The role of the calf muscle venous pump in exercise hyperaemia

Arterial leg blood flow response to rhythmic calf exercise in the supine and head-up tilted positions in healthy subjects, and in patients with venous insufficiency

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Preface

This work was carried out at the Institute of Basic Medical Sciences, Department of Physiology, University of Oslo and at the Department of Vascular and Thoracic Surgery, Akershus University Hospital and Institute of Clinical Medicine, Campus Ahus, University of Oslo, during the period 2007 to 2011.

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List of papers

Paper I:

Nådland IH, Walløe L & Toska K (2009). Effect of the leg muscle pump on the rise in muscle perfusion during muscle work in humans. *European Journal of Applied Physiology* **105**, 829-841.

Paper II:

Nådland IH, Wesche J, Sheriff DD & Toska K (2011). Does venous insufficiency impair the exercise-induced rise in arterial leg blood flow? *Phlebology* **26**, 326-331.

Paper III:

Nådland IH, Wesche J, Sheriff DD & Toska K (2011). Does the great saphenous vein stripping improve arterial leg blood flow during exercise? *European Journal of Vascular and Endovascular Surg*ery **41**, 697-703.

Abbreviations

AVH, ambulatory venous hypertension

AVP, ambulatory venous pressure

CEAP, clinical, etiological, anatomical, pathophysiological

CO, cardiac output

FF, femoral artery flow

FPC, femoral peripheral conductance

GSV, great saphenous vein

HR, heart rate

HUT, head-up tilted

MAP, mean arterial pressure

MVC, maximal voluntary contraction

SFJ, saphenofemoral junction

SPJ, saphenopopliteal junction

SSV, small saphenous vein

SV, stroke volume

The muscle pump debate

The muscle venous pump is well known to be important in promoting the venous return of blood from the legs towards the heart. In addition to the promotion of venous return, the muscle venous pump has a local mechanical pumping effect on arterial blood flow during rhythmic muscle activity. However, this effect is complex and less well established. During the last 40 years, a number of investigators have examined the part played by the mechanical pumping effect of muscle contractions and relaxations in exercise hyperaemia. Divergent conclusions have been reached. Some authors have proposed that the muscle venous pump is an important determinant of the rise in blood flow to exercising muscles. On the other hand, others have claimed that its contribution to exercise hyperaemia is the scientific equivalent of the Emperor's new clothes, and is merely an illusion. In other words, the muscle venous pump is not an important determinant of muscle blood flow during muscle work. In this thesis, the effect of the muscle venous pump on the rise in arterial leg blood flow was examined using a specially designed experimental calf exercise model. This was first examined in healthy subjects, and then in patients with incompetent venous valves, who should not have an optimally functioning muscle venous pump.

Introduction

Cardiovascular responses to dynamic exercise

There is a wide range of cardiovascular responses to exercise, including but not limited to central circulatory adjustments to exercise and local control of blood flow to active muscles. I will start with a brief description of the central circulatory adjustments to dynamic exercise. The mechanisms responsible for these are not examined in the papers for this thesis, and I will therefore not discuss them in any great detail here.

Central circulatory responses to dynamic exercise

As early as the 19th century Chaveau and Kaufmann (1887) measured changes in blood flow to dynamically active skeletal muscles. They measured the venous outflow of blood from a lip lifting muscle in horses chewing oats, and found higher values in horses that where hungry, and thus chewing more vigorously. Barcroft and Dornhorst (1949) measured blood flow during rhythmic leg exercise in humans using air-plethysmography, and found it to be higher during exercise than during rest. When Walløe and Wesche (1988) measured leg blood flow continuously during rhythmic quadriceps exercise using ultrasound Doppler, they found it to increase to a surprisingly high level. There is general agreement that muscle blood flow increases rapidly during exercise, as reviewed by Rowell (2004). As the blood flow to active skeletal muscles increases, the amount of blood pumped by the heart must adjust to the demands of the periphery. Cardiac output (CO) usually increases during exercise, and the rise in CO may be caused by an increase in heart rate (HR) or stroke volume (SV), or both. HR is under the control of both the parasympathetic and the sympathetic branches of the autonomic nervous system. The effect of activity in parasympathetic nerves to the heart is to lower HR. The initial tachycardia during exercise is brought about by the withdrawal of parasympathetic activity (Pickering & Davies, 1973;Ogoh *et al.*, 2005). Further increase in HR is caused by sympathetic activity (Levick, 2003). SV is generally thought to increase during exercise (Levick, 2003). The most important factors leading to increase in SV are an increase in venous return of blood from the periphery and increased contractility (controlled by the sympathetic branch of

the autonomous nervous system). Mean arterial pressure (MAP) increases during dynamic exercise, but only moderately, because the rise in CO is almost offset by the fall in total peripheral resistance (caused by vasodilation in the exercising muscle) (Levick, 2003).

Local adjustments in arterial blood flow to dynamically active muscles

Blood flow to skeletal muscles is closely regulated to match the muscle metabolic demand. The blood flow through a tissue is given by the product of perfusion pressure and the vascular conductance of the tissue vascular bed. This relationship can be expressed by the formula $Q = \Delta P \times C$, where Q is the flow of blood, ΔP is the perfusion pressure (the difference between the arterial and venous pressure), and C is the vascular conductance. Thus, blood flow to exercising muscle can be modified by a change in perfusion pressure or vascular conductance or both. Although it is clear that exercise hyperaemia is primarily a local phenomenon, there has been a long-running controversy regarding the mechanisms responsible for increasing blood flow to active muscles. Several factors leading to vasodilation have been proposed, including the following: the effects of metabolites from contracting muscle cells: mechanical influences on skeletal muscle vascular tone (compression of the intramuscular arterioles during muscle contraction could activate a mechanosensitive response by the vascular smooth muscle and /or endothelium to elicit rapid vasodilation): conducted and flow-mediated vasodilation: and vasodilators released from red blood cells (Joyner & Proctor, 1999;Clifford & Hellsten, 2004;Rowell, 2004;Tschakovsky & Sheriff, 2004;Clifford, 2007;Joyner & Wilkins, 2007;Saltin, 2007;Clifford, 2011). It has been argued against an important role of nerves in exercise hyperaemia (including sympathetic withdrawal, active vasodilation via sympathetic vasodilator fibres and vasodilation elicited by acetylcholine spillover from active motor nerves) (Joyner & Proctor, 1999;Clifford & Hellsten, 2004;Rowell, 2004;Clifford, 2007;Joyner & Wilkins, 2007).

It is several decades since it was suggested that the muscle venous pump, in addition to aiding the return of blood to the heart from the lower limbs, might increase local arterial blood flow to the active muscles themselves (Ludbrook, 1962;Folkow *et al.*,

1970;Folkow *et al.*, 1971). This mechanism is believed to increase muscle perfusion by increasing the perfusion pressure across the muscle, and will be explained in detail later in the introduction.

In summary, the rise in arterial blood flow to dynamically active muscles seems to be the result of several mechanisms acting in concert. It is a complex process, with a good deal of redundancy, which makes it challenging to study the contribution of each of the potential mechanisms separately. In the papers for this thesis, we did not distinguish between different vasodilatory mechanisms, and I will therefore only discuss them briefly. On the other hand, the papers do provide new information about the contribution of the mechanical actions of the calf muscle venous pump to the arterial leg blood flow response to rhythmical exercise, and this will be discussed in detail.

Lower limb venous anatomy

There can be considerable inter-individual variations in venous anatomy, but the main features are described below. The venous system in the lower limbs is divided into the superficial and the deep venous systems. They are separated by the muscle fascias, with the deep veins lying below the muscle fascia, and the superficial veins above it. Perforator veins pass through the muscle fascia, connecting the two venous systems.

The type of exercise described in the three papers for this thesis involves the calf muscles. The deep venous system in the calf can be separated into intermuscular veins (the posterior tibial, anterior tibial and peroneal veins) and intramuscular veins (the soleus and gastrocnemius veins). In the lower leg, the three groups of intermuscular deep veins are all paired, and they accompany the anterior tibial artery, the posterior tibial artery and the peroneal artery. The posterior tibial veins run up posteriomedially along the medial side of the tibia. The peroneal veins located behind the fibula pass upwards through the calf, where they join the posterior tibial veins in the upper calf. The anterior tibial veins run proximally in the leg between tibia and fibula, and join the tibial/peroneal trunk to form the popliteal vein in the upper calf. The calf muscles include the gastrocnemial and the soleal muscle groups. The soleus veins drain mainly

into the posterior tibial and peronal veins (Reis *et al.*, 2008). The gastrocnemius veins within the medial and lateral gastrocenmius muscles drain into the popliteal vein (Meissner, 2005).

The deep veins of the calf join to form the popliteal vein, which passes proximally behind the knee and then upwards in the distal thigh through the adductor canal, where it is called the femoral vein (also known as the superficial femoral vein, however, this term is no longer used to avoid confusion with the superficial venous system). Small muscle veins within the deep muscles of the lateral thigh join to form the profunda femoris vein. The latter joins the femoral vein to form the common femoral vein, which continues to form the external iliac vein.

The great saphenous vein (GSV) (also known as the long saphenous vein, the greater saphenous vein and the internal saphenous vein (Reich-Schupke & Stücker, 2011)) and the small saphenous vein (SSV) (also known as the short saphenous vein, the lesser saphenous vein and the external saphenous vein) are the main superficial veins. The GSV originates in the medial foot and passes upward along the medial leg and thigh. In the proximal thigh, it perforates the deep fascia to join the femoral vein at the saphenofemoral junction (SFJ). The GSV lies in a saphenous compartment, with the deep muscle fascia and the saphenous fascia as boundaries (Meissner, 2005). Duplication of the GSV (identified by splitting of the vein into two channels, both lying on the muscular fascia and which later rejoin) is present in the thigh in 8% of cases and in the calf in 25% of cases (Meissner, 2005). The SSV originates in the lateral foot and passes upward along the middle of the back of the leg, usually in an inter-facial compartment consisting of the deep muscular fascia and a superficial fascia. It perforates the muscle fascia and passes between the two heads of gastrocnemius in the lower part of the popliteal fossa, and joins the popliteal vein at the saphenopopliteal junction (SPJ). However, the mode of ending of the SSV is variable. It may join the GSV in the upper third of the thigh, or may bifurcate, one branch joining the GSV and the other the popliteal or deep posterior veins of the thigh, occasionally it ends, below the knee joint, in the GSV or in the deep muscular veins of the calf. A cranial extension of the SSV, often referred to as the vein of Giancomini,

may ascend posteriorly in the thigh to communicate with the GSV through the posterior thigh circumflex vein. The GSV and SSV deliver blood into the deep veins at the SFJ and SPJ. The deep and superficial venous systems are also connected by perforator veins, as shown in Figure 1.

The primary function of the venous system is the return of blood to the heart. The return of blood from the lower limbs to the heart relies on one-way venous valves. Properly functioning venous valves ensure unidirectional flow from the lower limbs to the heart. Perforating veins usually contain valves that prevent venous flow of blood from the deep veins into the superficial venous system during muscle contraction (Almen & Nylander, 1962). The density of venous valves seems to increase from the thigh to the lower leg. The iliac veins contain few valves. Valves are present in 1.2% of common iliac veins, 27% of external iliac veins, and in 10.1% of internal iliac veins (Meissner, 2005). A review of the location, number and consistency of location of venous valves in the femoral and popliteal veins suggest that the femoral vein contain between one and six valves, and the popliteal vein contain between zero and four valves (Moore *et al.*, 2011). Further it suggest that deep valves are consistently located in the common femoral vein (within 5 cm of the inguinal ligament), the femoral vein (within 3 cm of the deep femoral vein tributary) and in the popliteal vein near the adductor hiatus. The main trunk of the GSV usually has at least six valves and the SSV usually has 7 to 10 closely-spaced valves (Meissner, 2005).

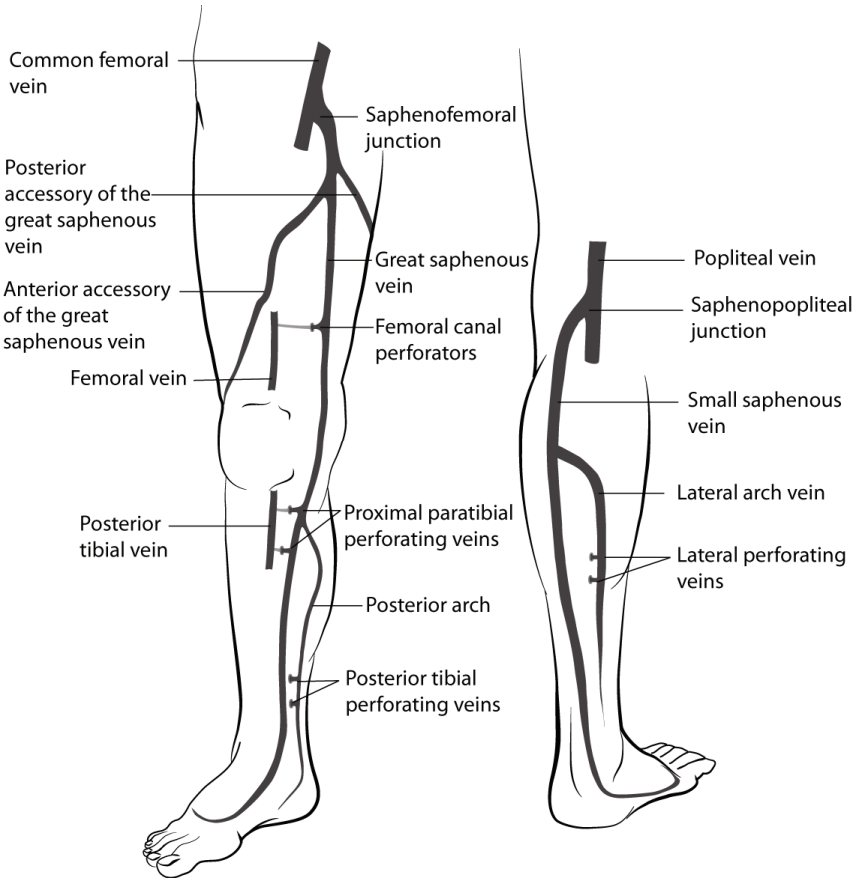

Figure 1. The main superficial veins of the lower limb and some of the connections to the deep venous system. The figure shows the connection of the GSV and SSV with the deep venous system at the SFJ and SPJ. The deep and superficial venous systems are also connected by perforator veins. The femoral canal perforators (Hunterian perforators, Dodd perforators) connect the GSV and femoral vein or the popliteal vein; the proximal paratibial perforating veins (Sherman and Boyd perforators) connect the GSV and posterior tibial veins; and the posterior tibial perforating veins (Cockett perforators) connect the posterior arch (Leonardos vein) and posterior tibial veins. The lateral arch vein communicates with the peroneal veins through the lateral calf perforators. (Adapted from Meissner, Lower Extremity Venous Anatomy (2005) and Norgren, Vensjukdomar, page 39-40, (2004)).

Venous pumps

The venous pumps of the foot and the calf

The venous pumps in the lower leg are the foot pump, the distal calf muscle venous pump and the proximal calf muscle venous pump. They were thoroughly described by Gardner and Fox (2001):

The most distal pump is the foot pump. This pump is activated by putting weight on the foot, which leads to a longitudinal stretching and emptying of the lateral plantar veins of the foot (Fig. 2B). Gardner and Fox identify the possible paths for outflow as the posterior tibial, the anterior tibial or the peroneal veins amongst the deep veins, or the superficial GSV and SSV. They further indicate that the foot pump plays an important role in venous return from the lower limb, and that its action is coordinated with the actions of the venous pumps in the calf.

There are two calf pumps, proximal and distal. The distal calf muscle venous pump is activated by dorsiflexion of the ankle, which causes the muscles to descend within the fascia and thus increases pressure in the distal compartment (Fig. 2A). Upon activation of the distal calf pump, anterior tibial muscle contraction is likely to obstruct the anterior tibial veins. In addition, the rise in distal calf pressure impairs posterior tibial blood flow, thus leaving the peroneal conduits as the paths of least resistance. The proximal calf muscle venous pump is activated upon plantar flexion of the ankle, emptying the proximal calf pump into the popliteal and femoral veins (Fig. 2C).

Gardner and Fox further describe the actions of the three pumps in the leg as coordinated during normal ambulation. Activation of the distal calf muscle venous pump by dorsiflexion of the ankle empties the anterior tibial veins, the distal part of the peroneal and the posterior tibial veins simultaneously. The subsequent weight bearing empties the foot pump (the plantar veins of the foot), and activation of the proximal calf muscle venous pump by plantar flexion of the ankle empties the proximal calf pump (both the muscle veins and adjacent deep veins) into the popliteal and femoral veins. Thus, in ambulation, each muscle pump empties its contents into the next more proximal one that has previously been emptied to receive it.

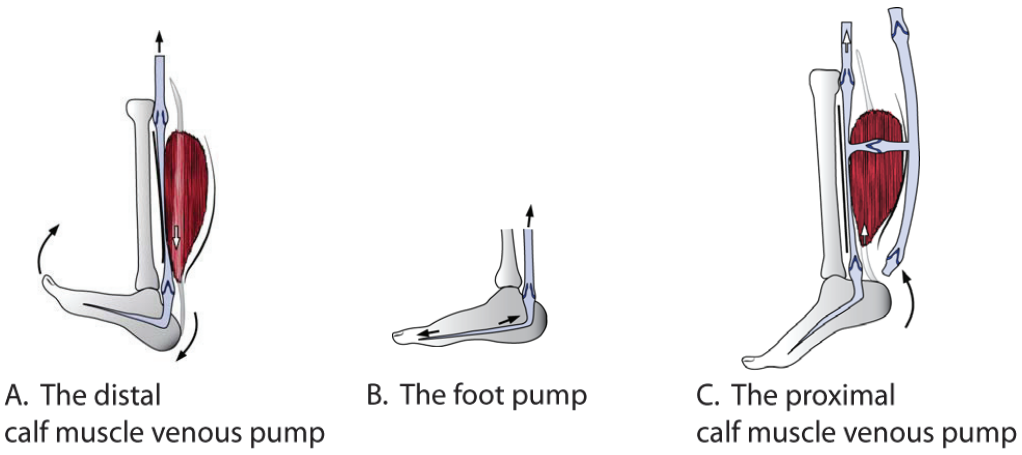

Figure 2. The distal and proximal calf muscle venous pumps and the foot pump. Each muscle pump empties its contents into the next more proximal one that has previously been emptied to receive it (as described in the text above). (Adapted from Norgren, Vensjukdomar, page 49-50, (2004)).

Muscle venous pumps in the thigh and arm

Other muscle venous pumps exist in addition to those described above. They include the quadriceps muscles, which during contraction compress the femoral veins, and push blood toward the inguinal vessels. Less is known about the muscle venous pumps in the arm, but Fox and Gardner (2001) indicate that they exist in the arms as well, and that they are necessary for the return of blood toward the heart. They explain the actions of the pumps as follows. When the fist is clenched, blood is ejected upwards from the forearm. At the same time, blood is pushed from the hand into the distal forearm. On extension of the fingers, the blood in the distal forearm is pushed more proximally. Thus, also in the arm, each muscle venous pump empties its contents into the next more proximal one that has previously been emptied to receive it. Other venous pumps exist in addition to those described above (Gardner & Fox, 2001), but they will not be discussed here.

How could a muscle venous pump increase arterial blood flow?

Muscle contractions may compress the arterial blood vessels to a greater or lesser degree, thus impeding arterial inflow (Anrep & Von Saalfeld, 1935;Folkow *et al.*, 1970;Wesche, 1986;Walløe & Wesche, 1988;Lutjemeier *et al.*, 2005). Contractions also squeeze the veins and push blood towards the heart. With properly functioning venous valves preventing retrograde flow of blood, the subsequent reduction in deep venous pressure is believed to widen the perfusion pressure (the arterio-venous pressure gradient) across the muscle, thus facilitating arterial inflow and muscle perfusion (Folkow *et al.*, 1970;Folkow *et al.*, 1971;Laughlin, 1987;Sheriff *et al.*, 1993;Sheriff & Van Bibber, 1998).

The terms “muscle pump” and “muscle venous pump” will be used synonymously throughout this thesis. The term “muscle pump effect” refers to the arterial flow promoting effect of the muscle pump, which is described above and illustrated in Figure 3.

It has been proposed that the effect of a muscle pump on the rise in arterial blood flow during exercise may be more efficient when a significant hydrostatic column is present, and that humans should therefore be studied in an upright position (Laughlin & Joyner, 2003). The explanation for this is that a larger hydrostatic column results in a larger arterial and venous pressure, thus facilitating a larger rise in perfusion pressure elicited by the muscle pump. Figure 3 shows how the muscle pump is believed to increase perfusion pressure and facilitate arterial blood flow.

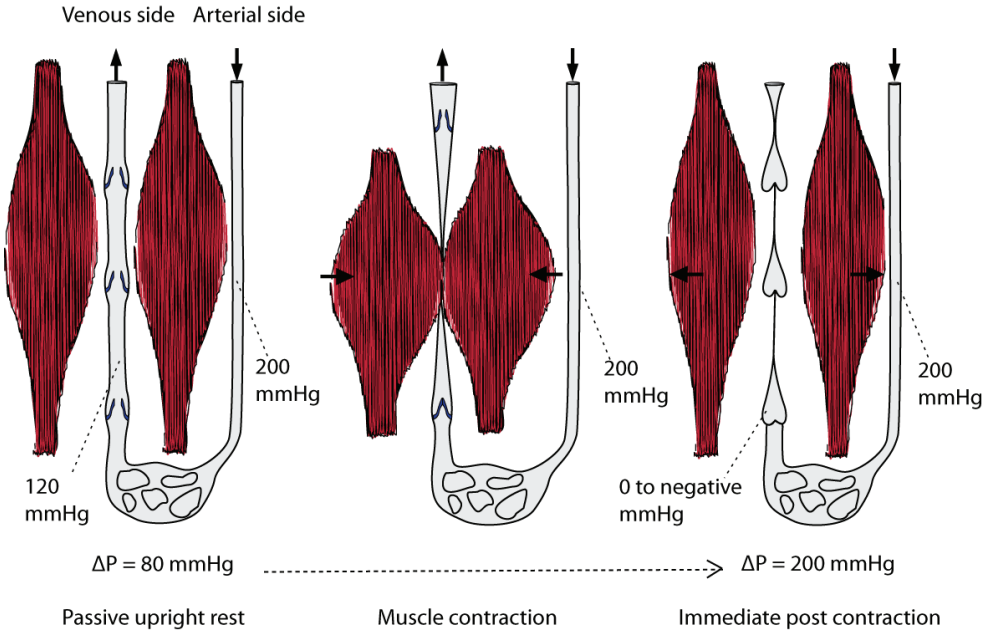

Figure 3. The perfusion pressure in this example is 80 mmHg in the passive resting upright position. During muscle contraction, blood is pushed from the veins towards the heart, and provided that there are competent venous valves, the retrograde flow of blood is prevented. The subsequent reduction in deep venous pressure is believed to widen the perfusion pressure (the arterio-venous pressure gradient) across the muscle, thus facilitating arterial inflow and muscle perfusion (the muscle pump effect). In this example, the perfusion pressure is increased from 80 to 200 mmHg. (Adapted from Rowell, Human Cardiovascular Control, page 29 (1993)).

Venous pressure in the lower limbs of healthy subjects in an upright position has been found to be roughly equal to the pressure of the corresponding hydrostatic column (the pressure exerted by the column of blood between the measurement site and the heart) (Pollack & Wood, 1949;Højensgard & Sturup, 1953;Arnoldi, 1965;Groothuis *et al.*, 2008). For the muscle pump to increase the perfusion pressure, the venous pressure must be reduced. It has been shown that muscle activity results in a reduction of the venous pressure in the lower limbs of healthy subjects to below the hydrostatic pressure, both the superficial GSV (Pollack & Wood, 1949;Højensgard & Sturup,

1953;Arnoldi, 1965) and in the deep posterior tibial vein (Højensgard & Sturup, 1953;Arnoldi, 1965).

When venous transmural pressure is elevated about 25 mmHg or more, the venoarteriolar reflex leads to local vasoconstriction (Henriksen & Sejrsen, 1977). Thus, in addition to increasing the arterio-venous pressure gradient, the mechanical muscle pump may also increase arterial leg blood flow through suspension of the venoarteriolar reflex, when this is activated in the upright resting position.

It has been proposed that the effect of the muscle pump on the rise in arterial blood flow during exercise may be absent in patients with incompetent venous valves (Folkow *et al.*, 1971). This is because retrograde blood flow is permitted and venous pressure is not lowered as efficiently as in healthy subjects: as a result, the gain in perfusion pressure and regional blood flow will also be lower (Folkow *et al.*, 1971).

Even though any muscle pump effect in exercise hyperaemia should be more important in an upright position, as argued above, the possibility that the muscle pump has an effect even in a situation with a smaller hydrostatic column cannot be ruled out. In resting supine humans, venous pressures of 6-16 mmHg have been reported in the GSV at the ankle (Pollack & Wood, 1949;Groothuis *et al.*, 2008), which should reflect the pressure in the deep veins (Groothuis *et al.*, 2008). Thus, it is possible that the muscle pump contributes to exercise hyperaemia also in this situation.

A further extension of the muscle pump theory is that the muscle pump can increase arterial inflow to active muscles even without the presence of a hydrostatic column. If the vessels inside the muscle are tethered to the surrounding tissue, the mechanical forces developed by the active skeletal muscle could be transmitted to the vasculature as well, creating negative venous pressure during the relaxation phase that would augment the perfusion pressure in the absence of a significant hydrostatic component (Laughlin, 1987;Sheriff *et al.*, 1993;Tschakovsky & Sheriff, 2004). Several laboratories have attempted to quantify the venous pressure required for the muscle pump alone to achieve the augmented blood flow observed during muscle work, providing estimates ranging from -30 mm Hg in dogs to -400 mm Hg in exercising

humans (Laughlin, 1987;Sheriff *et al.*, 1993;Toska & Eriksen, 1994;Naik *et al.*, 1999). It is not known whether negative pressures of this magnitude can in fact be produced.

Previous studies on the effect of the muscle pump on arterial blood flow

The following section focuses on some previous studies of the effect of muscle pumps on arterial blood flow, representing some of the important concepts regarding a muscle pump effect on arterial blood flow.

All studies examining the muscle pump effect in exercise hyperaemia have one thing in common. They examine the effect indirectly, because current methodologies do not permit direct measurement of venous pressure within muscle (Hamann *et al.*, 2003). Several different experimental models and approaches have been used, ranging from muscle preparations and in situ animal studies with contractions induced by electrical stimulation, via animals exercising voluntarily to several different human exercise models. As Don Sheriff nicely pointed out in a Point-Counterpoint discussion regarding the muscle pump effect in exercise hyperaemia, "There are nearly as many models of exercise as there are investigators examining response to muscular activity……The contribution of the muscle pump to muscle perfusion is likely to differ among all of these models" (Sheriff, 2005).

A muscle pump effect on arterial blood flow is proposed

Although Ludbrook (1962) proposed that the muscle pump might increase perfusion pressure and facilitate arterial blood flow, I would highlight the work of Folkow and colleagues (1970) as the pioneering study in this field.

They examined the effect of rhythmical muscle contractions on muscle blood flow using a feline (cat) calf muscle preparation. Venous outflow and arterial inflow was measured using electromagnetic flow probes. The muscle work was induced by electrical stimulation, and because the authors reasoned that increases in muscle perfusion pressure would influence muscle perfusion markedly only in a situation where the muscle resistance vessels were maximally dilated (in all other

circumstances, local adjustment of the resistance vessels would be the dominating factor determining muscle perfusion), the effect of rhythmical muscle contractions on muscle blood flow was examined during maximal exercise vasodilation. Venous pressure was measured to find the spacing of contractions that induced the largest reduction in venous pressure despite the large arterial inflow.

One of the main findings of this study was that contractions had an impeding effect on the arterial inflow to the muscle, and most of the venous outflow occurred during the relaxation phase. Secondly, it was found that the most effective reduction in venous pressure was obtained when contractions lasted 0.2-0.3 seconds with a relaxation phase of 0.7 seconds. With this spacing the venous pressure remained depressed between contractions despite the high arterial inflow. If the interval between contractions was longer, venous pressure increased before the next contraction. Thirdly, blood flow through the rhythmically working muscles was higher in a "leg-down" position, than if the legs were at heart level, where the net effect of rhythmical muscle work was to reduce the blood flow, relative to the flow through the maximally dilated vascular bed in the post-exercise situation. Folkow and colleagues suggested that the increased flow in the leg-down position was due to the muscle pump, which created a gain in effective perfusion pressure and a corresponding increase in muscle blood flow. This is the muscle pump effect described earlier in the introduction (see Fig. 3).

However, they argued that their use of a calf muscle preparation and the relatively abnormal type of exercise, in which all muscle groups of the calf contracted simultaneously, did not mimic natural movement. Further, they considered that it would be of interest to examine whether there is a muscle pump effect on arterial blood flow in the dependent limbs of humans, because the hydrostatic column is larger than in a short-limbed animal.

With this in mind, they investigated the effect of leg position on blood flow in the calf muscles of humans during heavy rhythmic calf exercise (Folkow *et al.*, 1971). In this study, subjects performed plantar flexions using the optimal spacing found in the

previous study. To give complete vascular relaxation the intensity of the muscle work performed was such as to rapidly produce pain. Blood flow during rest and during heavy rhythmic exercise was measured (133 Xenon clearance) in the supine and "leg-down" tilted positions (60° angle to the horizontal plane). They demonstrated that calf muscle blood flow during exercise was markedly increased when the subject was tilted from the supine position to the "leg-down" position. The authors concluded that heavy rhythmic exercise can maintain a marked reduction in mean venous pressure, through the effect of the muscle pump, provided that the venous valves are competent, and can do so despite the large inflow into the venous capacitance vessels during the relaxation phase.

Thus, as early as the beginning of the 1970s, Folkow and colleagues suggested some important factors relating to the effect of the leg muscle pump on arterial blood flow during exercise; the large hydrostatic column in dependent limbs of humans, the importance of the nature of the exercise, and the spacing of the rhythmical contractions.

Is the muscle venous pump alone responsible for the rapid rise in arterial blood flow at the onset of exercise?

Sheriff (1993) rekindled the muscle pump debate again in the 1990s by questioning whether the rise in muscle blood flow and vascular conductance at the onset of dynamic exercise is in fact due to the muscle pump. He and his colleagues considered vasodilation to be too slow to account for the rapid rise in flow at onset of exercise. They measured CO (electromagnetic blood flow measurements), arterial pressure and central venous pressure in dogs running on a treadmill. CO was kept constant by pacing with an atrioventricular block. The main findings were a sudden rise in total vascular conductance, CO and central venous pressure and a reduction in arterial pressure. The time course of these changes was the same with and without pacing of the heart. The reduction in arterial pressure was greater when CO was kept constant by pacing, but total vascular conductance and central venous pressure rose to the same extent as when CO increased normally. Sheriff and colleagues proposed that the reduction in arterial pressure was due to muscle relaxation drawing blood from arteries

into veins at the onset of exercise, and that the venous blood was pumped centrally, raising central venous pressure. Furthermore, the resulting increase in muscle blood flow, coupled with falling systemic arterial pressure and rising central venous pressure markedly increased calculated conductance, which is actually "virtual conductance" across a pump.

Sheriff and Van Bibber (1998) sought to test directly whether the mechanical forces produced during muscle contractions and relaxations could act on the muscle vasculature in a manner sufficient to initiate and sustain blood flow. To isolate the muscle pump, they made a shunt between the aorta and vena cava in anaesthetised supine pigs. They could thus study blood flow driven through the hindlimb muscles (measured by electromagnetic flowmetry) either in the normal heart-perfused manner (shunt clamped), or with the muscle pump isolated, where the only force was provided by the active muscles themselves (aorta and vena cava clamped). Muscle contractions were induced by electrical stimulation (contraction rate was 1 per second, with the contraction phase lasting 0.2 seconds) of the quadriceps bilaterally. The authors found no statistical differences between the size of the initial increase in blood flow between the heart-perfused and the shunt-perfused situation. They concluded that mechanical forces produced during rhythmic muscle contractions and relaxations act on the vasculature in a manner sufficient to generate blood flow. Further, they suggested that the muscle pump can initiate and sustain blood flow without a pressure difference across the active muscles, when the energy imparted by the heart and a significant hydrostatic component to blood pressure are both lacking.

In both of the above-mentioned studies, it was assumed that vasodilation is too slow to account for the rapid rise in blood flow at the onset of rhythmic contractions, and can therefore be eliminated as a factor contributing to the rise in flow at exercise onset. However, today there is evidence that rapid vasodilation at the onset of exercise does occur, and some of this is presented below. The mechanisms that may be responsible for rapid vasodilation are beyond the scope of this thesis, and are not discussed in detail.

Evidence of rapid vasodilation at the onset of exercise

Observations of a delay in vasodilation in response to muscle stimulation (Gorczynski *et al.*, 1978) or direct application of vasodilator agents (Wunsch *et al.*, 2000) have commonly been cited as indicating that vasodilator mechanisms are too slow to account for the immediate rise in blood flow at the onset of exercise (Sheriff *et al.*, 1993;Sheriff & Van Bibber, 1998;Clifford & Hellsten, 2004;Tschakovsky & Sheriff, 2004). However, evidence from previous studies carried out by our research group indicates that rapid vasodilation occurs at the onset of exercise (Wesche, 1986;Walløe & Wesche, 1988;Kiens *et al.*, 1989;Wesche, 1989;Eriksen *et al.*, 1990). These are some of the earlier studies in which ultrasound Doppler was used to measure arterial blood flow during muscle work. This technique allows for continuous measurement of blood flow, and has good enough time resolution to measure rapid changes in blood flow.

In addition, several other studies of the arterial blood flow response to exercise in dogs (Valic *et al.*, 2002;Valic *et al.*, 2005) and humans (Leyk *et al.*, 1994;Tschakovsky *et al.*, 1996;Shoemaker *et al.*, 1998;Tschakovsky *et al.*, 2004;Saunders & Tschakovsky, 2004) support the occurrence of rapid vasodilation at the onset of exercise.

These indications of rapid vasodilation are also supported by direct observations of blood vessels (Mihok & Murrant, 2004;Armstrong *et al.*, 2007). Furthermore, evidence has emerged supporting the idea of mechanically induced vasodilation, in which compression of the intramuscular arterioles during muscle contraction activates a mechanosensitive response by the vascular smooth muscle and /or endothelium to elicit rapid vasodilation (Clifford & Hellsten, 2004;Clifford *et al.*, 2006;Clifford, 2007;Kirby *et al.*, 2007;Clifford & Tschakovsky, 2008). Thus, there is now convincing evidence that there is rapid vasodilation at the onset of exercise. However, as emphasised by Tschakovsky and Sheriff (2004), data demonstrating rapid vasodilation does not provide an argument against the existence of a muscle pump effect on arterial blood flow. The muscle pump effect could be one of several mechanisms contributing to the rise in muscle blood flow during exercise, and could act in combination with the rapid vasodilator mechanisms to increase blood flow to active muscles.

Studies indicating that the muscle venous pump does not enhance arterial blood flow during exercise

The results of the animal studies presented below indicate that the muscle pump does not contribute to the exercise hyperaemia observed. The authors of the first two studies reasoned that the influence of the muscle pump on muscle perfusion could best be determined after maximal vasodilation, and therefore increased the resting blood flow artificially.

In the first study, Dobson and Gladden (2003) examined the effect of electrically induced rhythmic muscle contractions on peak muscle perfusion in canine gastrocnemius in situ (contraction rate 1 per second, with a contraction lasting 0.2 seconds). Maximal vasodilation was elicited by adenosine /sodium nitroprusside and occlusion of the popliteal artery and vein, and venous blood flow was measured using a flow-through type transit ultrasound probe. The results indicated that the overall effect of rhythmic contractions was to reduce muscle blood flow in the maximally vasodilated muscle.

Hamann and colleagues (2003) examined whether the muscle pump could increase blood flow to exercising skeletal muscle during artificially induced vasodilation elicited by infusion of adenosine. They measured hindlimb arterial blood flow in dogs running on a treadmill, using an ultrasound transit time probe. They did not observe any increase in blood flow in the vasodilated limb during treadmill exercise, which indicated to them that any change in venous pressure elicited by the muscle pump was not sufficient to elevate hindlimb blood flow while arterial inflow was high. They concluded that the hyperaemic response to exercise is primarily attributable to vasodilation in skeletal muscle vasculature.

Valic and colleagues (2005) used single contractions lasting 1 second (induced by electrical stimulation) in anaesthetised dogs to study the effect of postural changes on blood flow. Arterial inflow and venous outflow in a single hindlimb was measured with ultrasound transit time flow probes. To alter hindlimb venous pressure, the dogs were tilted head-up (hindlimb below the heart) and head-down (hindlimb above the

heart). The arterial pressure remained constant with postural changes. The venous volume expelled in the head-up position was larger than in the head-down position, but the increase in arterial flow was similar regardless of position. The authors concluded that the muscle pump is not a major contributor to the hyperaemic response to skeletal muscle contraction.

It has been argued that high arterial flow might influence the effectiveness of the muscle pump: For the muscle pump to increase the perfusion pressure, a reduction in venous pressure is necessary, and venous pressure must be kept lowered between contractions. In a situation with an artificially high arterial inflow, the veins may refill faster from the arterial side, thus shortening the period during which perfusion pressure is increased (Tschakovsky & Sheriff, 2004). It has also been argued that the instrumentation used to measure blood flow invasively may actually limit blood flow (Laughlin & Joyner, 2003;Sheriff, 2005).

Laughlin and Joyner (2003) discussed the studies of Dobson and Gladden (2003) and Hamann and colleagues (2003). Their review emphasised that the findings of these studies may be influenced by the lack of a significant hydrostatic column. The importance of a significant hydrostatic column was indicated by Folkow and co-workers in their pioneer study in the early seventies, "a muscle pump effect on muscle blood flow would be potentially important only in situations where transmural venous pressure is high, so that the possibility of a quantitatively marked reduction is present" (Folkow *et al.*, 1970). Thus the muscle pump effect on arterial blood flow may be more effective in a situation where the hydrostatic column is large, as is the case in the dependent limbs of humans in an upright position (Folkow *et al.*, 1970;Laughlin & Joyner, 2003).

Although Valic and colleagues (2005) did try to alter hindlimb venous pressure by tilting the dogs, they did not observe any muscle pump effect on arterial blood flow. The muscle work in their study was elicited by the use of electrical stimulation. It has been questioned whether the use of contractions induced by electrical stimulation is an adequate model of exercise (Lauglin, 1987). The higher blood flow observed during

natural exercise than when contractions are electrically evoked may indicate that the type of contractions produced by electrical stimulation influence the efficacy of the muscle pump (Laughlin, 1987;Tschakovsky & Sheriff, 2004;Sheriff, 2005). It is possible that the muscle pump is more effective during voluntary dynamic exercise than during stimulated contractions because the muscle fibres are activated sequentially rather than simultaneously (Folkow *et al.*, 1970;Laughlin, 1987;Laughlin & Joyner, 2003;Tschakovsky & Sheriff, 2004).

Since Folkow's study (1971), several human studies have been performed in which blood flow has been measured in naturally contracting muscles, and venous pressure has been manipulated by altering the position of the exercising limb relative to the level of the heart (Leyk *et al.*, 1994;Tschakovsky *et al.*, 1996;Shoemaker *et al.*, 1998;Shiotani *et al.*, 2002;Lutjemeier *et al.*, 2005;Walker *et al.*, 2007). Both arm and leg exercise models have been used, and in all cases, blood flow has been measured noninvasively by ultrasound Doppler, which permits continuous measurements and the detection of rapid changes in arterial blood flow. The techniques used to measure blood flow in the studies presented earlier in the introduction all have the disadvantage of being invasive (^{133}Xenon wash-out, electromagnetic flow measurements and the flow-through ultrasound transit time technique). In addition, Folkow and colleagues (1971) who used wash-out of ^{133}Xenon, frequently observed that the rate of wash-out from two consecutive injections of ^{133}Xenon in the same muscle could differ considerably during otherwise identical experimental conditions. Electromagnetic blood flow measurements were used in three of the studies described earlier in the introduction (Folkow *et al.*, 1970;Sheriff *et al.*, 1993;Sheriff & Van Bibber, 1998). With this technique, the electromagnetic transducer is wrapped around the exposed vessel, and the passage of blood induces a voltage which is proportional to flow. Drift of the zero flow voltage is a frequent problem with this method.

Reintroducing a larger hydrostatic column in human studies

Studies using upper extremity exercise models

Tschakovsky and colleagues (1996) measured brachial artery blood flow in supine subjects with the arm positioned above or below heart level. They stimulated the

muscle pump with repeated inflation/deflation (1 second inflation and 2 seconds deflation) of a forearm cuff to achieve mechanical emptying of forearm veins. They also examined brachial artery blood flow response during single handgrip contractions (8.6 Kg weights) and single cuff inflations. Their main findings were that rhythmic cuff inflations increased blood flow when the arm was below heart level, but not when it was above the heart. The immediate rise in brachial flow in response to both single contractions and single cuff inflations was greater with the arm below heart level. They concluded that a functional muscle pump does exist in the human forearm, but that rapid vasodilation also contributes to the early exercise hyperaemia.

Shoemaker and colleagues (1998) also used rhythmic handgrip exercise to study the role of the muscle pump and early vasodilation in producing an increase in arterial blood flow in healthy subjects. With the subject supine, the position of the arm was adjusted so that it was above or below heart level. Two different weights (corresponding to 4.9 and 9.7% of maximal voluntary contraction (MVC)) were raised and lowered according to two different contraction schedules (1-second contraction phase: 1-second relaxation phase, and 2-second contraction phase: 2- second relaxation phase). Blood velocity in the brachial artery was measured. In all cases, the rise in brachial blood velocity was greater when the arm was below the heart than when it was above the heart. The authors interpreted these findings as indicating a contribution by the muscle pump to the immediate flow increase following muscle contraction. Because the heavier workload resulted in larger increases in mean blood velocity as early as the first contraction compared to the lighter work load, despite similar reductions in forearm volume, they concluded that vasodilation must act together with a reduction in venous pressure to increase forearm blood flow at the onset of muscle work.

In the study by Walker and colleagues (2007), supine subjects performed rhythmic handgrip contractions (2 seconds contraction: 2 seconds relaxation, at a force corresponding to 30% of MVC). The brachial artery blood flow response was measured during steady-state exercise. Moving the arm between the above-heart and below-heart positions during contraction in the course of exercise caused sudden

changes in forearm arterial perfusion pressure (they argue that the veins were already emptied in the steady-state phase of exercise). The main finding was that the immediate changes in forearm blood flow following a change in arm position during steady-state exercise exceeded the level of change in forearm arterial perfusion pressure that is consistent with an immediate transmural pressure distension effect on resistance vessel calibre (conductance) in exercising human skeletal muscle. Forearm vascular conductance, but not forearm blood flow, was rapidly restored, within seconds of the alteration in distension. This is consistent with a rapid myogenic vasoregulatory compensation that is able to restore forearm vascular conductance but not forearm blood flow. The authors argued that these findings provide evidence of rapid myogenic regulation of vascular conductance in exercising human muscle, but with incomplete flow restoration via slower acting mechanisms. They conclude that local arterial perfusion pressure is an important determinant of steady-state blood flow in the exercising human forearm.

In this study, Walker and colleagues (2007) attributed the higher flow in the below-heart position to the fact that local arterial pressure was higher in this situation. The results indicate that there also must be a functional muscle pump that reduces forearm venous pressure sufficiently. The importance of competent venous valves and the reduction in venous pressure could be examined in patients with incompetent venous valves using a lower extremity exercise model, as was suggested by Folkow and colleagues (1971).

Studies using lower extremity exercise models

Leyk and colleagues (1994) examined early leg blood flow response to dynamic plantar flexion (0.5-0.8 second contraction, 30 cycles per minute) in an upright seated position, and a tilted seated position with the lower limbs above heart level. Exercise intensity corresponded to 5-10% and 25-30% of MVC. They measured femoral artery blood flow. During the initial phase of exercise, blood flow increased by a factor of 2.5 (5-10% of MVC) and 3.1 (25-30% of MVC) in the upright seated position. The corresponding values in the tilted seated position were 1.7 and 1.9 respectively. Although the results indicate that the muscle pump contributes to the rise in muscle

blood flow, the authors concluded that additional factors that cause rapid vasorelaxation must contribute to the initial changes in flow, because the rise was too large to be accounted for by the muscle pump alone.

Shiotani and colleagues (2002) examined the role of the muscle pump effect on arterial blood flow by analysing the response of femoral artery blood flow to bicycling exercise (at 5 W, 60 cycles per minute) in the upright and supine positions. The rise in blood flow was larger in the upright than in the supine position. They also measured pressure in a superficial vein at the ankle (without specifying which vein), and found it to be nearly zero in the supine position. In the upright position the pressure was reduced from 69 to 24 mmHg by exercise. They concluded that the muscle pump has the ability to increase femoral artery blood flow via the reduction in venous pressure.

Lutjemeier and colleagues (2005) studied femoral artery blood flow response during steady-state upright seated knee extension exercise in humans. Subjects performed knee extensions at light, moderate and high exercise intensities (corresponding to 4-5% of MVC at the lightest intensities, to 30-40% of MVC at the highest intensities, with 0.16 second contraction phase and 40 kicks per min). Assuming that the first cardiac cycles in early recovery reflect the level of vasodilation and vascular conductance during exercise, without the influence of muscle contractions, they estimated the net contribution of the muscle pump by the difference between the exercise blood flow and early recovery blood flow.

The main findings were that for the light exercise, blood flow fell from exercise to early recovery. For the moderate and higher exercise intensities, blood flow was similar to early recovery blood flow. From measurements of MAP, they observed that for the lighter and moderate levels of exercise, there was no difference between exercise and early recovery MAP. At the highest intensities MAP was higher during exercise and fell in the early recovery period.

From these results, the authors concluded that during the lightest exercise intensity, the muscle pump had a net positive effect on exercise blood flow. During moderate exercise, it had no net effect (the impedance due to contractions was offset by the

enhancement during the relaxation phase), and during the higher exercise intensities, any increase in blood flow during relaxation was insufficient to fully compensate for the contraction-induced impedance to muscle perfusion. A higher MAP was necessary to achieve the exercise blood flow observed at the higher exercise intensities.

Thus, in this study the authors emphasised that there are both negative and positive effects of dynamic muscle contractions and relaxations on muscle perfusion, and that it is necessary to consider the overall effects on exercise blood flow (Laughlin & Joyner, 2003;Lutjemeier *et al.*, 2005;Sheriff, 2010).

Is there any muscle pump effect on arterial blood flow in patients with venous insufficiency?

As previously indicated by several authors, if the muscle pump is to contribute to the rise in arterial blood flow during exercise, deep venous pressure must be reduced between contractions (Folkow *et al.*, 1970;Laughlin, 1987;Sheriff *et al.*, 1993;Sheriff & Van Bibber, 1998). Thus, the muscle pump effect on arterial blood flow should be dependent on competent venous valves (Ludbrook, 1962;Folkow *et al.*, 1971;Laughlin, 1987;Laughlin & Joyner, 2003). In their human study, Folkow and colleagues (1971) specifically emphasised the importance of the venous valves, suggesting that the less efficient lowering of venous pressure in patients with varicose veins could reduce the gain in perfusion pressure and regional blood flow, as compared with the situation in healthy subjects. The authors intended to test this hypothesis, but the experiments were not completed (personal communication). Folkows colleague Arenander (1960) examined arterial blood flow in the calf in patients with varicose veins by occlusion plethysmography in the recumbent position. However, the patients were not examined in the upright position or during dynamic exercise.

Venous valve incompetence and ambulatory venous hypertension

The pressure in the lower limb veins in the resting situation in patients with venous valve incompetence has been found to be roughly equal to the corresponding hydrostatic column (Pollack *et al.*, 1949;Sturup & Højensgard, 1950;Arnoldi, 1966),

as has also been found in healthy subjects (Pollack & Wood, 1949;Højensgard & Sturup, 1953;Arnoldi, 1965;Groothuis *et al.*, 2008). During muscle activity in the upright position, a reduction in lower limb superficial and deep venous pressure has been observed in healthy subjects (Pollack & Wood, 1949;Højensgard & Sturup, 1953;Arnoldi, 1965). However, in patients with valvular incompetence of the veins of the lower limb, the subsequent retrograde flow of blood can cause ambulatory venous hypertension (AVH), which is defined as an absence of the physiological fall in venous pressure in the veins of the lower leg and foot during activity of the calf muscle pump (Recek, 2006). Venous valve incompetence is closely linked to venous hypertension, and AVH was demonstrated by direct measurement of venous pressure several decades ago (Pollack *et al.*, 1949;Sturup & Højensgard, 1950). Retrograde venous flow was demonstrated by electromagnetic flow measurements by Bjordal (1970;1972a) about forty years ago. It has also been known for a long time that in patients with venous valve incompetence, the time needed to refill the veins after muscle activity is considerably shorter than in healthy subjects (Pollack *et al.*, 1949;Arnoldi, 1966).

The technique of measuring ambulatory venous pressures (AVP) is frequently used in diagnosing venous hypertension and assessing its severity. AVP is defined by Nicolaides and Zukowski (1986) as the venous pressure in a vein on the dorsum of the foot after 10 tiptoe movements (rising and lowering of the heel). Table 1 shows the range of AVP values in normal limbs and in limbs with venous disease as presented by Nicolaides and Zukowski (1986).

Table 1. AVP values in normal limbs and in limbs with venous disease

Type of limb	AVP (mmHg)
Normal	15-30
Varicose veins with competent perforators	25-40
Varicose veins with incompetent perforators	40-70
Deep valvular incompetence	55-80

The Value of Dynamic Venous Pressure Measurements.
(Adapted from Nicolaides & Zukowski (1986)).

In the study of Pollack and Wood (1949) the average pressure found in the GSV in healthy subjects was 86,8 mmHg in the quiet standing position, and the pressure decreased to a stable value of 22 mmHg after about 7 steps when walking on a treadmill at 1.7 mph. In patients with GSV insufficiency the average pressure found during quiet standing was 81.1 mmHg, and after treadmill walking at 1.7 mph the pressure was 43,7 mmHg (Pollack *et al.*, 1949). In the same study, the average pressure found for patients with a history of ilio-femoral trombophlebitis in addition to GSV insufficiency, was 87.7 mmHg in the quiet standing position and 76.6 after treadmill walking. These values correspond well with the AVP values from Table 1, although the pressure found in the patients with GSV insufficiency was slightly higher in the study of Pollack and colleagues (1949).

It is important to note that Stranden (1986) has recommended recording pressure in veins proximal to the ankle (Nicolaides and Zukowski (1986) measure on the dorsum of the foot), when evaluating chronic venous insufficiency in the calf. This is because pressure changes during ambulation may differ between a dorsal foot vein and calf veins, depending on the existence of a foot pump, and sufficiency of venous valves at the level of the ankle. In other words, sufficient venous valves at the ankle in

combination with the foot pump may mask proximal venous insufficiency. Pollack and colleagues (1949) did actually measure the GSV pressure proximal to the ankle.

The patients included in the second and third paper for this thesis all had competent venous valves in the deep veins of the lower limb. However, all had GSV incompetence resulting in symptomatic varicose veins. Earlier in this introduction, it is explained how a muscle pump effect is thought to increase perfusion pressure and facilitate muscle blood flow (see Fig. 3, page 20), and that this mechanism relies on a reduction in venous pressure, which in turn depends on competent venous valves. Importantly, it is the reduction in deep venous pressure that is thought to widen the arterial-venous pressure gradient and increase arterial blood flow.

How may insufficiency of superficial veins influence the muscle pump effect on arterial blood flow?

As described earlier in this thesis, the calf perforator veins provide a link between the deep and superficial veins (see Fig. 1, page 16), enabling equilibration of pressure changes between the deep and superficial venous systems (Recek, 2006).

Simultaneous measurements of the deep tibialis posterior vein and superficial GSV during muscle activity have shown that during the contraction phase, there is a larger rise in deep venous pressure than in the superficial GSV both in healthy subjects (Højensgard & Sturup, 1953;Arnoldi, 1965) and in patients with insufficiency of the GSV (in combination with incompetent ankle perforating veins) (Arnoldi, 1966). The pressure gradient is reversed in the relaxation phase, with a larger reduction in deep venous pressure than in the superficial veins (Højensgard & Sturup, 1953;Arnoldi, 1965;Arnoldi, 1966). These findings indicate that the direction of blood flow is from the deep veins to the superficial veins during contraction, and from the superficial veins through the perforator veins into the deep venous system between contractions (Recek, 2006). Arnoldi, who carried out measurements both in healthy subjects and in patients with GSV insufficiency and incompetent ankle perforating veins, found that for healthy subjects, the minimum pressure in the deep posterior tibial vein after 4 contractions was 30 mmHg, whereas the corresponding pressure in the GSV was 41

mmHg (Arnoldi, 1965). In patients with GSV insufficiency and incompetent ankle perforating veins, the corresponding values were 30 and 43 mmHg in the deep posterior tibial and the superficial GSV respectively (Arnoldi, 1966). These findings indicate that there may be a larger pressure gradient between deep and superficial veins between contractions in patients with superficial venous insufficiency. Phlebography of the normal lower leg during muscular contraction and relaxation (Almen & Nylander, 1962) has shown that during contraction, the perforator veins are closed, and blood is forced proximally into the popliteal vein. However, it has been demonstrated by colour-coded ultrasound imaging that some blood tends to escape from the deep veins into the superficial veins, both in healthy subjects and in patients with superficial venous insufficiency (Sarin *et al.*, 1992). The findings of Almen and Nylander (1962) further indicate that the direction of blood flow during the relaxation phase is from superficial veins, through the perforator veins into the deep veins. This was also the conclusion drawn by Bjordal (1986), based on combined Doppler velocity and venous pressure measurements in the saphenous system in normal individuals. This means that deep veins refill from both the arterial side and the superficial veins during the relaxation periods. Both paths of deep venous refill will increase deep venous pressure.

The bidirectional flow within calf perforators has also been demonstrated by electromagnetic flow measurements in patients with primary varicose veins (Bjordal, 1970;Bjordal, 1972a;Bjordal, 1972b). However, the inward component of bidirectional flow within calf perforators prevails over the outward component, and the larger the saphenous reflux, the larger is the inward vector (Recek & Koudelka, 1979).

The observations discussed above suggest that in patients with GSV insufficiency, the deep veins are more influenced by venous refill from superficial veins between contractions than in healthy subjects. This could restrict the widening of the arterial-venous pressure gradient and subsequent increase in arterial blood flow, and thereby impair the muscle pump effect on arterial blood flow.

What is the physiological importance of the calf muscle pump on arterial blood flow?

The strongest evidence for a muscle pump effect on arterial blood flow comes from human studies where there is a substantial hydrostatic column present and the exercise performed involves voluntary contractions (Folkow *et al.*, 1971;Leyk *et al.*, 1994;Tschakovsky *et al.*, 1996;Shoemaker *et al.*, 1998;Shiotani *et al.*, 2002;Lutjemeier *et al.*, 2005). Although studies using arm exercise models are excellent as proof of principle, the largest hydrostatic column is present in the lower extremities in the upright position, as during ordinary locomotion.

We wanted to study the physiological importance of the calf muscle venous pump on arterial leg blood flow in humans. Because we considered it important to investigate this in a model with a large hydrostatic column, we used a calf exercise model involving rhythmic plantar flexions (activation of the proximal calf muscle pump, see Fig. 2C), and compared the rise in femoral artery flow (FF) on transition from rest to rhythmic exercise in the supine and 30-degree head-up tilted (HUT) positions.

The ultrasound Doppler method used to measure blood flow has the advantage of being non-invasive, and the high temporal resolution allow for detection of rapid changes in blood flow.

Bearing in mind the evidence indicating rapid vasodilation at the onset of exercise, the calf exercise model was developed to ensure that the muscle work was the same in the supine and HUT positions so that the level of exercise-induced metabolic demand for the change in perfusion should be the same in both cases. It was also important to ensure that any impeding effect of muscle contractions on arterial blood flow was equal in the two positions. The muscle pump effect (i.e. the increase in perfusion pressure and arterial blood flow) varies with the height of the hydrostatic column. The combination of the flow-hindering effect of contractions and the flow-promoting muscle pump effect is in paper I (and also in the summary of paper I, page 53) described as the net contribution of the muscle pump to exercise hyperaemia. This was also done by Lutjemeier and colleagues (2005). However, as the muscle pump effect

refers to the ability of rhythmic muscle contractions to lower venous pressure and thereby widen the arterial–venous pressure gradient across muscle, the flow-hindering effect of contractions is not described as part of the muscle pump in this thesis (see definition in the introduction, page 19).

Assuming that the response of MAP to dynamic muscle work is small and of the same magnitude in the two positions, we reasoned that if the exercise-induced rise in FF was any larger in the HUT position than in the supine position, this would indicate a contribution of the muscle venous pump to exercise hyperaemia (that is large enough to overcome the flow-hindering effect of contractions).

While most previous studies have focused on either the onset of or steady-state exercise, we intended to quantify both the onset and the steady-state flow responses to a series of rhythmic contractions in humans when venous and arterial pressure were altered by a change in body position.

In order to test for an impeding effect of the calf muscle contractions on arterial blood flow, we examined the immediate post-exercise changes in FF, reasoning that immediately post-exercise the impeding effect of muscle contractions would suddenly be eliminated, whereas the muscle pump effect (i.e. the increase in perfusion pressure and arterial blood flow elicited by the muscle pump) will subside more slowly, because it takes some time for the veins to refill (Pollack *et al.*, 1949;Arnoldi, 1965).

To further examine the role of the calf muscle pump in exercise hyperaemia, patients with incompetent venous valves were studied. The patients included for the second (pre-operative) and third (post-operative) papers for this thesis had isolated GSV insufficiency. Thus they should lack an optimally functioning muscle pump. However, they had competent venous valves in the deep veins and they did not have any large incompetent perforator veins. Thus, we would expect the muscle pump effect on arterial blood flow to be impaired, but not totally eliminated in these patients.

Aims

The aim of this work was to study the physiological importance of the calf muscle venous pump in exercise hyperaemia in humans, where a significant hydrostatic column exists. Using a specially designed calf exercise model, the FF response at the onset of and during steady-state rhythmic exercise was followed in the supine and HUT positions.

The specific aims of each paper were as follows:

Paper I) Does the calf muscle pump contribute to the exercise-induced rise in arterial blood flow during rhythmic exercise in healthy subjects?

Paper II) Is the contribution of the calf muscle pump to the exercise-induced rise in arterial blood flow impaired in patients lacking an optimally functioning muscle pump because of incompetent venous valves?

Paper III) Does the impaired contribution of the calf muscle pump to the exercise-induced rise in arterial blood flow improve after patients undergo surgical treatment for venous insufficiency?

Discussion of methods

Subjects

Healthy subjects

The first study (paper I) was based on repeated measurements on 19 healthy (mean age 23), non-smoking subjects. The study included both men and women. One subject who was originally included was later excluded from the study because of difficulties in obtaining good quality ultrasound Doppler signals.

Patients with incompetent venous valves

All patients were recruited from Akershus University Hospital, Department of Vascular and Thoracic Surgery. Prior to inclusion, standard duplex ultrasound of the lower limb arterial system was performed to confirm the absence of lower extremity arterial disease. The superficial venous system, the femoral vein and popliteal vein were examined by duplex ultrasound in the upright position. The examination was performed by one of the co-authors of papers II and III, Jarlis Wesche. Ten patients (mean age 46) with insufficiency of the GSV participated in the experiments for the second study (paper II). In the third study (paper III), 9 patients (mean age 48) who were treated for GSV insufficiency by GSV stripping were included. All the patients were non-smokers and healthy apart from their GSV insufficiency, although one patient participating in both the pre- and post-operative study was taking antihypertensive medication (acetylcholine esterase inhibitor). Both studies included men and women. One patient was excluded from the studies because of difficulties in obtaining good quality ultrasound Doppler signals.

Pre-operative data from 8 of the 10 patients in paper II was included in paper III. Given the results in paper I, we wanted to examine the muscle pump effect on arterial blood flow in patients with incompetent venous valves, and this was the background for recruiting patients for the next study. We also wished to do a post-operative study, but not all of the patients who participated in the pre-operative study also took part in the post-operative study. 2 patients were lost to follow-up. One new patient was

therefore included in the postoperative study. In paper III, the novel findings are the postoperative results. However, both pre- and post-operative results are presented to permit comparisons.

CEAP classification of the patients studied for papers II and III

The CEAP (clinical, etiological, anatomical, pathophysiological) classification for chronic venous disorders (CVD) was developed in 1994 by an international committee of the American Venous Forum. In 2004 it was revised and the changes were presented in a consensus statement (Eklöf *et al.*, 2004). The patients who participated in the second (paper II) and third (paper III) studies were classified as $C_{2\text{-}3s}$,E_p,A_s,P_r. According to the CEAP classification, this means that the following description applies to the patients in studies II and III:

The $_S$ indicates that they all had symptomatic chronic venous disorder (examples of symptoms are ache, pain, tightness, skin irritation, heaviness, and muscle cramps). A clinical classification of C_3 indicates that the patients had oedema, in addition to varicose veins (C_2) and telangiectasies and/or reticular veins (C_1). None of the patients participating in our studies showed any skin changes, skin discoloration (C_4) or venous ulcers (C_5). They all had a primary etiology (E_p) with no history of deep vein thrombosis. They were anatomically classified as A_s, where s indicates that the superficial venous system was affected. The pathophysiological classification P_r indicates venous reflux. Reflux lasting up to 0.5 seconds is considered normal, because a short period of retrograde flow is needed for the venous valves to close (Meissner, 2005). The patients participating in the second and third study (papers II and III) had venous reflux duration of at least 1 second, but not more than 2 seconds.

Operation procedure

The patients participating in the post-operative study (paper III) were operated under total intravenous anaesthesia (TIVA) by division of all tributaries of the SPJ, division of the GSV and flush suture ligation to the femoral vein. Subsequent stripping of the GSV was performed to approximately 10 cm below the knee. Patients also underwent stab phlebectomy of tributaries of the GSV.

Experimental methods

Calf exercise model

The rhythmic exercise performed in the three studies involved lifting a weight by pressing a pedal connected to the end of a tilting bed (Fig. 4). Subjects performed active plantar flexions with passive transport back to the starting position. The pedals were constructed with heel supports adjustable in two dimensions to make sure that the rotational axis of the pedal movement was exactly the same as the rotational axis of the ankle in all subjects, thus facilitating contraction of the calf muscles only (Fig. 4). The tilting bed and pedal system are described in detail in paper I (page 830-831). The same muscle work was performed in the supine and 30 degree HUT position (0.5 G). The experimental protocol was the same in the three studies, and is described in detail in the papers for this thesis (Fig. 2, page 831 in paper I, and Fig. 1 in papers II and III, page 327 and 698 respectively). Importantly, the bed was tilted with the subject at rest, and the subjects rested for 2 min before the start of each bout of muscle work. By this time, any of the measured variables that were influenced by the change in body position should have adjusted and stabilized (Sheriff *et al.*, 2007), making it possible to study the effect of muscle work alone. The changes upon tilting were not discussed in the papers for this thesis, and will not be discussed any further here.

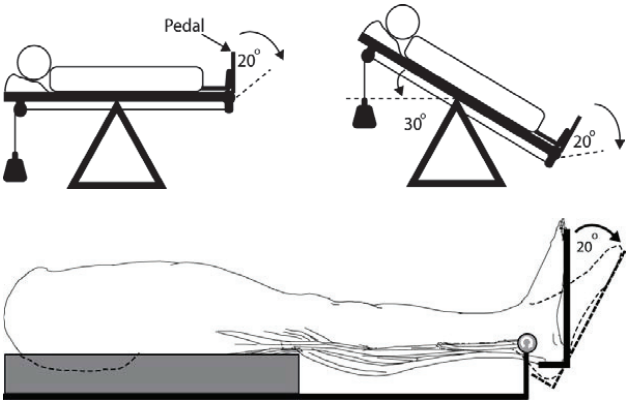

Figure 4. The tilting bed in the supine and HUT positions, and construction of the pedals to provide the correct rotational axis of movement. The weights are attached under the bed.

Isolation of the proximal calf muscle pump

As described earlier in this thesis, the proximal calf muscle pump is activated upon plantar flexion, which empties the proximal calf pump (both the muscle veins and the adjacent deep veins) into the popliteal and femoral vein. The exercise performed in our studies did not involve activation of the distal calf muscle pump, which is activated by dorsiflexion of the ankle. Whether the foot pump was activated during this exercise is uncertain. However, the foot pump is activated by weight-bearing (Gardner & Fox, 2001), so that the involvement of the foot pump in our exercise model is probably small, since the participants did not support their own body weight during the experiments. This was accomplished using a seat and a vacuum mattress preformed to the body, which ensured that any muscular activity other than in the calf muscles was minimised. For the foot pump to have been activated, the participants would have had to support their own body weight in the HUT position. This in turn would have added muscle work in the HUT position. In order to study the effect of the mechanical muscle pump on the exercise-induced rise in arterial blood flow, it was important to ensure that the muscle work and thus the level of exercise-induced metabolic demand for the change in perfusion was the same in the supine and HUT positions.

The terms “calf muscle pump” and the “proximal calf muscle pump” will be used synonymously throughout the rest of the thesis.

The calf contractions corresponded to 30% of the participants’ individual MVC

Each participant performed rhythmic plantar flexions corresponding to 30% of their individual MVC. The average weight lifted was 6 (range 3.5-8) kilograms for the healthy subjects, 4 (range 3-6) kilograms for the patients in paper II, and 4.4 (range 3-6) kilograms for the patients in paper III. At this exercise intensity, the subjects were able to perform two minutes of exercise without any exhaustion or use of accessory muscles in addition to the calf muscles. Furthermore, this exercise intensity did not lead to any large exercise-induced changes in MAP. This was important, because large changes in MAP would in itself influenced the exercise-induced rise in FF. This in turn, would complicate the ability to study the effect of the calf muscle pump on arterial blood flow.

Ideally, venous pressure should be measured within the muscles, but current methodologies do not permit direct measurements of pressure in the small veins and venules within the muscles. Additionally, such measurements would probably interfere with the actions of a muscle pump. Therefore we do not know exactly how venous pressure changed during the muscle work performed in the three studies. Arnoldi (1965) observed that it took four forceful muscle contractions to reduce venous pressure maximally in the deep tibialis posterior vein and the superficial GSV, while in the study of Pollack and Wood (1949), it took about seven contractions to reduce venous pressure maximally in the GSV during treadmill walking at 1.7 mph. Additional increases in speed or incline did not reduce venous pressure any further. Consequently, it seems, at least during leg muscle work, that several contractions are necessary to reduce venous pressure maximally, depending on the intensity of the exercise. Thus, the venous pressure in deep and superficial veins should be reduced during the rhythmic calf exercise performed by the healthy subjects (paper I). However, in the patients with GSV insufficiency, we expect less efficient emptying of the GSV, as was shown by Pollack and colleagues (1949), where the pressure reduction in GSV was 37 mmHg, compared to 64 mmHg in healthy subjects (Pollack & Wood, 1949).

The optimal spacing of rhythmical contractions may depend on contraction force

Folkow and colleagues (1970) observed that the most effective reduction in venous pressure in a feline calf muscle preparation was obtained when contractions lasted 0.2-0.3 seconds with a relaxation phase of 0.7 seconds. With this spacing, the venous pressure was kept lowered between contractions despite the high arterial inflow. Using this spacing, they demonstrated a muscle pump effect on arterial blood flow in humans during heavy rhythmic exercise, despite the large inflow into the venous capacitance vessels during the relaxation phase. Thus, their study indicates that the spacing of contractions may need to be adjusted to the level of arterial inflow; high arterial inflow would require a shorter time between contractions to avoid an increase in venous pressure.

The spacing of contractions in our three studies (1 second contraction and 1 second relaxation) was not identical to that recommended by Folkow and colleagues (1970). Both the contraction phase and relaxation phase lasted longer. A 50% duty cycle (contraction-phase) could lead to impedance of arterial blood flow for 50% of the contraction-relaxation cycle. Thus, the duration of the contraction phase could probably have been reduced. A relaxation phase of 1 second is closer to the recommended 0.7 second relaxation phase. We did not measure venous pressure, and therefore do not know how the arterial inflow at the 30% of MVC calf exercise performed in our three studies would influence venous pressure. However, using contraction and relaxation phases lasting 1s, we were able to provide evidence for a muscle pump effect in exercise hyperaemia.

Heart rate and beat-by-beat recording method

In all experiments, the RR interval from a three-lead electrocardiogram (SD100, GE Vingmed, Horten, Norway) was used to calculate HR. A recording program (program for real-time data acquisition, (version REGIST3), Morten Eriksen, Oslo, Norway) detects the R waves, and the time interval between them is measured and used to calculate the HR. The R detection of the program is very robust (Toska, 1995), which is important, as many of the other variables are processed in relation to HR (see Fig. 5).

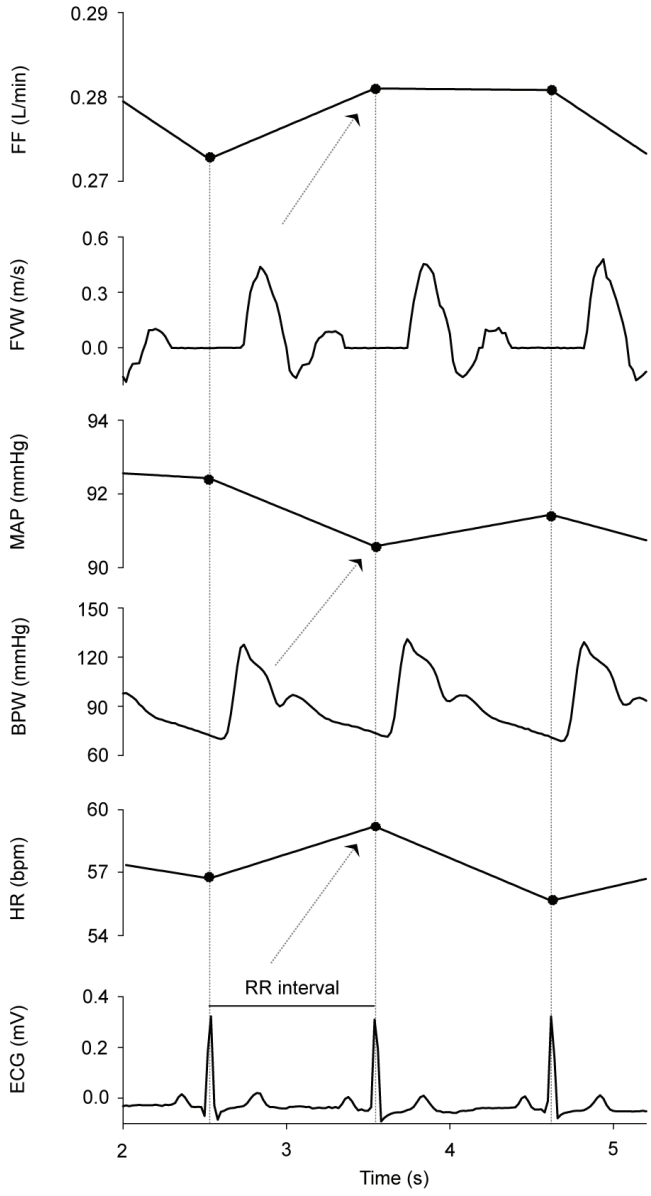

Figure 5. REGIST was used to record all data from the experiments. The RR interval from ECG was used to calculate HR, the blood pressure wave (BPW) to calculate MAP, and the femoral velocity wave (FVW) to calculate FF, as described in the methods section.

Femoral artery blood flow by ultrasound Doppler

In all papers, FF was continuously recorded by ultrasound Doppler (Wesche, 1989). SD 100 (GE Vingmed, Horten, Norway) was in the pulsed mode with an operating frequency of 3 MHz. The ultrasound transducer was handheld over the common femoral artery, and aimed at an angle of 45 degrees between the sound beam and the direction of the blood stream in the femoral artery. Because this method uses the spatial average velocity, care was taken to ensure that the ultrasound beam covered the vessel cross section, and to avoid signals from the femoral vein. The cross-sectional instantaneous intensity-weighted mean velocity signal was calculated by the velocimeter and on-line interfaced to a recording computer where the velocity integral was calculated over each RR interval. Importantly, the calculation of the cross-sectional instantaneous intensity-weighted mean velocity signal takes into account the existence of several velocities present simultaneously across a vessel, and the number of red blood cells that travel at the different velocities. The calculated velocity integral was angle-corrected, and assuming that the femoral artery was circular, it was multiplied by the vessel cross sectional area of the femoral artery to give the beat-to-beat femoral artery 'volume'. Beat-to-beat FF was calculated from the corresponding HR and femoral 'beat volume' values.

The diameter of the femoral artery was measured (CFM 750 and Vivid 7, GE Vingmed, Horten, Normay) in the supine position, assuming that the diameter would not change in the HUT position. A few pilot experiments were performed to examine whether any changes in femoral artery diameter in fact occurred when changing from the supine to the 30-degree HUT position. However, as our assumption was confirmed by Groothuis and colleagues (2007), who found little or no change in the femoral artery diameter in the transition from supine to 70 º HUT, we did not continue these experiments.

Furthermore, the diameter measurements were made in the resting situation, on the assumption that there are no changes in femoral artery diameter between rest and exercise. We did not measure femoral artery diameter during exercise. However, the diameter of the femoral artery does not change between rest to exercise, and the

diameters do not differ between different exercise intensities (Lutjemeier *et al.*, 2005). We therefore believe that the diameter in the resting situation also reflects the diameter during exercise in our studies.

Continuous measurements of finger arterial pressure

Finger arterial blood pressure was measured continuously and non-invasively by Finapres (2300 BP monitor, Ohmeda, Madison, WI USA) (Imholz *et al.*, 1998;Bogert & van Lieshout, 2005). A small finger cuff was applied on the middle phalanx of the third finger of the left hand. This is a photoplethysmographic method based on a volume clamp technique where blood volume in the finger is kept constant throughout the cardiac cycle, and the pressure in the cuff is converted into finger arterial blood pressure. This non-invasive technique measures MAP as reliably as intra-arterial devices (Imholz *et al.*, 1998). Because the peripheral blood pressure measurement is sensitive to changes in hydrostatic pressure, care was taken to ensure that the hand was at heart level in both the supine and the HUT positions. In all papers, MAP was calculated by integration of the blood pressure wave and divided by the RR interval.

In paper II and III, the calibration signals from the Finapres were recognised and filtered by a program in MATLAB. Interpolation was made between the last and first successful measurement. Each filtration was visually checked. In paper I each filtration was tracked manually, and interpolation was made between the last and first successful measurement.

Mathematical calculations and data analysis

Calculation of derived variables

Femoral peripheral conductance (FPC) was calculated by dividing FF by MAP in all three studies. In this calculation we assume that the venous pressure is zero. However, venous pressure was not measured, and as discussed in paper I (page 837), the exact calculation of peripheral conductance during exercise is very complicated, because the venous pressure is changing and unknown (Laughlin, 1987;Sheriff *et al.*, 1993;Rowell, 2004).

Calculation of femoral artery flow responses to rhythmic plantar flexions

In order to determine the muscle pump effect on arterial blood flow, the transient and steady-state FF responses to calf exercise performed in the supine and HUT positions were studied. With the intention of testing for an impeding effect of calf contractions on arterial blood flow, we also examined the immediate post-exercise response in FF in paper I. The papers for this thesis describe in detail how the FF responses and the corresponding changes in HR, MAP and FPC were calculated (Fig. 3, page 832 in paper 1, Fig. 2 in papers II and III, page 328 and 699 respectively). In paper I, the transient response to exercise performed in the HUT position was calculated using a slightly different time interval from that used to calculate the transient response in FF in the supine position. This was done to ensure that the calculated value included peak FF at the onset of exercise for all subjects, both in the supine and in the HUT position. However, no statistical difference was found between the supine and HUT positions in the time it took to reach half the peak FF. To be sure that the difference in timing did not contribute to the findings in paper I, the data was reanalysed using the same time intervals in the supine and HUT positions. This did not change the results. For the second and third papers, the same time intervals for the calculations of the FF response were used in the supine and HUT positions.

Statistical methods

Coherent averaging and non-parametric Wilcoxon signed rank test

In order to determine the muscle pump effect on arterial blood flow, the transient and steady-state FF responses to exercise performed in the supine and HUT positions were studied. The immediate post-exercise responses in FF were examined in paper I, in order to test for an impeding effect of calf contractions on arterial blood flow. As a response to a certain input (for example exercise) can be influenced by random noise and variation in the recorded variables throughout an experimental session (Tschakovsky *et al.*, 2006), coherent averaging were used (Toska, 1995). Calculating the median responses to several identical experiments in one subject partly eliminates variations in the variables not related to exercise, and makes it easier to highlight

similar responses to the exercise stimulus. The arithmetic median of the responses in several identical experiments in one subject/patient were calculated to achieve the individual responses. Coherent averaging was also used to present averaged group responses, where the individual responses were coherently averaged.

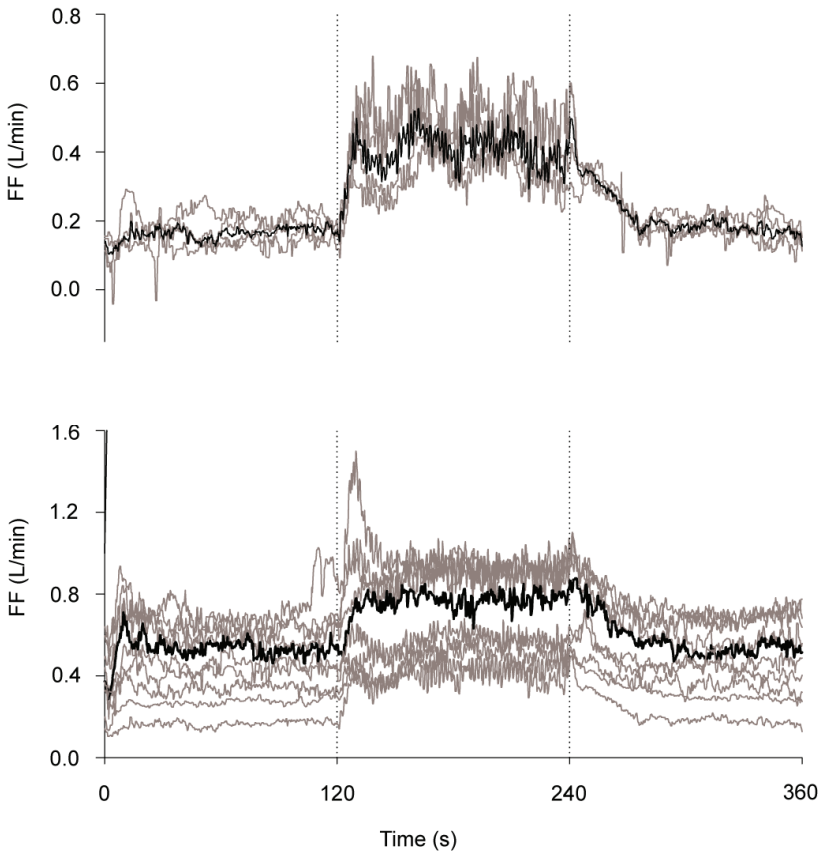

Figure 6. Above: Coherent averaging for one subject. Grey lines indicate four exercise bouts (in the supine position), while the solid black line indicates the median response for that subject. The vertical dashed lines mark the beginning and end of calf muscle work. Below: Coherent averaging for the group. Grey lines indicate individual median responses for nine different subjects, and the solid black line indicates the median for the group.

In all three papers, the statistical significance of differences between the onset and steady-state changes in FF, HR, MAP and FPC found in the supine and HUT positions was analysed by two-sided paired Wilcoxon tests, as were the immediate post-exercise responses in the supine and in the HUT positions (paper I). The significance level was set at 0.05.

Summary of papers

Paper I: Effect of the leg muscle pump on the rise in muscle perfusion during muscle work in humans

In the first study, the effect of the calf muscle pump on the transient and steady-state rise in FF during rhythmic plantar flexions was studied. Because a larger hydrostatic column results in a higher arterial and venous pressure, which should allow the muscle pump to elicit a larger rise in perfusion pressure, the FF response to calf exercise was compared in the supine and HUT positions. Because muscle contraction in itself may impede arterial blood flow, we hypothesised that if the rise in FF from rest to exercise is larger in the HUT position than the rise in the supine position, this would indicate that the calf muscle pump makes a positive net contribution to exercise hyperaemia. At the onset of muscle work, FF rose 0.37 L/min above rest in the supine and 0.5 L/min above rest in the HUT position. Thus, the rise in the HUT position was 35% larger, and the difference was statistically significant ($P < 0.05$). During steady-state exercise, FF was stable at 0.38 and 0.39 L/min above rest in the supine and HUT positions, respectively. MAP changed only by a few mmHg and was similar in the supine and HUT positions, which indicates that it has little influence on the FF responses to exercise.

These results indicate that the muscle pump increases muscle perfusion during the initial phase of muscle work, but that vasodilation is a more important determinant of muscle perfusion during steady-state muscle work.

Paper II: Does venous insufficiency impair the exercise-induced rise in arterial leg blood flow?

For paper II, the transient and steady-state effects of the calf muscle pump on the rise in FF during rhythmic plantar flexions were studied in patients with GSV insufficiency. Because they have incompetent venous valves, these patients should not have an optimally functioning muscle pump. Using the same experimental calf exercise model as in paper I, FF responses to calf exercise were compared in the

supine and HUT positions. We hypothesised that given that the calf muscle pump contributes to the rise in muscle perfusion during muscle work in healthy subjects, its effect should be absent or reduced in patients with venous insufficiency. Neither the transient nor the steady-state rise in FF showed any difference between positions (the transient rise in FF was 0.21 L/min in the supine position and 0.24 L/min in the HUT position, and the corresponding figures for the steady-state rise were 0.20 and 0.22 L/min).

These results indicate that the muscle pump effect in exercise hyperaemia is impaired in these patients.

Paper III: Does the great saphenous vein stripping improve arterial leg blood flow during exercise?

In the third study, we examined whether the contribution of the calf muscle pump to the rise in FF improves after patients undergo surgery as a treatment for their venous insufficiency. Patients were investigated both before and after SFJ ligation and GSV stripping. The transient and steady-state rise in FF in response to calf exercise were compared in the supine and HUT positions. Prior to GSV stripping, neither the transient nor the steady-state rise in FF showed any difference between the supine and HUT positions (the transient rise in FF was 0.25 L/min in the supine position and 0.25 L/min in the HUT position, and the steady-state changes were 0.20 and 0.21 L/min respectively). After GSV stripping the immediate rise in FF was 30% larger in the HUT position than in the supine position (0.26 vs. 0.20 L /min) ($P < 0.05$), but there was no difference between positions in the steady-state rise in FF (0.21 and 0.20 L/min in the supine and HUT positions respectively).

These results indicate that the muscle pump effect in exercise hyperaemia is improved in these patients after venous surgery.

General discussion

The rise in arterial blood flow to dynamically active muscles is the result of several mechanisms acting in concert. These include vasodilator mechanisms that increase conductance across the muscle vascular bed, and the mechanical muscle pump, which is thought to facilitate arterial inflow by increasing perfusion pressure. All three papers for this thesis examine the role of the calf muscle pump on the rise in FF during rhythmical calf muscle work. Below, I discuss this mechanism in healthy subjects and in patients with incompetent venous valves.

Effect of the calf muscle pump on arterial blood flow during calf exercise

Rythmical calf exercise both enhances and impedes arterial blood flow

During dynamic muscle work there is an increased metabolism, and thus an increase in arterial blood flow and oxygen delivery is required. Blood flow increases rapidly at the onset of muscle work. In fact, it has been shown to increase more than 2 times the resting flow already during the first relaxation phase during rhythmic quadriceps exercise at 10, 30 and 75 % MVC (Walløe & Wesche, 1988). The increase in arterial blood flow is partly caused by exercise-induced vasodilation. However, dynamically active muscles also have direct mechanical effects (other than the mechanical influences on skeletal muscle vascular tone), both positive and negative, on their own arterial blood supply. The negative effect is when contractions compress the vessels and impede perfusion by increasing resistance to arterial inflow. The positive effect is the muscle pump effect (see Fig. 3, page 20) where the muscle pump facilitates arterial inflow by increasing perfusion pressure across the muscle. In the first paper, the role of the calf muscle pump in exercise hyperaemia in healthy subjects was examined by comparing the exercise-induced rise in FF in the supine and HUT positions. Because the muscle work performed was the same in both positions, the impeding effect of contraction should also be the same in both positions, whereas the muscle pump effect will vary with the height of the hydrostatic column. If the rise in FF from rest to

exercise was larger in the HUT position than in the supine position, this would indicate that the muscle pump contributes to exercise hyperaemia. We observed a statistically significant larger rise in FF in the HUT position compared to the supine position at the onset of muscle work (0.50 L/min versus 0.37 L/min, Table 3, page 836 in paper I). Thus, the rise was 35% larger in FF in the HUT position than in the supine position, indicating that the calf muscle pump contributes to the transient rise in FF. No difference between positions was found for the steady-state rise in FF, which indicates that the muscle pump effect on the rise in FF is less important during the steady-state phase of muscle work. However, a calf muscle pump contribution to the rise in FF during steady-state muscle work cannot be ruled out completely, as explained in the following. An increase in metabolic demand is matched closely by an increase in muscle blood flow. The muscle pump effect is mechanical and varies with the height of the hydrostatic column. If the calf muscle pump contributes to the steady-state rise in arterial blood flow, this may reduce the extent of the metabolic vasodilation necessary to achieve the specific level of blood flow required. In other words, the same rise in blood flow is reached in the supine and HUT positions, but it seems likely that the way the flow is reached differs somewhat, and that the muscle pump is more important in the HUT position.

To examine the impeding effect of calf contractions on arterial blood flow, the immediate post-exercise changes in FF were examined. We reasoned that immediately after exercise, the impeding effect of muscle contractions would suddenly be eliminated, whereas the muscle pump effect (i.e. the increase in perfusion pressure and arterial blood flow) would subside more slowly, because it takes some time for the veins to refill (Pollack & Wood, 1949;Arnoldi, 1965). Immediately after exercise, there was a tendency for FF to rise in the supine position (Table 3 in paper I), although it was not statistically significant. There was no such trend in the HUT position. There was a statistically significant rise in FPC in the supine position (Table 3 in paper 1), but no rise in the HUT position. Thus, the impeding effect of the calf contractions on arterial blood flow is more evident in the supine position, where the muscle pump effect will be small because the hydrostatic column is smaller. Interestingly, a substantial rise in FF was seen immediately after the end of rhythmic quadriceps

exercise (25% of MVC) in healthy supine subjects (Elstad *et al.*, 2009). It is worth noting that after supine rhythmic quadriceps exercise, there was a corresponding increase in CO that may have been partly responsible for the observed rise in FF after the end of quadriceps muscle work in that study. The increase in CO after rhythmical quadriceps exercise was also observed in the study of Eriksen and colleagues (1990). During upright rhythmic quadriceps muscle work, the muscle pump effect (in this case the quadriceps muscle pump) on arterial blood flow should be larger because of the larger hydrostatic column. Lutjemeier and colleagues (2005) examined the net effect of the muscle pump on arterial blood flow during upright rhythmic quadriceps exercise in healthy subjects. At a force corresponding to 30-40% of MVC, any enhanced flow during the relaxation phase was not enough to fully compensate for the impedance to flow during the contraction phase. This meant that MAP had to increase during the exercise to maintain FF. However, at lower forces (corresponding to 4-5% of MVC), the impeding effect of contractions was less, making the effect of the muscle pump on arterial blood flow more evident.

In summary, the muscle pump effect will vary with the height of the hydrostatic column. The impeding effect of contractions will vary with the force of contractions (and may depend on the specific muscle group involved). During the 30% of MVC calf exercise performed by the healthy subjects in paper I, there was a physiologically evident effect of the muscle pump on arterial flow at the onset of exercise in the HUT position. The hydrostatic effect in the 30 degree HUT position is only 50% of the effect in the standing position ($\sin 30° = 0.5$), and thus the muscle pump effect should be even greater in the upright position.

The calf muscle pump contributes to the rise in arterial blood flow at the onset of exercise

In the introduction, a number of studies examining the part played by the mechanical muscle pump in exercise hyperaemia were presented. However, few of these have looked at both the onset and steady-state phases of exercise. We examined the effect of the calf muscle pump on arterial blood flow both at the onset of exercise and during

steady-state exercise, and below I focus on the findings from the healthy subjects in paper I.

FF clearly increases very rapidly during the first few seconds of exercise in both the supine and the HUT positions (Fig. 5, page 835 in paper I). This is expected because the exercise-induced vasodilation has been shown to occur as early as the first 1-2 seconds of exercise (Saunders & Tschakovsky, 2004). The impeding effect of the calf contractions on arterial blood flow is present from the very first contraction in both the supine and in the HUT position. The larger hydrostatic column in the HUT position allows the calf muscle pump to elicit a larger rise in perfusion pressure. However, one would expect the rise in perfusion pressure to build up as the veins are emptied during subsequent muscle contractions. It took four muscle contractions to reduce venous pressure maximally in the tibialis posterior vein and the GSV in the study of Arnoldi (1965), and about seven contractions in the study of Pollack and Wood (1949), who measured venous pressure in the GSV during treadmill walking. The FF response to exercise performed by the healthy subjects in paper I peaked after ~ 4 contraction-relaxation cycles, and during this period the rise in FF was 35% greater in the HUT position than in the supine position. Although we did not measure venous pressure, this indicates that the calf muscle pump did elicit a rise in perfusion pressure and contributed to the rise in FF at the onset of calf exercise. As was mentioned earlier in the discussion, the calf muscle pump may also contribute to the rise in FF in the HUT position during the steady-state phase of exercise, even though no difference was found in the FF response between the two positions.

In summary, the findings in paper I indicate that in situations where there is a significant hydrostatic column, the muscle pump contributes to exercise hyperaemia at the onset of exercise. However, as pointed out in the introduction, the rise in arterial blood flow to dynamically active muscles is the result of several mechanisms acting in concert. Both during the onset of exercise and during steady-state exercise, exercise-induced vasodilation contributes to the rise in FF. Vasodilation seems to be a more important determinant of steady-state blood flow than the muscle pump, although it is

possible that the muscle pump contributes to the rise in FF in the steady-state phase of muscle work as well.

Venous insufficiency impairs the effect of the calf muscle pump on arterial blood flow

By studying patients with incompetent venous valves, we were able to expand our insight into the physiological importance of a mechanical muscle pump effect on arterial blood flow during exercise further in paper II and III. We expected the muscle pump effect to be impaired in these patients, whereas any impeding effect of the calf contractions would not be affected by the incompetent venous valves. We hypothesised that, given that the calf muscle pump contributes to the rise in muscle perfusion during muscle work in healthy subjects, its effect should be absent or reduced in patients with venous insufficiency. We used the same experimental calf exercise model as for the healthy subjects in paper I, and found that neither the transient nor the steady-state rise in FF was different between positions (Table 1, page 329 in paper II, Table 2, page 701 in paper III) in the patients with venous insufficiency. During steady-state exercise, we did not observe any difference between positions in FF response in healthy subjects either. Thus, to be able to compare the muscle pump effect in healthy subjects and patients with incompetent venous valves, the onset of exercise should be examined. The lack of a difference between positions in the FF response at the onset of exercise indicates that the calf muscle pump does not contribute to the rise in FF in these patients with venous insufficiency. In other words, GSV insufficiency can impair the muscle pump effect.

We do not expect the impeding effect of contractions on arterial blood flow in the patients to be any larger than in the healthy subjects, because the patients reached their 30% of their MVC when lifting a smaller weight than the healthy subjects in paper I. Thus, we did not test whether calf exercise had an impeding effect on arterial blood flow in these patients.

Although our results indicate that the effect of the calf muscle pump effect is impaired in patients with GSV insufficiency, FF nevertheless rose in these patients during

muscle work. None of the patients had a history of peripheral arterial disease, and it seems likely that several vasodilator mechanisms contribute to the transient and steady-state rise in FF in these patients.

The effect of the calf muscle pump on arterial blood flow improves after venous stripping

The patients studied for paper III had incompetent venous valves, resulting in an impaired contribution by the calf muscle pump to the rise in FF during calf exercise. We therefore reasoned that venous stripping improves the situation. Prior to GSV stripping, the transient rise in FF was 0.25 L/min above rest in both supine and HUT positions (Table 2 in paper III). After GSV stripping, however, the transient rise in FF at the onset of exercise was 30% larger in the HUT position than in the supine position (0.26 vs. 0.20 L /min). This is close to the 35% difference found in the healthy subjects in paper I. There was no difference between positions in the steady-state rise in FF. The 30% larger transient rise in FF in the HUT position indicates that the calf muscle pump contributes to the rise in FF. Consequently, the effect of the calf muscle pump on arterial blood flow is strengthened in these patients after GSV stripping.

Limitations

There was a difference between the weight lifted by the patients (paper II and III), and the healthy subjects (paper I). Additionally, the patients were older than the healthy subjects, which could influence our findings. Both limitations are discussed below.

Each participant performed rhythmic plantar flexions corresponding to 30% of their individual MVC. This was important to avoid the use of any muscles in addition to the calf muscles, since this would have made it more difficult to study the isolated effect of the calf muscle pump on arterial flow. The patients in papers II and III reached 30% of their MVC by lifting a smaller weight than the healthy subjects in paper I (average weights lifted were 4, 4.4 and 6 Kg respectively), and this is reflected in the larger exercise-induced changes in FF in the healthy subjects than in the patients (Fig. 5c, page 835 in paper I, Fig. 3, page 329 in paper II, and Fig. 3c and g, page 700 in paper III). It is possible that the smaller weight lifted by the patients explains why no

difference was found between the supine and HUT positions in the rise in FF (prior to venous surgery). However, this is unlikely since the muscle work was the same in the supine and HUT positions for both the healthy subjects and the patients. This made it is possible to study the mechanical muscle pump effect on arterial blood flow in both groups, even though the patients reached 30% of their MVC by lifting a smaller weight than the healthy subjects. This was confirmed in paper III. The patients lifted exactly the same weight prior to and after GSV stripping. The 30% larger rise in FF in the HUT position than in the supine position after GSV stripping indicates that the effect of the calf muscle pump on arterial blood flow was in fact impaired prior to GSV stripping.

The 'Edinburgh vein study' (a study of the prevalence of varicose veins and chronic venous insufficiency in the general population) show that chronic venous insufficiency in men and women under the age of 35 years is rare (Evans *et al.*, 1999). The patients discussed in papers II and III were older (mean age 46 and 48 years respectively) than the healthy subjects in paper I (mean 23 years). It has been suggested that there is an age-related effect on vasodilator responsiveness to exercise (Carlson *et al.*, 2008), and age-matched healthy controls was not included in our studies. Carlson and colleagues observed that peak vasodilatory responses to single, 1-s dynamic forearm contractions were impaired approximately 40-45% in older healthy adults (mean 62 years) at 10, 20, and 40% of MVC, as were the total vasodilatory responses (Carlson *et al.*, 2008). However, it is unlikely that an age-related impairment in vasodilator responsiveness explains the lack of a difference in the rise in FF between the supine and HUT positions in the patients prior to venous surgery. Firstly, the patients in our studies were relatively young, compared to those studied by Carlson and colleagues. Secondly, if there is an age-related impairment in vasodilator responsiveness in our patient groups, it should be equally apparent in both the supine and the HUT positions and therefore should not obscure the mechanical effect of the calf muscle pump on arterial blood flow. This was confirmed in paper III, where the 30% larger rise in FF in the HUT position than in the supine position after GSV stripping indicates that the effect of the calf muscle pump on arterial blood flow in fact was impaired prior to GSV stripping. In addition, Saunders and Tschakovsky (2006) suggest that

vasodilatory mechanisms that contribute to the early rise in arterial blood flow at the onset of exercise are blunted with age in the forearm, but that they are preserved in the legs.

Taken together, the findings of paper II and III indicate that incompetent venous valves in the superficial venous system can influence arterial blood flow during rhythmic calf exercise. The effect of perforator and/or deep venous insufficiency would be expected to have a more marked effect. However, our study indicates that GSV insufficiency caused a physiologically evident impairment in the contribution of the muscle pump to rise in FF that was improved after venous surgery.

Clinical implications and future research

Although the effect of the muscle pump on arterial flow is impaired in patients with GSV insufficiency, several vasodilator mechanisms ensure that FF increases in response to the calf exercise performed in these patients. However, the effect of the muscle pump could be essential for sufficient arterial blood supply during muscle activity in patients with peripheral arterial occlusive disease (with or without concomitant venous insufficiency) and also in patients with small vessel disease. In these patients the mechanical muscle pump could increase perfusion pressure and facilitate increased arterial blood flow, when other mechanisms are compromised.

Intermittent pneumatic compression devices have been widely used for treatment of chronic venous insufficiency and venous ulcers (Nelson *et al.*, 2008). This therapy has also been shown to augment blood flow to the limbs in patients with peripheral arterial occlusive disease (Delis & Nicolaides, 2005;Kavros *et al.*, 2008;de *et al.*, 2010). The mechanisms responsible for the augmentation in arterial flow have not been examined in detail, but the results in the three papers for this thesis suggest that a mechanical pumping effect may be partly responsible the observed rise in arterial blood flow. This therapy could be especially useful in patients with peripheral arterial occlusive disease who are unable to walk normally because of claudication pain, or who for other reasons are unable to activate the calf muscle pump. Further research is needed to

evaluate the clinical importance of the mechanical muscle pump and intermittent pneumatic compression therapy in these patients.

Conclusions

I) The calf muscle pump contributes to the exercise-induced rise in arterial blood flow at the onset of rhythmic exercise in healthy subjects.

II) The contribution of the calf muscle pump to the exercise-induced rise in arterial blood flow is impaired in patients lacking an optimally functioning muscle pump because of incompetent venous valves.

III) The impaired contribution of the calf muscle pump to the exercise-induced rise in arterial blood flow improves after patients undergo surgical treatment for venous insufficiency.

The fact that the muscle pump effect on the rise in arterial blood flow is impaired in patients with incompetent venous valves, and that an improvement is seen after venous surgery, provides confirmation that the calf muscle pump contributes to exercise hyperaemia. This mechanism acts together with exercise-induced vasodilation to increase arterial blood flow to dynamically active muscles at the onset of exercise.

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Errata

Paper 1: Effect of the leg muscle pump on the rise in muscle perfusion during muscle work in humans

Page / paragraph / line	**Original text**	**Corrected text**
834 / 2 (right column) / 9	The transient rise in heart rate at the onset of muscle work was significantly larger ($P < 0.05$) in the horizontal than in the tilted position, whereas the steady-state responses in the horizontal and tilted positions showed no difference (Table 3)	The steady-state rise in heart rate at the onset of muscle work was significantly larger ($P < 0.05$) in the horizontal than in the tilted position, whereas the transient responses in the horizontal and tilted positions showed no difference (Table 3)
836 / Table 3 / Immediate post-exercise, tilted rise, Femoral peripheral conductance, 95% CI	(-0.0007 to – 0.0004)	(-0.000 – 0.0004)

Paper 2: Does venous insufficiency impair the exercise-induced rise in arterial leg blood flow?

Page / paragraph / line	**Original text**	**Corrected text**
330 / 2 (left column) / 9	The patients were older than the healthy subjects (mean age 46 versus 23) and lifted less weights (mean 4 versus 6 kg, range 3–6 versus 6.5–8 kg).	The patients were older than the healthy subjects (mean age 46 versus 23) and lifted less weights (mean 4 versus 6 kg, range 3–6 versus 3.5–8 kg).

Paper 3: Does the great saphenous vein stripping improve arterial leg blood flow during exercise?

Page / paragraph / line	**Original text**	**Corrected text**
701 / Table 2 / Onset responses, Difference, Femoral flow (the error occurred when the numbers were rounded to fewer decimals during the review process)	0.09* (0.0-0.18)	0.08* (0.002-0.181)
703 / reference number 20	Pollack AA, Wood EH. Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. J Appl Physiol 1949;1:649-62.	Pollack AA, Taylor BE, Myers TT,Wood EH. The effect of exercise and body position on the venous pressure at the ankle in patients having venous valvular defects. J Clin Invest 1949;28:559–63

Papers

I

II

III