrome. The model employing venous obstruction of a casted leg with high intracompartmental pressure seems suitable to induce the kind of microcirculatory impairment seen in patients with chronic compartment syndrome.

5. Effects of limb elevation and increased IMP on neuromuscular function

Ischemia of the tissues in the anterior leg compartment is characterised by pain, weakness of dorsiflexion of the ankle and toes, and a variable degree of sensory loss over the first interdigital cleft. In the present study, abnormally increased pressure to 40 mmHg was induced in the anterior compartment both when the limb was kept at heart level and when it was elevated. However, a gradual loss of sensation in the foot and leg muscular weakness occurred only in the elevated limb. This was because limb elevation above heart level combined with venous obstruction of a casted leg further reduced perfusion pressure to 17 mmHg. According to Heppenstall and co-workers perfusion pressure must exceed 30 mmHg in a non-traumatised compartment for adequate neuromuscular function Heppenstall et al. (1988). Stainsby and Otis suggested that mean arterial perfusion pressure is reduced below 25-30 mmHg before cell oxygen delivery falls below normal levels (Stainsby & Otis 1964). This may explain why elevating IMP pressure to 40 mmHg with the limb at heart level did not significantly change muscle and nerve function, since mean perfusion pressure was 43 mmHg. The results indicate that about 35 cm of elevation of a leg above heart level lowers its tolerance for increased tissue pressure. These findings are in agreement with results which show that IMP values between 30 and 50 mmHg and perfusion pressure below 30 mmHg may impair function and even threaten the viability of muscle and nerve tissue (Ashton 1975; Hargens et al. 1979; Matsen et al. 1979b; Heppenstall et al. 1988; Wiger & Styf 1998; Wiger et al. 2000).

Elevation of a limb above the heart may decrease local venous pressure. However, elevation cannot decrease local venous pressure below the level of local tissue pressure. Thus elevation of a limb with increased tissue pressure reduces local perfusion pressure. In the present study, decreased perfusion pressure and decreased MBF reduced the tolerance of the leg to abnormally increased IMP when the leg was elevated. If increased intramuscular pressure alone was the primary factor, one would expect the pressure tolerance in the leg to be similar at the heart level and in an elevated position. The possible pathogenesis of symptoms and signs of the elevated vein-obstructed leg may be a combination of increased intramuscular pressure, decreased perfusion pressure and decreased blood flow of the involved limb.

Findings from the present study are clinically important. They suggest that limbs with increased tissue pressure should not be elevated. In this situation, a diminution of the tolerance of the leg to increased tissue pressure is directly related to the reduction in perfusion pressure, which reduces MBF and induces sensory dysfunction and muscular weakness.

6. Suppressing vibration-induced artefacts in a PPG signal

The filter technique used here resulted in a remarkable reduction of vibration-induced artefacts in PPG signal (*Study IV*). The distortion of the PPG signal was analysed strictly using the ratios of PPG data originating from PPG signals with and without the artefact suppressing filter. As a second indication, the correlation between the PPG data originating from PPG signals with and without the artifact suppressing filter was calculated. The correlation coefficient was 0.99. The filtering distortion was found to be marginal. Thus the filter technique allows for measuring muscle blood flow by PPG during vibration. This

finding extends the possibilities of evaluating changes in muscle blood flow under different physiological and ergonomic conditions.

However, the type of vibration applied is an important factor. As the acceleration of the vibration has its main power between 5 and 2000 Hz, and the PPG signal spectrum decreases considerably above the third harmonic of the fundamental frequency, i.e. the heart rate, a sharp low-pass filter was found to be suitable in this case. Other vibrations will have different demands. Furthermore, the filter technique used is applicable when the vibration exposure is stationary. Transient artefacts, i.e., due to the subject's movement, will not be fully suppressed. The biomechanical properties of the limb and limb system being tested will also influence the choice of filter.

CONCLUSIONS

1. Post-occlusive and post-exercise hyperaemia can be measured in human anterior tibial muscle using the photoplethysmography (PPG) technique. This technique enables non-invasive, continuous assessment of relative changes in muscle blood flow (*Study I*)

2. Increased IMP, induced by venous obstruction of a casted leg, reduces perfusion pressure and MBF, and results in a diminished amplitude and area of the compound muscle action potentials. Limb elevation above heart level combined with venous stasis of a casted leg further reduces perfusion pressure and MBF, and induces sensory dysfunction and muscular weakness (*Study II*).

3. Abnormally elevated IMP induced by venous obstruction of a casted leg reduces local perfusion pressure and MBF at rest after exercise. PPG appears to be a promising non-invasive tool for measuring MBF in clinical conditions with impaired microcirculation and abnormally elevated intramuscular pressure (*Study III*).

4. MBF can be measured using PPG during vibration exposure with a filter, thus extending the possibilities of applying this technique to different physiological and ergonomic conditions (*Study IV*).

5. Concentric muscular activity produces greater MBF than leg activation with the venous foot pump during venous obstruction. Thus, in this perspective, it is better to advise patients to move their feet than to use the passive venous foot pump in the postoperative period after major surgery on a lower limb (*Study V*).

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