POSTURAL VASOCONSTRICTION IN THE HUMAN FOOT

By

AHMAD ABUL-ANWAR KAMAL HASSAN MOHAMMAD

(M.B.B.Ch., M.Sc.)

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Department of Physiology,

Charing Cross & Westminster Medical School.

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ABSTRACT

This thesis describes some of the physiological factors influencing the postural control of foot skin blood flow in healthy subjects, using laser Doppler flowmetry. In one study, video-television microscopy was used to assess the postural changes in capillary blood flow. In 2 of the studies, foot swelling rate was also measured using strain gauge plethysmography.

During the release of sympathetic vasoconstrictor tone induced by indirect heating, postural vasoconstriction was impaired in areas with arteriovenous anastomoses (plantar surface of the big toe) but preserved in areas without anastomoses (dorsum of the foot) including maintenance of the postural fall in capillary blood flow. These experiments suggest that the postural regulation of flow takes precedence over thermoregulation in foot skin capillaries. A positive correlation was also found between the flow values measured by laser Doppler flowmetry and television microscopy. This validates the laser Doppler technique as a reliable method capable of recording reflex changes in superficial skin microcirculation.

When changes in skin temperature were locally induced, postural vasoconstriction in both skin areas was preserved at temperatures of 26-36°C, partially attenuated at 38-40°C and totally abolished at 40-44°C. The failure of vasoconstriction

at higher temperatures might explain postural intolerance and swelling of the extremities commonly observed in hot environments.

In women with normal ovulatory menstrual cycles, the postural vasoconstrictor response was partially augmented during the follicular phase associated with reduced foot swelling rate, but impaired during the luteal phase with a higher foot swelling rate. Women taking combined oral contraceptive steroids also showed impaired postural response and increased foot swelling during the latter part of the cycle. These results might explain the incidence of premenstrual oedema in some women, and suggest that female sex hormones have a strong modulating influence on peripheral blood flow and vascular tone.

Using lumbar sympathetic and local nervous blockades, the postural vasoconstrictor response in the foot has been shown to be mediated mainly by a local neurogenic mechanism supplemented in part by a local myogenic response, together with a minor contribution from a central sympathetic reflex mechanism.

These findings are discussed in relation to the role played by the postural increase in precapillary resistance in oedema prevention in health.

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INTRODUCTION

In order to gain a better understanding of the pathophysiology of peripheral vascular diseases, a proper understanding of the physiological mechanisms regulating blood flow and vascular resistance in the extremities during health is required. The investigation of these mechanisms in man, relies upon the application of physiological stimuli which are known to be associated with changes in peripheral vascular tone; for example a change in skin or body temperatures or a change in the height of an extremity in relation to the heart i.e. a change in vascular transmural pressure.

This thesis is concerned with the study of the mechanisms of the postural changes in skin blood flow of the human foot with special reference to their interaction with the vasomotor responses associated with the thermoregulatory function of the cutaneous circulation, as well as their interaction with the sex hormone induced changes in the peripheral vasculature in women. The significance of the postural vasoconstrictor response as an oedema preventing mechanism, and its pathophysiological implications will also be discussed.

In the studies to be described, the foot was chosen to examine the physiological blood flow changes in the skin

microcirculation, since not only does it represent the commonest site of clinical oedema but it is also the commonest site for clinically significant peripheral ischaemia. For example, it has been shown that an abnormal blood flow velocity in the foot could provide a sensitive index of the severity of peripheral arterial occlusive disease of the lower extremities (Shionoya et al, 1981). Furthermore, in diabetic patients with peripheral neuropathy, trophic lesions (including ischaemic ulceration and oedema) manifest themselves more frequently in the foot and toes (Watkins & Edmonds, 1983).

In the literature review to be presented, Section 1 deals with the anatomy, physiology and control of the cutaneous microcirculation with particular emphasis on the role of skin blood flow in human thermoregulation. In Section 2, the cardiovascular responses associated with changes in posture are discussed with special emphasis on the role of the peripheral postural vasoconstriction in the homeostatic control of the capillary exchange function and its relation to orthostatic oedema.

Section 1

THE CUTANEOUS CIRCULATION IN MAN

A. ANATOMICAL CONSIDERATIONS

The skin blood vessels are arranged in such a way as to facilitate heat exchange between the blood and external environment (Nelms, 1963). The cutaneous arteries arise from the subcutaneous arterial plexus, penetrate the dermis and run obliquely towards the skin surface giving off arteriolar branches to supply adnexal structures (e.g. sweat glands, sebaceous glands and hair follicles). They finally terminate forming the superficial horizontal subpapillary arteriolar plexus from which arise the capillary loops supplying the dermal papillae. These loops drain into the superficial horizontal subpapillary venous plexus (Figure 1). In the plantar skin of the human foot, there are at least 2-3 additional venous plexuses in the mid- and lower dermis (Conrad, 1971) which drain into the subcutaneous venous plexus.

In most skin areas the capillary loops lie almost perpendicular to the surface except in the nailfolds where the loops lie nearly parallel to the surface (Davis & Lawler, 1958). The arterial limb of the capillary loop is usually narrower than the venous limb (7-12 and 10-20 μm in diameter, respectively) (Fagrell, 1984).

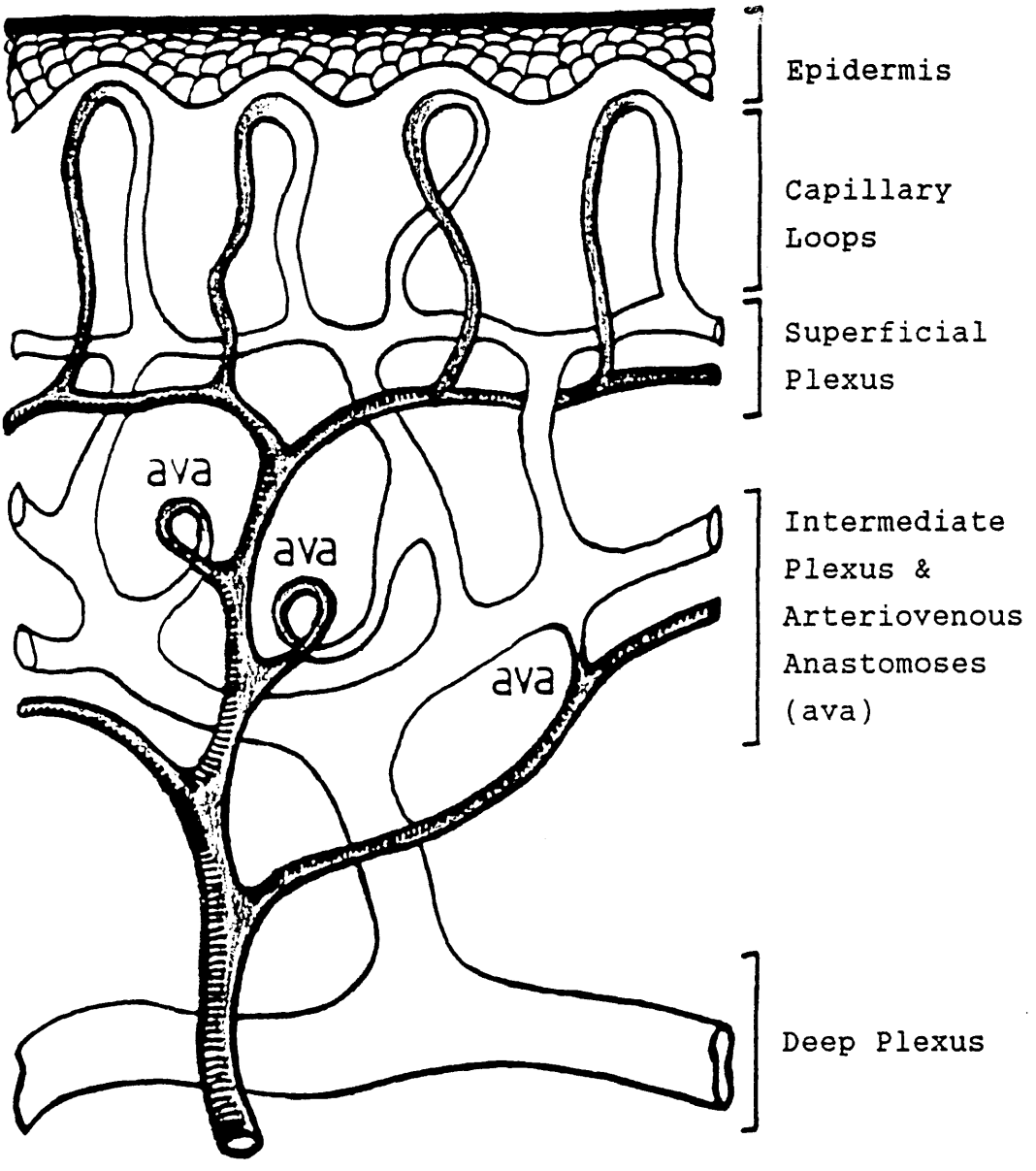


Figure 1: Schematic diagram of the blood vessels in the skin on the plantar aspect of the foot (not to scale).

The number of capillaries varies considerably in different skin areas. In the nailfolds, there are about 20 capillaries/mm^2 (Davis & Lawler, 1958). In the plantar skin, there are about 60 capillaries/mm^2, whereas on the dorsum of the foot the capillary density may gradually decrease to be less than one half that observed on the plantar surface (Conrad, 1971). In the fingers and toes, the terminal capillary loops are located in the skin papillae. One to three capillaries can be seen by direct microscopy in each papilla (Fagrell, 1984). In old age, large areas of skin have few capillaries (Ryan, 1983).

The gravitational stasis associated with the standing position in man is thought to be responsible for many changes in the blood vessels of the foot and lower leg as compared with those in the upper limb and trunk. The capillary loops are less in number and their supporting basement membrane is thicker, with a high proportion of dermal vessels showing dilatation, elongation and tortuosity. In addition, the cutaneous veins of the foot have twice as much smooth muscle in their walls compared to those in the skin of the forearm (Ryan, 1983).

Arteriovenous Anastomoses

One of the characteristic features of the cutaneous circulation in man is the presence of a large number of arteriovenous anastomoses particularly in acral skin areas

(e.g. the sole of the foot and the plantar surface of the toes, the palm of the hand and the tips of the fingers, as well as the nose, lips and ears). These shunt vessels are defined as naturally occurring precapillary vascular channels connecting the arterial and venous sides of the circulation; thus the blood passing through these channels bypasses the high resistance terminal arterioles and the capillary exchange vessels (Sherman, 1963).

Arteriovenous anastomoses arise from small arteries or arterioles usually at the level of, or slightly superficial to, the eccrine sweat glands (Conrad, 1971), at a depth of about 1 - 1.5 mm from the skin surface (Mescon et al, 1956). They are found sometimes as straight but mostly as coiled channels (Figure 1). In the pad of the toe, a more complex type of anastomoses may occur, and is known as a glomus. The digital glomus consists of an afferent artery that gives rise to smaller arterioles and continues as one to four anastomotic channels surrounded by a dense nerve plexus. These channels empty into a collecting venule which tends to arch over and encircle the glomus before joining a sub-papillary vein. The entire glomus is surrounded by a lamellated collagenous tissue (Popoff, 1934, Mescon et al, 1956).

Arteriovenous anastomoses have an average lumen diameter of 35μm, but can vary in width from 20 to 70μm depending on their state of constriction or dilatation (Grant & Bland,

1931). Each anastomosis is composed of 3 parts: an arterial segment with a smooth muscle wall and a narrow lumen, a thick-walled intermediate portion with inner longitudinal and outer circular muscle layers and a rich network of nerves, and a thin-walled funnel-shaped venous portion. In the human foot, Grant and Bland (1931) found the anastomoses to be more concentrated in the nail beds (593/cm^2), numerous in the toe pad (293/cm^2), less numerous in the sole near the heel (197/cm^2) and absent on the dorsum of the foot. Other workers, however, have reported much lower numbers of the glomus type arteriovenous anastomoses (18-25/cm^2) in the pads of the digits (Popoff, 1934; Mescon et al, 1956).

Arteriovenous anastomoses are more richly supplied with nerves than arterioles and they frequently exhibit rhythmical contractions and dilatations under comfortably warm conditions. Moreover, their response to a given stimulus is quicker and more powerful than neighbouring arterioles (Clark, 1938).

Folkow (1955) stated that cutaneous arteriovenous anastomoses are extensively supplied with sympathetic vasoconstrictor fibres and are predominantly controlled by the hypothalamic heat regulating centre. Later, it has been confirmed that arteriovenous anastomoses in the hindlimb of dogs (Spence et al, 1972) and sheep (Hales et al, 1982) receive alpha adrenergic innervation. In man, Coffman (1972) has demonstrated that arteriovenous shunt flow in the finger was

reduced far more with body cooling or intraarterial norepinephrine infusion than capillary blood flow, suggesting that the sympathetic nervous system might exert a greater influence on arteriovenous shunt flow than on nutritional flow. Other workers, however, have provided evidence for cholinergic innervation of the digital arteriovenous anastomoses. Hurley and Mescon (1956) studied skin biopsies taken from the plantar surface of the great toes of healthy male subjects and were able to localize specific cholinesterase enzyme in many nerve fibres surrounding the anastomoses. Nonspecific cholinesterase activity has also been shown to be localized to nervous structures supplying the glomus anastomoses of the human finger (Beckett et al, 1956).

Arteriovenous anastomoses are thought to be primarily involved in local and systemic temperature regulation. In the human foot, the greatest change in skin temperature with local cooling was found to occur in areas having the greatest density of shunt vessels (Grant & Bland, 1931). However, the anastomoses appear to be more sensitive to changes in central body temperature than to local temperature alterations (Zanick & Delaney, 1973). It has been argued that opening of the anastomoses could transfer large volumes of warm arterial blood directly into the capacious superficial venous plexuses, thus helping dissipation of heat (Conrad, 1971). It has been estimated that an arteriovenous anastomosis 40μm in diameter would pass 256 times as much blood as a capillary

(of the same length) 10μm in diameter (Molyneux, 1977). Hales and Iriki (1977) concluded that the specific thermoregulatory requirements of the cutaneous circulation are mainly effected by alterations in flow through arteriovenous anastomoses mediated by central mechanisms, whereas the nutritional capillary blood flow appears to be primarily under local control mechanisms. On the other hand, the close proximity of the superficial capillary loops to the skin surface and their relatively large surface area could place these vessels among the most efficient heat exchangers in peripheral tissues. However, the composite surface area of the 4 venous plexuses in the plantar skin is almost twice that of the capillaries and the total blood volume in these plexuses is about 30 times that in the capillaries. Therefore, the presence of these plexuses in the plantar skin, in addition to arteriovenous anastomoses, provides this area with a much greater capacity for varying the rate of heat exchange with the external environment than dorsal skin (Conrad, 1971).

B. CONTROL OF SKIN BLOOD FLOW

Skin blood flow under resting conditions varies in different skin areas, with higher flows being reported in the face and digits than in most other skin areas (Tur et al, 1983). In any particular skin area, however, the flow usually shows intermittent fluctuations even in the comfortable range of environmental temperature, especially in the fingers and

toes. These fluctuations in flow are thought to be produced mainly by modification of the cutaneous vascular tone in accordance with the requirements of body temperature regulation (Burton & Taylor, 1940). Using finger pulse plethysmography simultaneously with direct recording of sympathetic impulses in human skin nerves, it has been shown that these variations in flow were synchronous with the spontaneous rhythmic activity in the cutaneous sympathetic nerves (Hagbarth et al, 1972). It is, therefore, believed that the autonomic vasomotor regulation of the cutaneous circulation provides a strong support to the metabolic and sweating mechanisms involved in human thermoregulation (Benzinger, 1969).

Skin blood flow appears to be under the control of a wide variety of neurohumoral and local regulatory mechanisms:

i. Nervous Control Mechanisms

The regulation of skin blood flow is thought not to be primarily directed at matching the metabolic needs of the tissue, but rather at matching the requirements of the body for heat conservation or dissipation (Wiedeman et al, 1981). The temperature-regulating centres of the hypothalamus have been suggested to exert a direct control over resistance vessels, venous vessels and arteriovenous anastomoses via the efferent sympathetic vasoconstrictor fibres. The frequency of impulses along these fibres, therefore, determines to a

large extent the level of cutaneous vascular resistance. The low discharge rate normally prevailing in the cutaneous vasoconstrictor sympathetic fibres (Hagbarth et al, 1972) would keep skin blood flow well below its maximum, so that changes in body temperature could produce large variations in cutaneous flow by modifying the constrictor fibre activity (Mellander & Johansson, 1968).

The distribution of sympathetic fibres to the blood vessels in the extremities seems to be non-uniform in different skin areas. Woollard and Phillips (1933) have demonstrated that anaesthesia of the nerves to the upper and lower extremities resulted in higher skin temperatures in the hands and feet (particularly in the fingers and toes) than in more proximal parts of the limbs, with the toes showing greater increments in temperature than the fingers. The authors suggested, on the basis of this evidence, that under comfortable environmental conditions, the distal parts of the limbs receive more sympathetic vasoconstrictor fibres than do proximal parts, with greater sympathetic vasoconstrictor tone to the lower than the upper extremities. This conclusion has gained further support by the observation of earlier dilatation of the fingers than the toes during body heating (Pickering & Hess, 1933; Goetz, 1946). It has also been suggested that the cutaneous blood vessels in the hands and feet receive only sympathetic constrictor nerve supply, whereas the skin vessels in the more proximal parts of the limbs receive both constrictor and dilator nerve supply

(Hertzman, 1959).

In man, the reflex control of cutaneous resistance and capacitance vessels subserves the regulation of not only body temperature via thermoregulatory reflexes but also blood pressure and the distribution of blood flow and blood volume via non-thermoregulatory reflexes (Rowell, 1977):

a. Thermoregulatory Reflexes

One of the primary mechanisms by which humans adjust to a thermal stress is to alter the resting vasomotor tone of the skin blood vessels to facilitate heat exchange with the environment (Rowell, 1983). The alterations in skin blood flow of the extremities in response to changes in body temperature have been shown to depend on the integrity of sympathetic nerves (Freeman, 1935). The vasodilatation of the foot obtained during indirect heating (Gibbon & Landis, 1932) is thought to be principally, if not entirely, produced by the release of the sympathetic vasoconstrictor tone (Hertzman, 1959; Roddie, 1983). Although Lewis and Pickering (1931) suggested that active vasodilator fibres might be involved in this reflex vasodilatation, subsequent studies could not find any evidence for the existence of such dilator nerves in the human hand or foot (Warren et al, 1942; Sarnoff & Simeone, 1947; Arnott & Macfie, 1948; Gaskell, 1956; Roddie et al, 1957a & b). Furthermore, since Hurley and Mescon (1956) and Beckett et al (1956) have demonstrated the

presence of cholinesterase activity in the human digital skin, any proposed vasodilator nerve supply to the hand or the foot would probably be mainly cholinergic in nature. However, atropinization of the hand had no effect on the increase in hand blood flow produced by indirect heating (Gaskell, 1956; Roddie et al, 1957a). In contrast to the hand and foot, the increased flow in more proximal skin areas (e.g. forearm) is essentially mediated by an active vasodilator mechanism (Roddie et al, 1957a) probably involving bradykinin formed during increased sweat gland activity (Fox & Hilton, 1958). These two different mechanisms of vasodilatation in proximal and distal parts of the limbs have been confirmed by direct recording of skin sympathetic vasomotor and sudomotor activity in normal subjects exposed to high ambient temperatures (Bini et al, 1980)

Role of Central and Peripheral Thermoreceptors in Reflex Regulation of Skin Blood Flow

Gibbon and Landis (1932) found that when one arm was immersed in warm water at 44°C there was an increase in skin temperature of the fingers of the opposite hand within 15 minutes. However, when the circulation in the heated arm was arrested by a pneumatic cuff, the rise in finger temperature on the opposite side was delayed until the cuff was released. This indicates that the vasodilatation in the fingers in response to indirect heating depends on the return of warmed

blood from the heated limb to the body. Pickering (1933) suggested that the central temperature-regulating mechanism could respond to a change in temperature of less than 0.1°C.

However, the central thermoreceptors appear to be not only responsive to changes in core temperature but also to input from cutaneous thermoreceptors. Kerslake and Cooper (1950) found that application of radiant heat to the trunk or legs produced vasodilatation in the hand after a latency of 10-15 seconds. The vasodilatation was unaffected by arresting the circulation to the heated legs but was reduced or absent after lumbar sympathectomy (Cooper & Kerslake, 1953), suggesting that the reflex is mainly produced by stimulation of cutaneous thermoreceptors, and is somehow dependent on the integrity of sympathetic nerves to the heated area.

Despite the role played by peripheral thermoreceptors in temperature regulation, Rowell (1977) concluded that core temperature in man, acting mainly on hypothalamic thermoreceptors, constitutes the major drive to the cutaneous circulation unless great changes in skin temperature occur.

Other haemodynamic changes during heat stress

The reflex increase in limb blood flow in response to body heating seems to be confined to the skin (Edholm et al, 1956; Roddie et al, 1956). However, since the hands and feet and particularly the fingers and toes are composed mainly of skin

with little muscle (Greenfield, 1963), changes in the total flow to these regions are usually taken as an index of changes in skin blood flow. In contrast to the marked increase in skin blood flow during heat stress, splanchnic and renal blood flows are reduced. In addition, there is also an increase in cardiac output, heart rate and stroke volume, but a decrease in central venous pressure, central blood volume and total peripheral resistance. Diastolic blood pressure is usually reduced, systolic pressure does not show any consistent change, and although pulse pressure rises, mean arterial pressure usually falls slightly (Rowell, 1983).

b. Nonthermoregulatory Reflexes

Cutaneous sympathetic fibres not only mediate thermoregulatory reflexes but also constitute the efferent arm of nonthermoregulatory (e.g. baroreceptor) reflexes (Rowell, 1983). Although the skin blood vessels in acral areas (e.g. human hand) have been considered to be relatively unresponsive to baroreflexes (McNamara et al, 1969), the cutaneous vessels in nonacral areas (e.g. human forearm) have been shown to participate in such reflexes. Thus, application of lower body negative pressure (a manoeuvre which simulates gravitational pooling of blood in the legs) produces a strong baroreceptor-mediated vasoconstriction in the skin of the forearm (Zoller et al, 1972; Rowell et al, 1973). During body heating, despite the marked

thermoregulatory vasodilatation, the forearm skin retains its ability to vasoconstrict in response to lower body suction. However, the vasoconstriction could not completely override the heat-induced vasodilatation (Crossley et al, 1966; Johnson et al, 1973).

ii. Humoral Control Mechanisms

In addition to the above mentioned neural control mechanisms, the cutaneous vascular resistance could also be influenced by a number of circulating or locally produced vasoactive substances. For example, epinephrine, norepinephrine, angiotensin and serotonin produce cutaneous vasoconstriction, whereas acetyl choline, bradykinin, histamine, adenosine triphosphate and prostaglandin-E produce cutaneous vasodilatation (Roddie, 1983). However, the role played by humoral factors on vascular tone is thought to modify the primary influence exerted by sympathetic vasoconstrictor fibre activity (Mellander & Johansson, 1968). For example, sex hormones strongly modulate blood flow and vascular reactivity in women (Altura & Altura, 1977), as will be discussed later.

iii. Local Control Mechanisms

Since the metabolic rate of the skin is very low, the accumulation of vasodilator metabolites is thought to have little influence on skin blood flow (Wiedeman et al, 1981).

However, transient occlusion of the cutaneous circulation does result in a reactive hyperaemia which is believed to be primarily mediated by local myogenic and metabolic mechanisms (Shepherd, 1964), though the hyperaemia has been shown to be significantly reduced in conditions of peripheral vasoconstriction (Mosley, 1969; Hassan & Tooke, 1987).

Axonal reflexes might also be involved in the local regulation of skin blood flow. Stimulation of cutaneous nociceptive fibres by scratching the skin produces an arteriolar vasodilatation (flare) thought to be mediated mainly by a local somatic (antidromic) axon reflex (Lewis, 1927), whereas the vasoconstriction obtained in the hand in response to an increase in the local transmural pressure is thought to be primarily produced by a local sympathetic axon reflex (Henriksen & Sejrsen, 1976). Furthermore, the maintenance of a constant skin blood flow in the face of changes in perfusion pressure (autoregulation) has been suggested to be principally mediated by a local myogenic mechanism (Folkow, 1964; Henriksen et al, 1973).

Effect of Local Temperature on Skin Blood Flow

The effects of local temperature on the skin circulation are physiologically relevant since the skin is exposed to a greater range of temperature than any other organ in the body except, perhaps, the upper part of the alimentary tract (Greenfield, 1963). The responses of the different

components of the cutaneous vascular bed to localized temperature changes serve to assist body temperature regulation by vasoconstriction to moderate cold stimuli and vasodilatation to warm ones (Kunkel et al, 1939; Allwood & Burry, 1954; Hellon, 1963). The magnitude of the response to changes in local skin temperature is largely influenced by the general thermal state of the body. Thus, the increase in skin blood flow with local heating is augmented by body warming but partially depressed by body cooling. Conversely, the vasoconstriction produced by moderate local cooling is enhanced by body cooling but partially inhibited by body warming (Spealman, 1945). Although Abramson (1946) considered that local heating at about 40-46°C could produce maximal vasodilatation in the hand, a combination of local heating and the release of sympathetic vasoconstrictor tone by indirect heating can produce a greater vasodilatation in the hand than that produced by either procedure alone (Roddie & Shepherd, 1956).

The changes in local temperature involve not only the direct effect of heating or cooling on the vascular smooth muscle, but also effects on neurohumoral transmission and blood rheology (Svanes, 1980), as well as effects on capillary hydrostatic pressure (Levick & Michel, 1978) and capillary filtration rate (Landis & Gibbon, 1933). Most of these temperature-induced effects are thought to be mainly mediated by local mechanisms (Freeman, 1935; Doupe, 1943; Keatinge & Harman, 1980; Vanhoutte, 1980; Svanes, 1980; Vanhoutte et al,

1981).

Role of Cutaneous Veins in Human Thermoregulation

Cutaneous veins receive a rich sympathetic innervation (Hertzman, 1959), and are considered to play a significant role in the local and reflex control of the cutaneous circulation in man, particularly with respect to thermoregulation (Rowell, 1977). A reduction or an increase in central body temperature is accompanied by venoconstriction or venodilatation respectively.

Cutaneous veins are also sensitive to changes in local temperature. Moderate local cooling potentiates, whereas local heating attenuates the venomotor responses induced by reflex increase in sympathetic activity (Vanhoutte, 1980; Zitnik et al, 1971). It is thought that the changes in cutaneous venous reactivity in response to changes in local temperature can reinforce the reflexly-induced venomotor alterations in response to changes in body temperature (Rowell, 1977).

In summary, it may be concluded from the above discussion that the changes in skin blood flow in response to a particular stimulus could be determined by more than one control mechanism, with different skin areas probably having different control systems. However, the nature of the possible interactions of these mechanisms as well as their

relative contributions to the final response of skin blood flow to a certain stimulus remains to be elucidated, particularly in areas distinguished by the presence or lack of arteriovenous anastomoses.

Section 2

CARDIOVASCULAR ADJUSTMENTS TO CHANGES IN POSTURE

A change from the horizontal to the vertical position in humans, is accompanied by a number of circulatory reflex adjustments which tend to counteract the hydrostatic pooling of blood in dependent areas below the level of the heart, and hence, help to maintain arterial blood pressure (Abramson, 1946). In this section, the systemic and regional haemodynamic responses to postural change will be briefly reviewed, with particular emphasis on the mechanism and physiological significance of peripheral orthostatic vasoconstriction.

A. SYSTEMIC HAEMODYNAMIC RESPONSES TO POSTURAL CHANGE

On assumption of the upright position the stroke volume decreases, the heart rate increases but does not fully compensate for the reduction in stroke volume, and therefore, cardiac output usually falls. Systolic blood pressure, in most instances, does not show any consistent change, but diastolic pressure is increased. Because the mean arterial pressure is almost maintained, the total peripheral resistance must increase in direct proportion to the fall in cardiac output (Blomqvist & Stone, 1983). Similar responses are also obtained when the subject is passively tilted to a foot-down position on a tilt table (Tuckman & Shillingford, 1966).

The time course of the cardiovascular responses to passive upright tilting has been recently defined by Hainsworth and Al-Shamma (1988). They found that these responses almost reached a steady state within 4-6 minutes from the onset of tilt. They also found that the postural changes in heart rate, cardiac output and diastolic blood pressure declined with increasing age.

Although in the steady state systolic blood pressure remains almost unchanged, it has been shown that after a sudden change from the recumbent to the standing position, the brachial systolic blood pressure falls within the initial 10 sec. but returns to the original resting level within 30 sec. (Wald et al, 1937). A large transient increase in heart rate within the initial 12 sec. of standing has also been recently reported (Dambrink & Wieling, 1987).

The increases in heart rate, diastolic pressure and total peripheral resistance in orthostasis are thought to be largely produced by an increased sympathetic nervous activity mediated mainly via high-pressure or low-pressure baroreceptors. The high-pressure (carotid sinus and aortic arch) baroreceptor mechanism could be activated by the initial drop in systolic blood pressure (Amberson. 1943), whereas the low-pressure (cardiopulmonary) baroreceptor mechanism might be activated by the reduction in central blood volume on standing (Gauer & Thron, 1965).

Although the changes in arterial blood pressure and heart rate associated with the upright position appear to be rather well established, there have been no reports on the possible changes in these parameters when one extremity is lowered below heart level in supine subjects.

B. REGIONAL HAEMODYNAMIC RESPONSES TO POSTURAL CHANGE

On standing, the arterial and venous pressures below heart level increase in parallel; thus the arteriovenous pressure gradient remains unchanged, but the transmural pressure is increased (Rushmer, 1976). The increase in transmural pressure in the distensible resistance vessels might be expected to dilate these vessels, and hence produce a passive reduction in the resistance to blood flow. However, the rise in total peripheral resistance during orthostasis can only be ascribed to an active vasoconstriction. It appears that this compensatory vasoconstriction is widespread; involving not only the circulation to the limbs, but also the splanchnic, pulmonary and cerebral circulations (Gauer & Thron, 1965).

Changes in total limb blood flow and flow velocity with changes in body posture

It is believed that the total blood flow and the velocity of flow in the lower extremities are both reduced on acquiring the standing position. McDowall (1938) stated that the volume of blood flowing through the lower limbs may be reduced by 50 per

cent in the upright posture; this reduction is accompanied by a fall in the oxygen saturation of blood in the femoral vein. Thompson et al (1928a) found that when a dye was injected into an arm vein, it took a much longer time to appear in a foot vein when the subject was in the motionless standing position than when he was in the recumbent position, indicating that the velocity of blood flow was much reduced in the erect posture; a conclusion confirmed by Mayerson et al (1939). Using an ultrasonic Doppler flow sensor, Rushmer (1976) recorded diminished flow velocity and volume flow in the axillary and femoral arteries when the subject was tilted to 60° from the horizontal, indicating a prompt vasoconstriction in both the arm and leg.

Changes in Skin Blood Flow with Changes in Body Posture

Although the orthostatic reduction in skeletal muscle blood flow appears to be rather well established (Brigden et al, 1950, Amery et al, 1973), the postural changes in skin blood flow are less clearly defined and in many instances seem to be contradictory.

Using thermometry, Mayerson and Toth (1939) found that when the subject was tilted to 75° from the horizontal at a room temperature of 25-27°C, there was a sharp or a gradual drop in cutaneous and subcutaneous temperatures which was most marked in the lower extremities. These findings are somewhat at variance with those of Roth et al (1938) who observed mainly an

increase in the skin temperature of the toes with different degrees of postural change at a room temperature of 25°C in the majority with a decrease in toe temperature in only a minority of cases. At higher room temperatures (30 - 35.6°C), Nielsen et al (1939) found a decrease in mean skin temperature (measured at 15 different points), but an increase in only the toe temperature in subjects tilted to 45° from the horizontal. However, the relationship between skin blood flow and skin temperature is non-linear (Cooper et al, 1949), and skin temperature is not only a reflection of the local blood flow but is also thought to be influenced by flow to proximal and distal areas and by environmental temperature (Greenfield et al, 1963).

Using venous occlusion plethysmography, Beaconsfield and Ginsburg (1955a) recorded a transient reduction in hand blood flow in subjects tilted to 80° from the horizontal. Because the blood flow curves obtained using venous occlusion plethysmography are sometimes difficult to interpret (Greenfield et al, 1963) particularly when the local veins to the region are distended (e.g. during orthostasis), Scheinberg et al (1948) emptied the veins of the foot by transient application of an external pressure and found that blood flow into the emptied foot was greater in the upright than in the horizontal position. However, under these conditions, the increase in venous transmural pressure which normally occurs in the erect posture has been sacrificed.

When the calorimetric method was used, Kidd and McCready (1958) found that in the standing position the heat elimination from the fingers (kept at heart level or fully dependent) was not significantly altered from that measured in the horizontal position, whereas the dependent toes showed an increase in heat elimination. Conrad (1971) argued that the relation of heat loss to blood flow might be altered in the dependent position due to the marked change in distension and filling of the capillaries and venous plexuses in the skin.

More recently, Skagen (1983) used the rate of washout of subcutaneously injected ^{133}Xenon to measure the changes in subcutaneous blood flow during a postural stress. It was found that head-up tilting of 30-70° caused a reduction in subcutaneous blood flow in the arm kept at heart level as well as in the dependent leg.

It can be seen from the above discussion that the apparent lack of agreement, sometimes encountered, concerning the orthostatic changes in skin blood flow might be attributable, at least in part, to the different methods used for measurement of blood flow, and in some instances also to different environmental conditions. Therefore, a suitable blood flow measuring technique is required, which should be less affected by the distension of the vascular bed on standing. It is also obvious that whenever the postural changes in peripheral blood flow are to be examined, it is necessary to maintain a constant and comfortable environmental temperature.

In brief, it might be concluded that the bulk of available evidence is in favour of peripheral vasoconstriction on standing which involves not only the skin (Amberson, 1943) but also the subcutaneous and skeletal muscle tissues (Henriksen & Paaske, 1980).

Orthostatic changes in peripheral venous tone

Although peripheral veins are capable of forceful constriction when stimulated (Hertzman, 1959; Shepherd, 1963), it has been reported that the veins of the human limbs appear to be little influenced by the activity of the carotid sinus baroreceptors (Rothe, 1983; Tripathi et al, 1984). Assumption of the upright posture has been shown to be associated with increased peripheral venous tone (Page et al, 1955; Sharpey-Schafer, 1961; Newberry, 1970). However, other studies have provided evidence that the venoconstriction associated with upright tilting is largely of transient nature (Gauer & Thron, 1962) and is no longer elicited during general anaesthesia (Epstein et al, 1968), indicating that it is probably evoked by emotional influences due to the tilt procedure. Therefore, it has been suggested that humans adjust to a postural stress mainly by vasoconstriction rather than venoconstriction (Gauer & Thron, 1965).

Because the effective circulating blood volume is reduced during standing, it would seem of great importance that an active venoconstriction, by limiting the distension of the

capacitance vessels, could prevent excessive venous pooling, and hence might partially help restore the central blood volume. However, Hainsworth (1986) suggested that a lack of such venoconstriction in the limbs of upright man might be more beneficial. If the limb veins were to constrict powerfully, this would markedly increase the postcapillary resistance and would result in more fluid loss from the capillaries (Mellander & Johansson, 1968) which would eventually lead to a further reduction in the effective circulating blood volume.

Effect of Increased Transmural Pressure on Skin Blood Flow

The transmural pressure in the blood vessels of a limb can be increased by moving the limb from a horizontal to a dependent position, or by applying a venous congestion or a local negative pressure with the limb maintained horizontal.

i. Effects of Changes in Limb Posture on Skin Blood Flow

On lowering a limb below heart level, the intravascular pressure at any particular point along the limb increases by an amount equivalent to the height of a fluid column from that point to heart level. The increase in pressure is approximately equal in both arteries and veins, and although the perfusion pressure is unaltered, the transmural pressure is increased (Shepherd, 1963).

The changes in skin blood flow when the position of an

extremity alone is altered, with the rest of the body remaining in the horizontal position, have been the subject of disagreement, due probably to the different methods employed.

Wilkins et al (1950), using arteriovenous oxygen difference as a measure of blood flow, suggested that an increased flow occurs when the limb is dependent. This conclusion was based on the assumption that the oxygen saturation of arterial blood and oxygen consumption of the tissues under examination were unchanged by the change in limb posture.

Gaskell and Burton (1953), however, using venous occlusion plethysmography, have demonstrated a reduction in toe blood flow when the leg was lowered 15-30° from the horizontal, and suggested that the arteriolar constriction occurred probably as a result of distension of the walls of local veins in the dependent foot, and named the response "a veni-vasomotor reflex". Beaconsfield and Ginsburg (1955b) confirmed Gaskell and Burton's observations and found reductions in forearm, hand, calf and foot blood flows 3 minutes after the limb was lowered 45° from the horizontal.

Studies using calorimetry have shown increased heat elimination from the fingers and toes on lowering the arm or the leg in horizontal subjects (Roddie, 1956; England & Johnston, 1956), whereas authors who used ^{133}Xe clearance found that the subcutaneous blood flow in the distal part of the forearm or the leg was reduced when the area under study was lowered 30cm

below the jugular notch (Henriksen, 1977).

ii. Effect of Venous Congestion on Skin Blood Flow

When a pneumatic cuff is inflated around a limb to pressures less than diastolic blood pressure, the pressure in the veins distal to the cuff rises toward and eventually becomes approximately equal to the cuff pressure. The transmural pressure is increased most in the veins and, to a lesser extent, in all of the vessels back to the arteries, but the arteriovenous pressure difference (perfusion pressure) is reduced (Greenfield, 1964).

Friedland et al (1943), using several methods, demonstrated a reduction in blood flow when the venous pressure in the limb is raised. Their conclusion was based on the observation of reduced nailfold capillary blood flow velocity (by capillary microscopy), decreased oxygen content of blood from a forearm vein, decreased toe skin temperature, as well as a decrease in the plethysmographically recorded forearm blood flow when a cuff was inflated to 30-60 mmHg proximal to the site of measurement. Similar reductions in finger nailfold blood flow with venous occlusion have been recently demonstrated using laser Doppler flowmetry and vital capillaroscopy (Tooke et al, 1983).

However, Shanks (1955), using a calorimeter to measure heat elimination from the fingers, found no appreciable change in

the resistance to blood flow during venous congestion. On the other hand, Wallis et al (1963) demonstrated an increase in venous tone in an occluded segment of a forearm vein during a cuff inflation of 20-40 mmHg, which was abolished by local anaesthesia. Thus, it appears that elevation of local venous pressure might probably influence arteriolar as well as venous tone.

When blood flow was measured using the ^{133}Xenon clearance technique, it has been reported that an increase in venous transmural pressure by 25 mmHg or more elicits arteriolar constriction; a response which has been demonstrated in the skin and subcutaneous tissues (Henriksen & Sejrsen, 1976; Henriksen, 1977).

The Postural Changes in Peripheral Blood Flow in Women

In most of the studies describing the postural changes in peripheral blood flow, the experiments appear to be predominantly performed on male subjects. In one study in which some women were examined in addition to men, there was no mention of the menstrual cycle phase at the time of the study nor any difference in the postural vasoconstrictor response in the leg between men and women (Amery et al, 1973).

The importance of studying the postural changes in peripheral blood flow in women stems from the findings that skin blood flow exhibits large fluctuations at the different stages of the

normal menstrual cycle (Edwards & Duntley, 1949; Keates & Fitzgerald, 1969a), in addition to changes in venous distensibility (McCausland et al, 1963) and limb volume (Keates & Fitzgerald, 1969b). Moreover, female sex hormones or the use of oral contraceptive steroids have been suggested to produce profound influences on peripheral blood flow (Keates & Fitzgerald, 1975), peripheral venous distensibility (Goodrich & Wood, 1964), and vascular smooth muscle reactivity (Altura & Altura, 1977; Eccles & Leathard, 1985).

In view of these reports, it would seem likely that the reaction of the peripheral vasculature in women in response to a postural stress might differ from that in the men, or alternatively, this reaction might vary in the different phases of the menstrual cycle with the normal variations in the blood levels of the female sex hormones. This view is supported by the observations of Secher et al (1973) who, using intrauterine Xenon application, found that myometrial blood flow in women was reduced by an average of 44% on standing, but the reduction was much less (15%) in women studied during the luteal phase (secretory endometrium) than those studied during the follicular phase (proliferative endometrium) of the cycle (52%). These findings indicate that further studies are needed in order to elucidate the changes in the postural vasoconstrictor response throughout the normal menstrual cycle, and to clarify the possible role played by female sex hormones in modifying peripheral blood flow and vascular tone.

C. THE MECHANISM OF PERIPHERAL ORTHOSTATIC VASOCONSTRICTION

Central, humoral and local mechanisms have been suggested to contribute to the postural reduction in skin blood flow:

i. Central Nervous Mechanisms

Direct recording of sympathetic activity from human skin nerves showed a little change or only a transient increase in activity during upright tilting (Delius et al, 1972). This, perhaps, led to the belief that the cutaneous vasculature is little influenced by the arterial baroreceptor reflexes (Kirchheim, 1976). However, other workers have demonstrated that skin resistance vessels are involved in such reflexes (Beiser et al, 1970; Rowell et al, 1973).

It is thought that the reduced carotid sinus pressure in the erect position can cause a reflex increase in efferent sympathetic activity resulting in peripheral vasoconstriction (Amberson, 1943). It is also thought that the reduced central venous pressure can elicit a pressor reflex via the low-pressure baroreceptors (McDowall, 1924) even when the mean arterial pressure, the major determinant of the arterial (high-pressure) baroreceptor reflexes, remains unchanged (Zoller et al, 1972). Furthermore, it has been suggested, from animal studies, that a vestibular reflex via the fastigial nucleus of the cerebellum might be partly involved in the orthostatic fall in limb blood flow (Doba & Reis, 1974).

The involvement of a central sympathetic mechanism in the postural reduction in skin blood flow gains further support from the study of Beaconsfield and Ginsburg (1955a) who found that the initial fall in hand blood flow with upright tilting was absent in recently sympathectomized limbs. However, the contribution of such a mechanism to the postural fall in flow when a limb is lowered below heart level in supine subjects has not yet been fully elucidated.

ii. Humoral Mechanisms

Upright tilting has been shown to be associated with increased plasma levels of epinephrine and norepinephrine (Hickler et al, 1959), renin-angiotensin (Oparil et al, 1970), and arginine vasopressin (Davies et al, 1976) within the first few minutes after tilting. Although it might be anticipated that the increased plasma concentrations of these vasoconstrictor agents could play a role in the orthostatic vasoconstriction, their relative contribution to the postural increase in precapillary resistance is not completely understood (Mellander & Johansson, 1968). Furthermore, it is not known whether passive lowering of one extremity below heart level in supine subjects could produce significant changes in the blood levels of these agents. Also, it is not known whether humoral factors like female sex hormones, which influence vascular smooth muscle reactivity (Altura & Altura, 1977), could modify the constrictor response to postural change.

iii. Local Mechanisms

a. Local Neurogenic Mechanisms

It has been suggested that the reduction in digital blood flow on lowering the limb below heart level (Gaskell & Burton, 1953) or on application of a local subatmospheric pressure (Yamada & Burton, 1953-54) is mainly mediated by a local veno-arteriolar axon reflex elicited by venous distension. Beaconsfield and Ginsburg (1955b) found that the reduction in foot blood flow on lowering the extremity was still produced in sympathectomized limbs, indicating the presence of a local vascular response to the postural change. Henriksen (1977) provided a supportive evidence for this view and found that the reduction in subcutaneous blood flow on lowering the limb or during elevation of the local venous pressure was mainly induced by a local sympathetic axon reflex, since it was unaffected by spinal sympathetic blockade but abolished by local anaesthesia or alpha adrenergic blockade (Henriksen & Paaske, 1980).

It has been speculated that this local reflex is probably initiated by distension of the local veins, since the blood flow on lowering the leg remained constant when the increase in venous transmural pressure was prevented by activation of the "muscle vein pump" (Henriksen & Sejrsen, 1977) or by application of external counter pressure (Henriksen, 1977). However, the exact nature and the pathway of this local vascular reflex remains to be elucidated.

b. Local Myogenic Mechanisms

According to the myogenic theory, the vascular smooth muscle responds to stretching of arterial walls by contraction, thus resulting in increased arteriolar tone (Bayliss, 1902; Folkow, 1949 & 1964). Greenfield (1964) stated that, in response to a moderate rise in transmural pressure, the digital blood vessels might show an initial transient passive dilatation followed by an active constriction.

Although it has been suggested that the orthostatic increase in precapillary resistance in the feet of standing subjects might be mediated by a myogenic response (Mellander et al, 1964), it is not known to what extent this mechanism could be involved in the postural increase in precapillary resistance when a limb is lowered below heart level in horizontal subjects.

Thus, it appears from the above discussion that more than one mechanism could be involved in the postural reduction in peripheral blood flow. Indeed, it has been recently shown that both central and local mechanisms contribute to the orthostatic fall in skeletal muscle (Andersen et al, 1986) and subcutaneous (Skagen, 1983) blood flow in the human leg. However, the relative contribution of these mechanisms to the postural reduction in foot skin blood flow is still unclear.

D. THE PHYSIOLOGICAL SIGNIFICANCE OF POSTURAL VASOCONSTRICTION; AN OEDEMA PREVENTING MECHANISM

The orthostatic increase in precapillary resistance has been suggested to contribute not only to the rise in total peripheral resistance which helps maintain mean arterial pressure (Gauer & Thron, 1965) but also to the homeostatic control of capillary exchange function (Mellander et al, 1964). The precapillary vasoconstriction and the resulting fall in skeletal muscle and subcutaneous blood flow on lowering the limb below heart level, has been shown to be associated with a reduction in the expected increase in capillary filtration rate (Sejrsen et al, 1981a). Similar observations were obtained during venous stasis in the forearm and calf, and the response was absent in sympathetically denervated limbs (Henriksen et al, 1983a). These authors suggested that the precapillary vasoconstriction induced by the local sympathetic veno-arteriolar axon reflex might, therefore, act as an oedema protecting factor.

When a subject moves from the supine to the quiet standing position, about 500 ml of blood enter the legs (Gauer & Thron, 1965). The resulting increase in the volume of the lower extremities is probably due not only to increased vascular volume caused by venous distension (Abramson, 1946), but also to increased filtration of fluid from the blood stream into the tissue spaces as a result of the elevated capillary pressure (Waterfield, 1931a). The postural venous pooling and the

increased filtration are thought to contribute to the observed reduction in plasma volume (Thompson et al, 1928b; Waterfield, 1931b). However, a change from the supine to the upright position does not normally increase the volume of extracellular fluid in dependent regions enough to produce manifest oedema (Blomqvist & Stone, 1983). This is probably due to the existence of local regulatory mechanisms which tend to offset, at least partially, the effects of increased hydrostatic and transmural pressures in orthostasis (Mellander et al, 1964; Levick & Michel, 1978).

Since Starling (1896) pointed out that the net transcapillary fluid movement depends largely on the balance between the hydrostatic and osmotic pressure gradients across the capillary wall (Figure 2), the different forces involved in capillary fluid transfer constituted what has been known as the "Starling hypothesis" (Michel, 1984). Thus, a change in any of the Starling forces could result in a disturbance of the Starling equilibrium (Figure 2). Moreover, it has been suggested that the mean capillary hydrostatic pressure (Pc); the major force determining fluid filtration, depends not only on the values of mean arterial (Pa) and venous (Pv) pressures but also on the ratio of precapillary (Ra) to postcapillary (Rv) resistance (Pappenheimer & Soto-Rivera, 1948) according to the equation:

$$Pc = \frac{Pa + Pv\ (Ra/Rv)}{1 + (Ra/Rv)}$$

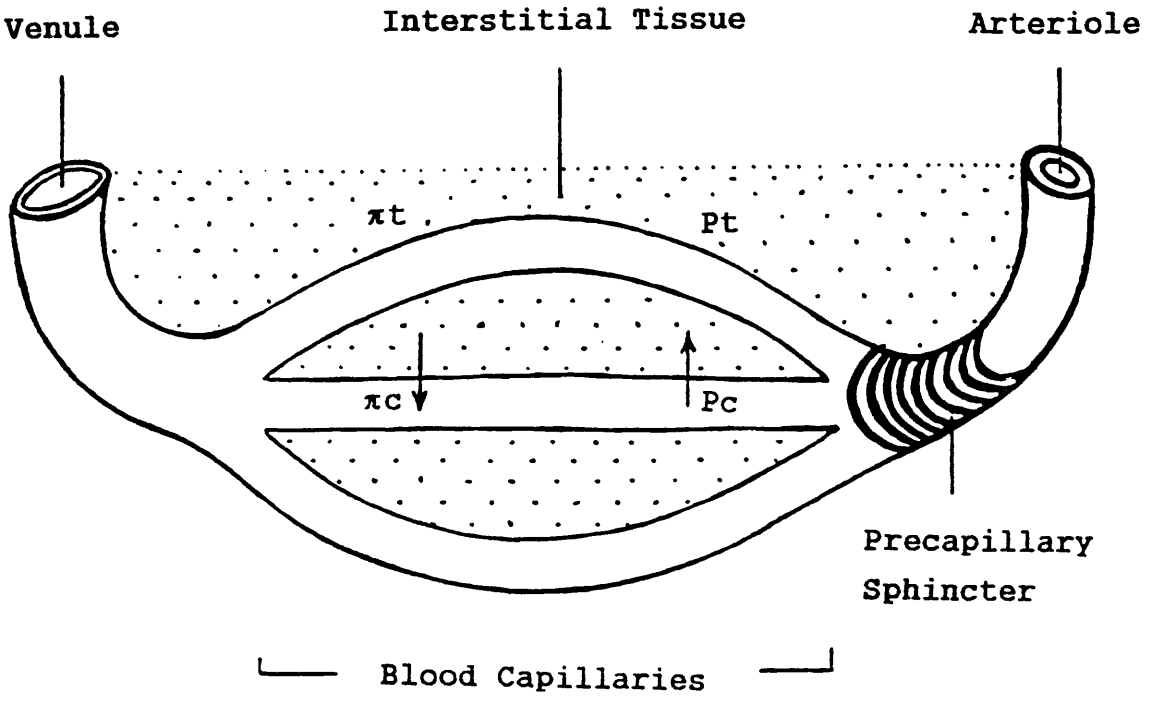


Figure 2: Diagram of a capillary bed illustrating the Starling forces involved in capillary fluid exchange. Arrows indicate the direction of fluid movement as a result of the hydrostatic or osmotic pressure gradients. The Starling hypothesis is represented by the equation:

$$Jv = Lp.\ A\ [(Pc - Pt) - \sigma\ (\pi c - \pi t)],$$ where:

Jv = Net transcapillary fluid flux
Lp = Hydraulic conductivity of the capillary wall (capillary filtration coefficient)
A = Capillary surface area
Pc = Capillary hydrostatic pressure
Pt = Interstitial fluid hydrostatic pressure
σ = Osmotic reflection coefficient of the capillary wall
πc = Capillary oncotic pressure
πt = Interstitial tissue protein oncotic pressure

The mechanism(s) by which "postural vascconstriction" might protect against excessive tissue fluid accumulation on dependency

Although the exact mechanism by which the increase in precapillary resistance limits the rise in capillary filtration is still unclear, several theories have been suggested. It has been thought that the increased activity of precapillary sphincters in response to a hydrostatic load might result not only in a reduction in capillary filtration coefficient and capillary surface area available for fluid exchange (Mellander et al, 1964; Mellander & Johansson, 1968) but also in an increase in the ratio of pre- to post-capillary resistance which could limit the rise in capillary hydrostatic pressure during orthostasis (Levick & Michel, 1978). These mechanisms, therefore, appear to maintain tissue homeostasis by reducing capillary filtration and hence preventing gross changes in interstitial fluid volume in dependent regions (Kitchin, 1963; Johnson, 1980; Aukland & Nicolaysen, 1981).

It is also possible that in the dependent limb, as filtration continues, the reduced blood flow could permit a build-up of capillary oncotic pressure (due to increased concentration of plasma proteins inside the capillaries) which would tend to oppose filtration i.e. a new steady state would probably be established, and further filtration would be reduced (Michel, 1984). This suggestion has been previously put forward by Krogh et al (1932) to explain the reduced filtration rate in

the forearms of standing subjects with venous pressure elevation. Indeed, the findings of increased plasma protein concentration and increased plasma oncotic pressure in the venous blood draining dependent regions lend much support to this view (Youmans et al, 1934; Noddeland et al, 1981; Moyses & Michel, 1984).

It should be stated that, in addition to the potential role of the postural increase in precapillary resistance, other oedema preventing mechanisms have been suggested to explain the reduction in the expected increase in filtration rate on dependency. These include the "muscle vein pump" which could reduce the rise in venous pressure in active standing (Pollack & Wood, 1948-49). Also, during quiet standing, as filtration continues in dependent parts, the rise in interstitial fluid pressure and the reduction in tissue protein oncotic pressure (by dilution of interstitial proteins) might tend to partially oppose further filtration (Aukland & Nicolaysen, 1981).

The above discussion emphasizes the need for further studies in order to elucidate the possible relationship between postural vasoconstriction and fluid filtration which might help disclose some of the underlying mechanisms of orthostatic oedema (Mellander, 1968; Kuchel et al, 1970).

Chapter II

METHODOLOGY

Chapter II

METHODOLOGY

A. SUBJECTS AND EXPERIMENTAL CONDITIONS

Experiments were performed on healthy subjects of both sexes ranging in age from 18 to 83 years. The subjects were lightly clad and were asked to wear similar clothing for repeat visits. Most subjects were recruited from academic, technical and nursing staff, and most of them were familiar with the environment and the equipment used. None of the subjects was taking any drug which might influence the measurements. After at least 10 minutes of supine rest brachial arterial blood pressure (using auscultation and sphygmomanometry) and heart rate (radial pulse) were measured for each subject as a routine.

Since changes in environmental temperature are accompanied by changes in skin temperature and skin blood flow (Winslow et al, 1937), all experiments were performed in a temperature-controlled clinical laboratory at an ambient temperature of 22 ± 0.5°C. These temperature limits were maintained by ceiling air conditioning augmented when necessary, by a special air conditioning system mounted on one of the side walls of the room. Room temperature was continuously checked by a minimum and maximum mercury thermometer (G H Zeal, London, England). The relative humidity in the room (as measured by a hygrometer) ranged

between 45 and 60%. This range is reported to have little effect on the skin temperature of the extremities if the study is conducted at a constant ambient temperature (Roth et al, 1939). No attempt was made to measure air velocity in the room. However, there were no noticeable air draughts.

Subjects were left, with the hands and feet exposed, to equilibrate in the constant-temperature room for at least 20 minutes prior to blood flow measurements. The subject was judged equilibrated when the observed skin temperature was fairly stable (variation not more than ± 0.5°C over a five minute period) as was skin blood flow. In order to examine the postural changes in foot skin blood flow, the subject lay down comfortably in the supine position on a specially constructed couch (Figure 3) so that either one or both feet could be placed at heart level (midaxillary line) or lowered passively to be positioned at a measurable distance below the heart.

Ingestion of food has been shown to influence not only arterial blood pressure, pulse rate and oxygen consumption but also the rate of peripheral blood flow especially in the hands and feet (Abramson, 1946). Smoking is also reported to cause a considerable reduction in hand and foot blood flow as measured by venous occlusion plethysmography in normal subjects (Abramson et al, 1939). Therefore, to permit valid comparisons within and between groups of subjects, each experiment was started at least 2 hours after the last

Figure 3: The experimental set-up used for measuring the postural changes in foot skin blood flow using a laser Doppler flowmeter. The fibreoptic probe carrying the laser beam fits in a light plastic probe holder which was attached to the skin by a double-stick disc. The couch was constructed with a jointed distal portion which can be screw-fixed horizontal or lowered to 53° from the horizontal. The feet were resting on pieces of soft rubber-foam extending up to the region of the hip.

consumption of food, drink or smoking.

B. LASER DOPPLER FLOWMETRY

This new technique was the principal method used in all the studies to be described for the measurement of foot skin blood flow.

Principle of Laser Doppler Flowmetry

The flowmeter contains a low power (2mW) Helium-Neon laser emitting red light at a wave length of 632.8 nm, (Figure 4a). The laser probe fits in a light plastic probe holder which was attached to the skin by a double-sided adhesive ring (Figure 4b). The laser beam is carried from the flowmeter to the skin by an efferent optical fibre. Within the skin the incident laser beam is scattered by both static (e.g. connective tissue) and moving (e.g. red blood cells) structures.

According to the Doppler effect, light striking static tissue is scattered unshifted in frequency, whereas light striking moving objects (principally red blood cells) is scattered shifted in frequency (Figure 4b); the degree of Doppler shifting being related to the red cell velocity. A portion of the shifted and unshifted backscattered light is carried by two afferent optical fibres and brought to impinge on the surface of two photodetectors (Figure 4a) where a

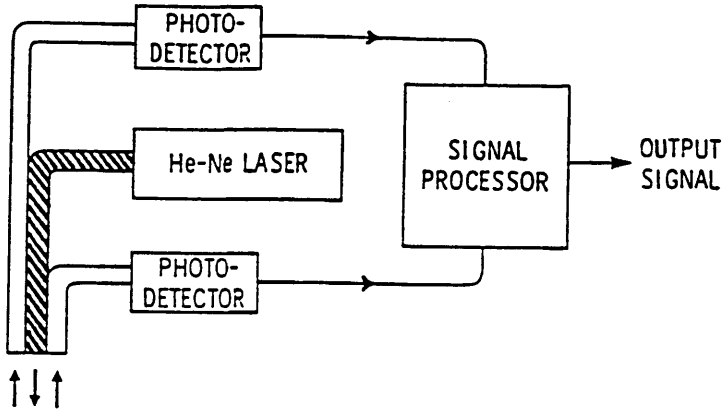


Figure 4a: Schematic diagram of the components of the laser Doppler flowmeter.

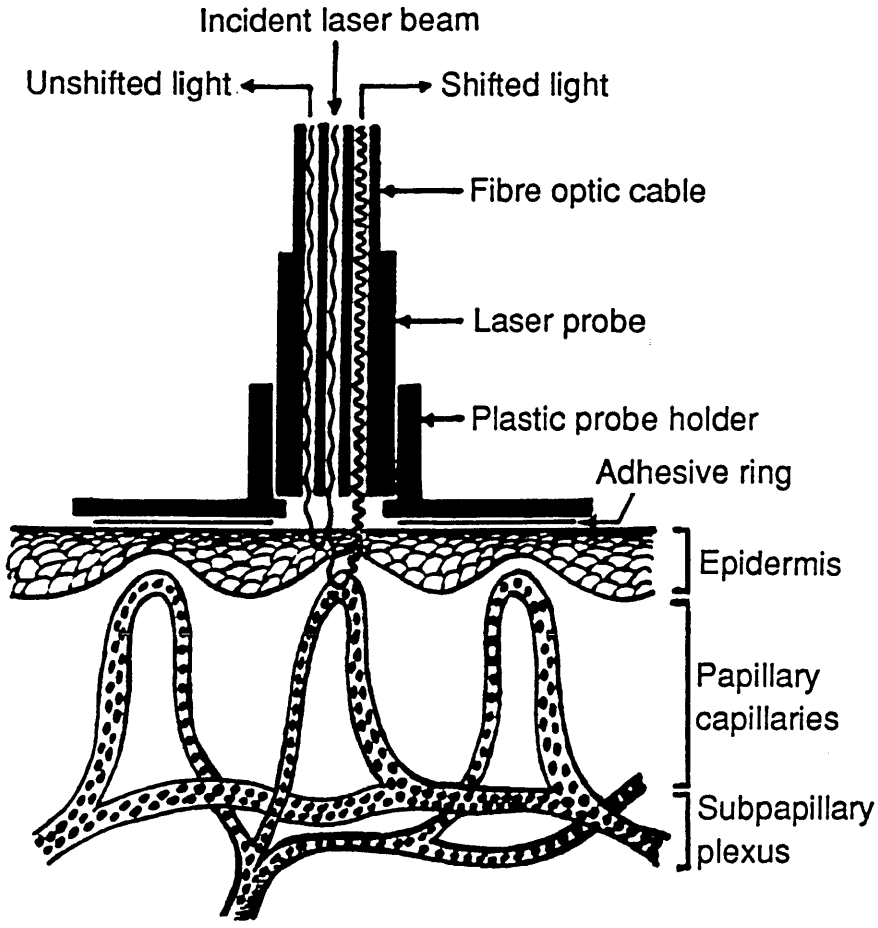


Figure 4b: The principle of laser Doppler flowmetry. The incident laser beam is backscattered partly unshifted in frequency by static tissue and partly shifted in frequency by the moving red blood cells. The laser probe and vascular bed are not to scale.

photocurrent is generated with a frequency equal to the Doppler shift. This photocurrent is then electronically processed to produce finally an output voltage signal which is related to the red cell flux (defined as the product of the number of red cells and their mean velocity in the measuring volume) (Oberg et al, 1984). The measuring volume is purported to be a hemisphere of cutaneous tissue with a radius of about 1 - 1.5 mm (Nilsson et al, 1980). However, Kolari (1985) showed that 5% of laser light could still penetrate the human skin to a depth of 1.77 mm, and 1% to a depth of 2.76 mm.

Rationale and Validation of Laser Doppler Flowmetry

Stern (1975) was the first to suggest that laser light scattering could be used for the assessment of skin microvascular blood flow in humans. In 1980, Nilsson et al evaluated the response of the laser Doppler flowmeter using a fluid model with red cell suspension. By using different concentrations and velocities of red cells, they found a linear relationship between the flowmeter response and the red cell flux. They also demonstrated the capability of the flowmeter to pick up rapid changes in skin blood flow in man in response to well known physiological stimuli. Since then, laser Doppler flowmetry has been used to assess the changes in skin blood flow in the extremities under various conditions (Svensson et al, 1983) and to evaluate skin vasomotor reflexes for the indirect assessment of peripheral

autonomic function (Low et al, 1983).

Moreover, laser Doppler flowmetry has been correlated with many of the conventional methods used to assess peripheral blood flow including ^{133}Xenon clearance (Holloway & Watkins, 1977), dynamic capillaroscopy (Tooke et al, 1983) and plethysmography and thermal clearance (Saumet et al, 1986).

Therefore, laser Doppler flowmetry was felt to be a particularly appropriate technique for examining the postural changes in skin blood flow because, in contradistinction to other blood flow measuring techniques (Greenfield et al, 1963; Challoner, 1976), the laser Doppler method is simple to use, completely non-invasive, applicable to any skin area, does not disturb the local circulation to the region under study, and gives a continuous record of flow (Oberg et al, 1984). It thus fulfills many of the criteria that make it ideally suited for clinical use, and in particular the study of skin blood flow in the limb in various postures.

Two laser Doppler flowmeters were used; Periflux MKVII, serial number 19, with a maximum output voltage of 4.77V, and Periflux PF1d, serial number 109, with a maximum output of 10V (Perimed, Stockholm, Sweden). Despite the difference in absolute voltage output, both flowmeters gave proportionately similar results when used to assess relative changes in flow from the same skin area. Therefore, for statistical reasons as well as comparative purposes, the flow values measured by

"Periflux PF1d" were normalised according to the flow values measured by Periflux MKVII.

The Recording Procedure

1. The flowmeter (placed on a vibration-free table, Figure 3) was switched on and left to warm-up for at least 15 minutes to obtain a stable baseline.
2. The probe holder (3 cm in diameter, with a central aperture 5 mm in diameter) was attached to the skin site from which blood flow was to be measured, with the laser probe fitted at a distance of 0.8 mm from the skin surface to prevent tissue compression (Figure 4b).
3. Foot skin blood flow was continuously measured (following the acclimatization period) with the subject lying supine first with both feet maintained at heart level for at laest two minutes, then with one foot placed passively 50 cm below the heart for at least 4 minutes and finally for at least 3 minutes, with the foot returned to heart level (Figures 3 and 5). It was noted that on placing the foot in the dependent position, there was a gradual reduction in skin blood flow within the first one or two minutes to reach a new steady low level by the 4th minute (Figure 5), after which there was no further appreciable reduction in the flow signal when recording continued for up to 30 minutes.
4. The flow was recorded on a 2-channel chart recorder with

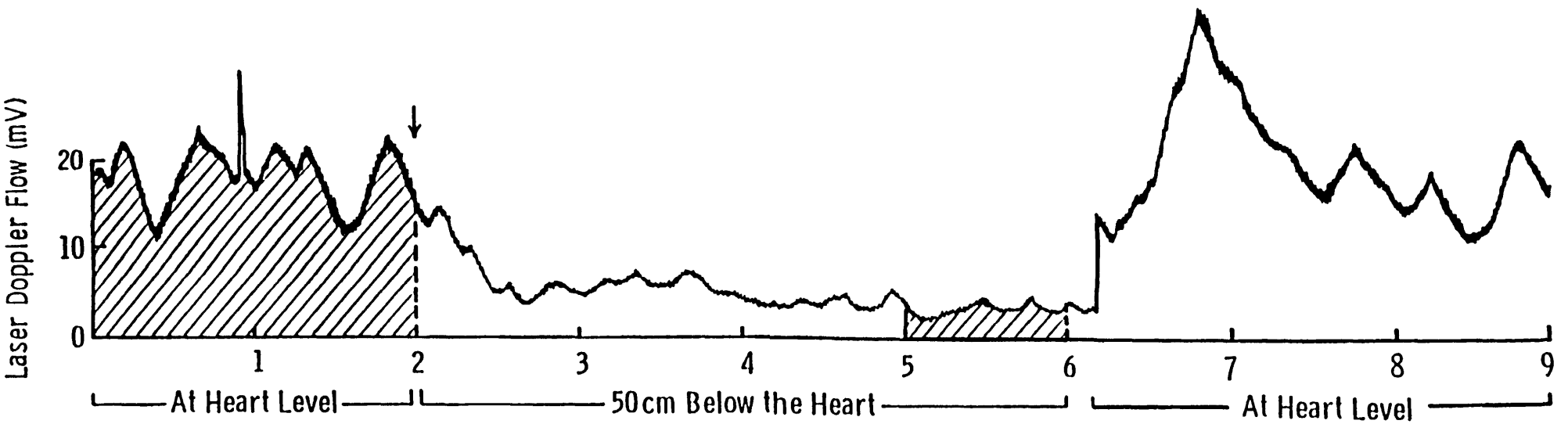


Figure 5: An example of the tracings obtained from a normal male subject aged 22 years showing the postural changes in skin blood flow measured on the dorsum of the foot using laser Doppler flowmetry. Mean flow values were calculated planimetrically during 2 minutes in the horizontal and the 4th minute in the dependent position. The arrow indicates the time when the foot was placed below heart level. The zero line represents the "biological zero" obtained when a cuff was inflated around the ankle at 250 mm Hg at the end of the experiment. The upward spike shown in the tracing obtained at heart level represents a movement artefact which was excluded from analysis.

a full scale deflection of 10V (Vitatron UK Ltd, Maidenhead). The flow showed rhythmical oscillations (vasomotion) with a frequency of 3-7 cycles/minute in the horizontal position which became less conspicuous in the dependent position. A pulsatile component equivalent to the heart rate was also apparent (Figure 5).

5. Recordings were made at a time constant of 3 seconds to avoid high-amplitude cardiac pulsations in the flow tracings, and in most studies, at a paper speed of 5 cm/minute.

6. The bandwidth filter of the instrument was selected at a frequency of 4 kHz when recording from low perfusion skin areas (e.g. dorsum of the foot) and at 12 kHz when recording from high perfusion skin areas (e.g. pulp of the big toe). If a bandwidth of 4 KHz was used when measuring from a highly perfused skin area, part of the signal would be cut out and so the voltage output would underestimate the blood flow. On the other hand, at low blood flow rates the Doppler shifts are usually less than 4 KHz, so, if a 12 KHz bandwidth was selected, the output flow signal would contain an unwanted noise generated in the photodetectors, bearing no relation to the actual blood perfusion (Rayman, 1987).

7. At the end of each experiment an arterial occlusion (a cuff inflated around the ankle at 250 mmHg) was applied (Figure 6). Under these conditions, the flowmeter often gave a voltage output (sometimes termed the biological zero) which was slightly higher than the level obtained

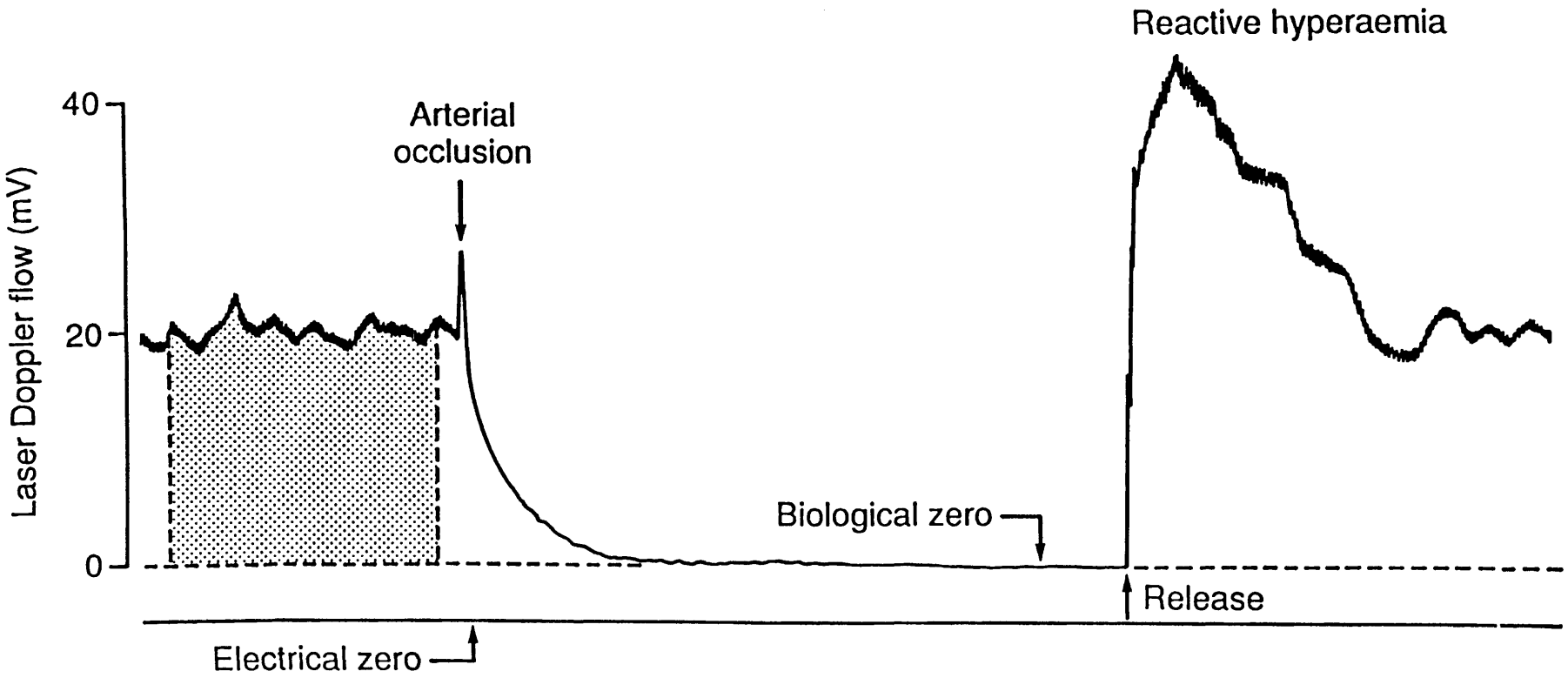


Figure 6: Application of an arterial occlusion of 250 mm Hg at the end of each experiment reduced the laser Doppler flow signal markedly to a level (Biological Zero) which was still slightly higher than the level obtained by backscattering of laser light from an inanimate stationary surface (Electrical Zero). The biological zero was used for the planimetric calculation of mean flow during any particular period (shaded area).

from backscattering of laser light from an inanimate stationary surface. This residual signal is thought to be related, in part, to small to-and-fro movements of red cells as fluid is exchanged across the microvessels, and in part to membrane, muscle fibre and macromolecular movements that are unrelated to blood flow (Rayman, 1987). Therefore, this "biological zero" was subtracted from the flowmeter output (Figure 6).

8. The tracings obtained were then analysed in order to obtain the mean flow values during two minutes in the horizontal and the 4th minute in the dependent position by measuring the area between the trace and the "biological zero" (Figures 5 & 6). The analysis was performed using a planimeter program and a HIPAD Digitizer (Bausch and Lomb, Texas, USA) connected with a microcomputer (380Z Disc System, Research Machines Ltd, Oxford, UK). The flow values were expressed in arbitrary units as millivolts (mV). Artefacts due to sudden involuntary movements of the subject were easily identified and excluded from analysis (Figure 5).

Factors Influencing Laser Doppler Measurements

1. Vascular Bed Geometry

Human skin blood flow is characterized by its large variations in different skin regions (Hertzman & Randall, 1948-49). Thus the skin of the palmar surface of the hand

and plantar surface of the foot exhibits relatively higher rates of blood flow than the skin of the trunk, forearm, thigh and leg. This is probably related to the relatively large number of arteriovenous anastomoses in the palmar surface of the hands and the plantar surface of the feet (Grant & Bland, 1931).

In the present study, preliminary experiments performed on 6 healthy male subjects (with a mean age of 28 years), showed that skin blood flow measured on the plantar surface of the big toe with the foot maintained at heart level was about 3-24 times higher than flow values recorded on the dorsum of the same foot.

Since laser Doppler flowmetry measures flow in a relatively small volume of cutaneous tissue (about 1 mm^3, Oberg et al, 1984), it might not necessarily reflect changes in total flow to the region. However, since total skin blood flow is extremely variable and usually in excess of the metabolic requirements of the cutaneous tissue (Greenfield, 1963), the relative changes in flow induced by different physiologic stimuli would seem to be far more important than the changes in the absolute flow itself. Moreover, when laser Doppler flowmetry was used to measure flow on the pulp of the toe during a maximal deep inspiration, the changes in the flowmeter output were found to closely parallel the changes in toe volume measured simultaneously by strain gauge plethysmography, suggesting that when the instrument is used

in this particular skin site it might reflect the fluctuations in the total blood flow to the region (Rayman, 1987).

2. Capillary Density

As the flowmeter signal is related not only to the mean velocity but also to the number of moving erythrocytes it would be reasonable to assume that the laser Doppler output from skin areas with high density of blood vessels is likely to be greater than the output from less well vascularised skin when examined under the same experimental conditions. Indeed, capillary density (measured by videomicroscopy) and laser Doppler flow were both found to be significantly higher when recorded on the dorsum of the finger than when measured on the dorsum of the foot (Rayman, 1987).

3. Blood Oxygen Tension

Changes in oxygen pressure within the physiological range have little effect on the flowmeter response under constant red cell velocity and concentration. In model studies, when oxygen tension was increased from 40 to 120 mm Hg the flowmeter output increased only by 4% (Nilsson et al, 1980).

4. Epidermal Thickness

The thickness of stratum corneum can vary from 40 μm on the

trunk to as much as 400 μm on the fingertips (Whitton & Everall, 1973). Thus, the thicker the epidermis the smaller will be the depth of vasculature represented within the measuring volume of the laser Doppler technique. However, despite this reasonable assumption, it was found in preliminary experiments that the mean flow values obtained from skin areas with relatively thick keratin layer (such as the plantar surface of the toes) were more than 10 times higher than the mean flow values recorded on areas with relatively thin stratum corneum (such as the dorsum of the foot). This is probably because of the relatively larger proportion of arteriovenous anastomoses in the former than in the latter areas. Moreover, when a section of skin (120 μm thick) was placed on the intact skin surface under the measuring probe on the dorsum of the foot, the flow signal was only attenuated by about 10% (Rayman, 1987).

5. Skin Temperature

In preliminary experiments, a significant positive correlation was found between skin temperature and the simultaneously recorded skin blood flow on the dorsum of the foot ($r = 0.948$, $P<0.01$) and on the plantar surface of the big toe ($r = 0.836$, $P<0.05$) (Figure 7). Therefore, it was felt necessary to measure skin temperature in all the studies involving measurement of skin blood flow. In these studies skin temperature was continuously measured using an adherent thermocouple of an electronic thermometer (Comark Electronics

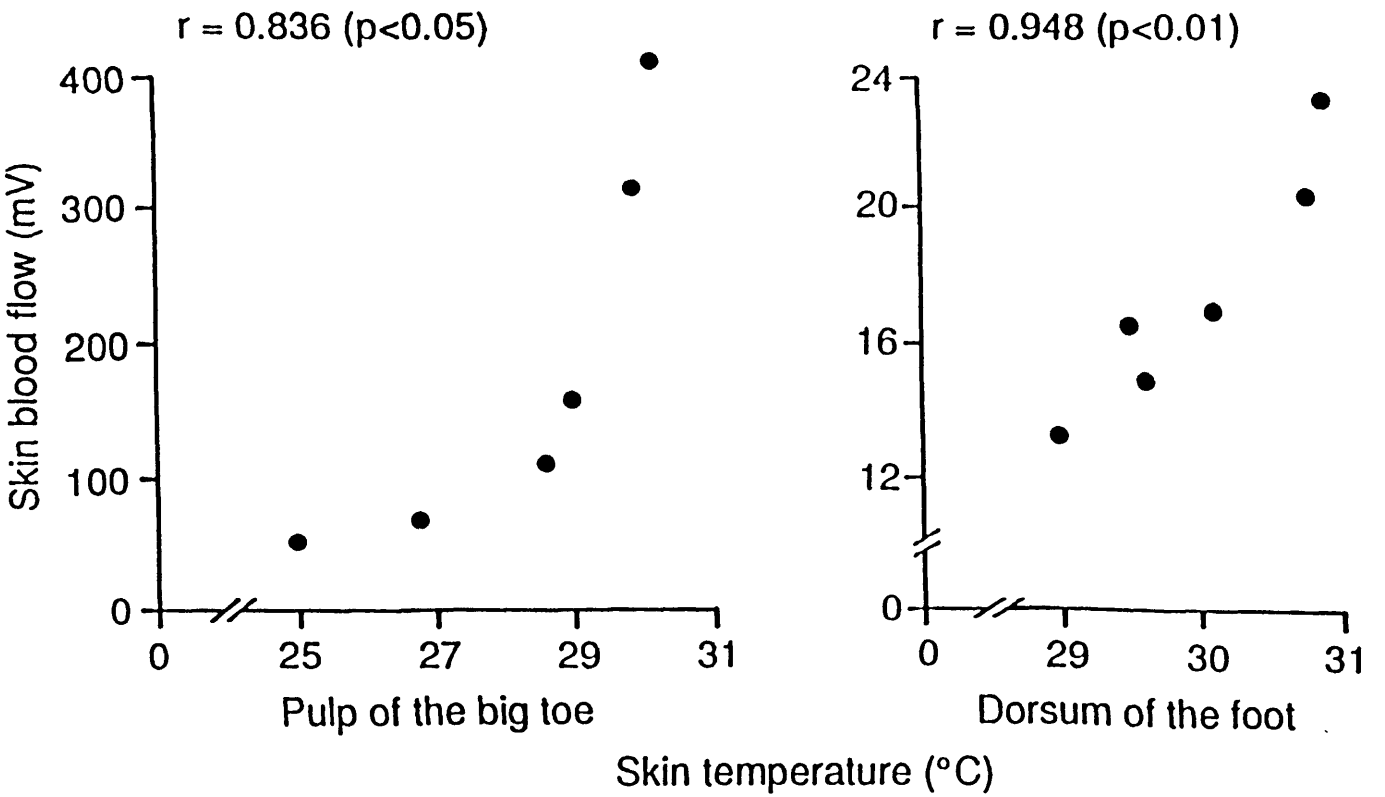


Figure 7: The relationship between skin temperature and skin blood flow measured at heart level on the dorsum of the foot and the pulp of the big toe using laser Doppler flowmetry in 6 healthy male subjects

Ltd, Littlehampton, Sussex, UK). The calibration of the thermometer was frequently checked in melting ice. The small sensitive end of the thermocouple was placed under the plastic probe holder just at the outer edge of the skin area from which skin blood flow was to be measured. Skin temperature mentioned in the text is the average temperature recorded to the nearest 0.1°C simultaneously during the time of the flow measurement. In a few number of experiments, another digital thermometer was used (type KTC, 871, Keithley, USA).

Reproducibility of the Postural Changes in Skin Blood Flow Using Laser Doppler Flowmetry

Experiments were performed on 6 normal male subjects aged 22-37 years (with a mean age of 28 years) after 20-30 minutes of acclimatization in the temperature-controlled room. Foot skin blood flow was measured with the subject lying supine first with the foot maintained at heart level (H), then with the foot lowered passively 50 cm below the heart (D). The magnitude of the postural fall in flow was expressed as a percentage relative to horizontal flow (H-D/H%). To examine the temporal variations in flow, measurements were repeated at 3 different times (9 am, 12 noon and 3 pm) on the same day (Tables 1 & 2) and at similar times on 3 different days within 2-3 weeks (Tables 3 & 4). To test the spatial variations in flow, measurements were performed at 2 different skin sites: (i) the dorsum of the foot, and (ii)

the plantar surface of the big toe of the same foot.

On the dorsum of the foot, the mean coefficient of variation of repeated measurements on the same subjects at 3 different times on the same day was 10.7% when flow was measured at heart level, 11.0% when flow was measured 50 cm below the heart, and 5.1% for H-D/H% (Table 1). On the plantar surface of the big toe, the corresponding mean coefficients of variation were 12.3%, 9.1% and 7.3% respectively (Table 2). When measurements were repeated at similar times on 3 different days, the mean coefficients of variation were 12.4%, 9.7% and 7.7% on the dorsum of the foot (Table 3), and 11.7%, 9.8% and 6.1% on the pulp of the big toe respectively (Table 4). Thus, when the same skin site was used for repeated measurements, the flow values recorded by laser Doppler flowmetry both in the horizontal and in the dependent positions were quite reproducible. Moreover, the magnitude of the postural reduction in flow as judged from H-D/H% was even more reproducible as indicated by its relatively lower mean coefficients of variation.

From Tables 1-4, it is also noted that the flow values measured both in the horizontal and dependent positions on the pulp of the toe are consistently higher than the corresponding values measured on the dorsum of the foot for the same subjects. These observations indicate the wide variability of skin blood flow in different skin regions and the marked dependence of the flow values on the level of the

Table 1: Reproducibility of the postural changes in skin blood flow (SBF, as mV) measured on the dorsum of the foot using laser Doppler flowmetry at 3 different times of the day in 6 healthy male subjects. C.V., coefficient of variation (calculated as SD x 100/mean), SEM, standard error of mean. H-D/H% refers to the percentage fall in flow on dependency.

		SBF at heart level [H]				SBF 50 cm below the heart [D]				H-D/H%			
Subject	Age	9 am	12 noon	3 pm	C.V.	9 am	12 noon	3 pm	C.V.	9 am	12 noon	3 pm	C.V.
1	22	16.5	21.5	19.2	13.1%	3.3	4.2	4.0	12.3%	80.0	80.5	79.2	3.3%
2	23	14.9	13.3	16.5	10.7%	2.8	2.5	3.1	10.7%	81.2	80.5	81.2	2.1%
3	24	23.3	25.0	21.7	7.1%	6.5	6.1	5.5	8.3%	72.1	75.6	74.7	7.0%
4	27	16.9	21.4	20.8	12.4%	5.4	5.8	6.7	11.2%	68.0	72.9	67.8	9.5%
5	36	13.2	16.3	14.7	10.5%	3.7	4.4	4.0	8.7%	72.0	73.0	72.8	1.9%
6	37	20.3	17.0	17.1	10.4%	7.1	5.3	6.0	14.8%	65.0	68.8	64.9	6.6%
Mean	28.2	17.5	19.1	18.3	10.7%	4.8	4.7	4.9	11.0%	73.0	75.2	73.4	5.1%
SEM	2.7	1.5	1.7	1.1		0.7	0.5	0.6		2.6	1.9	2.6	
Interindividual C.V.		21.1%	22.4%	14.7%		37.3%	28.0%	28.5%		23.9%	18.7%	23.8%	

Table 2: Reproducibility of the postural changes in skin blood flow (SBF, as mV) measured on the plantar surface of the big toe using laser Doppler flowmetry at 3 different times of the day in 6 healthy male subjects. C.V., coefficient of variation.

		SBF at heart level [H]				SBF 50 cm below the heart [D]				H-D/H%			
Subject	Age	9 am	12 noon	3 pm	C.V.	9 am	12 noon	3 pm	C.V.	9 am	12 noon	3 pm	C.V.
1	22	50	62	69	15.9%	9.1	10.5	11.4	11.2%	81.8	83.1	83.5	5.2%
2	23	110	105	93	8.5%	17.6	19.8	15.9	11.0%	84.0	81.1	82.9	8.4%
3	24	412	461	378	10.0%	81.0	87.2	76.0	6.9%	80.3	81.1	79.9	3.1%
4	27	157	139	191	16.3%	26.3	28.1	31.4	9.0%	83.2	79.8	83.6	11.7%
5	36	313	299	260	9.4%	39.9	41.7	37.0	6.0%	87.3	86.1	85.8	5.8%
6	37	68	59	78	13.9%	17.8	14.5	16.9	10.4%	73.8	75.4	78.3	9.4%
Mean	28.2	185.0	187.5	178.2	12.3%	32.0	33.6	31.4	9.1%	81.7	81.1	82.3	7.3%
SEM	2.7	59.5	65.5	50.3		10.7	11.6	9.8		1.9	1.5	1.1	
Interindividual C.V.		78.8%	85.6%	69.2%		82.0%	84.7%	76.2%		24.9%	18.8%	15.5%	

Table 3: Reproducibility of the postural changes in skin blood flow (SBF, as mV) measured on the dorsum of the foot using laser Doppler flowmetry at the same time of the day on 3 different days (within 2-3 weeks) in 6 healthy male subjects. C.V., coefficient of variation.

		SBF at heart level [H]				SBF 50 cm below the heart [D]				H-D/H%			
Subject	Age	Day 1	Day 2	Day 3	C.V.	Day 1	Day 2	Day 3	C.V.	Day 1	Day 2	Day 3	C.V.
1	22	16.5	18.1	21.8	14.5%	3.3	3.9	3.8	8.8%	80.0	78.5	82.6	10.6%
2	23	14.9	17.0	13.7	11.0%	2.8	3.1	3.0	5.1%	81.2	81.8	78.1	10.1%
3	24	23.3	25.8	22.4	7.4%	6.5	7.6	5.9	12.9%	72.1	70.5	73.7	5.7%
4	27	16.9	18.3	21.5	12.5%	5.4	6.1	6.0	6.5%	68.0	66.7	72.1	9.1%
5	36	13.2	16.7	17.8	15.1%	3.7	4.9	5.1	16.6%	72.0	70.7	71.3	2.3%
6	37	20.3	19.4	24.9	13.7%	7.1	6.3	7.4	8.2%	65.0	67.5	70.3	8.2%
Mean	28.2	17.5	19.2	20.4	12.4%	4.8	5.3	5.2	9.7%	73.0	72.6	74.7	7.7%
SEM	2.7	1.5	1.4	1.6		0.7	0.7	0.7		2.6	2.5	1.9	
Interindividual C.V.		21.1%	17.5%	19.5%		37.3%	31.3%	30.7%		23.9%	22.4%	18.7%	

Table 4: Reproducibility of the postural changes in skin blood flow (SBF, as mV) measured on the plantar surface of the big toe using laser Doppler flowmetry at the same time of the day on 3 different days (within 2-3 weeks) in 6 healthy male subjects. C.V., coefficient of variation.

		SBF at heart level [H]				SBF 50 cm below the heart [D]				H-D/H%			
Subject	Age	Day 1	Day 2	Day 3	C.V.	Day 1	Day 2	Day 3	C.V.	Day 1	Day 2	Day 3	C.V.
1	22	50	61	47	14.0%	9.1	10.8	9.7	8.7%	81.8	82.3	79.4	8.2%
2	23	110	98	116	8.5%	17.6	16.7	19.1	6.8%	84.0	83.0	83.5	3.0%
3	24	412	365	449	10.3%	81.0	73.6	94.2	12.6%	80.3	79.8	79.0	3.2%
4	27	157	183	143	12.6%	26.3	31.5	27.0	10.0%	83.2	82.8	81.1	6.3%
5	36	313	271	287	7.3%	39.9	42.3	37.1	6.5%	87.3	84.4	87.1	11.8%
6	37	68	81	57	17.5%	17.8	19.5	14.6	14.4%	73.8	75.9	74.4	4.3%
Mean	28.2	185.0	176.5	183.2	11.7%	32.0	32.4	33.6	9.8%	81.7	81.4	80.7	6.1%
SEM	2.7	59.5	49.4	63.8		10.7	9.4	12.7		1.9	1.3	1.8	
Interindividual C.V.		78.8%	68.5%	85.3%		82.0%	71.4%	92.8%		24.9%	16.5%	22.4%	

limb in relation to the heart.

There were also wide interindividual variations (bottoms of Tables 1-4) in the flow values measured at any particular time during repeated measurements. On the dorsum of the foot, the interindividual coefficient of variation ranged from 14.7% to 22.4% (mean 19.0%) with 5 repeated measurements in 6 subjects (Tables 1 & 3) when flow was measured at heart level, and from 28.0% to 37.3% (mean 31.2%) when flow was measured 50 cm below the heart, and from 18.7% to 23.9% (mean 21.5%) for H-D/H%. In the toe pulp, the corresponding values were 68.5% - 85.6% (mean 77.5%), 71.4% - 92.8% (mean 81.4%) and 15.5% - 24.9% (mean 19.6%) respectively (Tables 2 & 4). Thus, between subjects, there was much wider variations in horizontal and dependent flows when measured in the pulp of the toe (rich in arteriovenous anastomoses) than when measured on the dorsum of the foot, probably reflecting more variability in the resting vasomotor tone in skin areas rich in shunt vessels in different subjects. However, the interindividual variations in the ratio H-D/H% were relatively low and quite similar in both skin areas (mean 21.5% and 19.6%) which, in addition to being more reproducible as mentioned earlier, suggests that the value of this ratio could be used as an index of the magnitude of the postural response.

Marked spatial and temporal variations in skin blood flow using laser Doppler flowmetry have been previously reported

in the forearm and forehead, both when measured in the supine position (Tenland et al, 1983) as well as in the supine and sitting positions (Sundberg, 1984). As compared with these studies, the observations obtained from the present preliminary experiments on the foot suggest much less variations in this part of the body.

Taking all the above mentioned factors into account, the laser Doppler technique was considered appropriate especially for comparative studies, and was therefore chosen as the primary method in this investigation to study some of the physiological factors influencing the postural control of skin blood flow in the human foot.

C. VIDEO-TELEVISION MICROSCOPY

Since the laser Doppler flowmeter appears to record flow from capillaries as well as other superficial skin blood vessels as shown above, it cannot distinguish between nutritive and non-nutritive skin circulation in a particular skin region. Vital capillary microscopy, however, is the only direct technique which can show the functional behaviour and give a quantitative estimate of the nutritional capillary circulation of the skin (Fagrell & Östergren, 1987). This technique was used in one part of the present studies in order to : (i) examine the postural changes in capillary blood flow of the toe nailfold, and (ii) to compare the values obtained using this technique with the postural

changes in flow obtained using the laser Doppler method.

The capillary loops of the human skin are oriented more or less perpendicularly to the skin surface in the dermal papillae, except in the nailfolds, where the loops become nearly parallel to the surface and can be visualised by microscopy (Davis & Lawler, 1958).

Capillary blood flow velocity was measured in the nailfold of the right big toe using the television microscopy system shown in Figure 8. The terminal row of nailfold capillaries was visualised using a video camera (Model HV-65K, Hitachi Denshi Ltd, Japan) and X20 objective lens. The camera was mounted on a wild microscope (Heerbrugg, Switzerland) with a focusing system allowing both camera and lens to slide towards or away from the stationary nailfold to obtain the best focus. The nailfold was illuminated using a light source with a 50 Watt mercury vapour lamp (Leitz Instruments Ltd, Luton, UK) which has an emission spectrum similar to the absorption spectrum of haemoglobin to maximize the contrast between the red blood cells and the surrounding tissues. The light was carried to the skin by a fibreoptic light guide. To minimize skin heating, cold incident light was obtained by using a heat filter inserted between the light source and the fibreoptic light guide.

Prior to recording, the nailfold was painted with a clear nail varnish to prevent light reflection and allow better

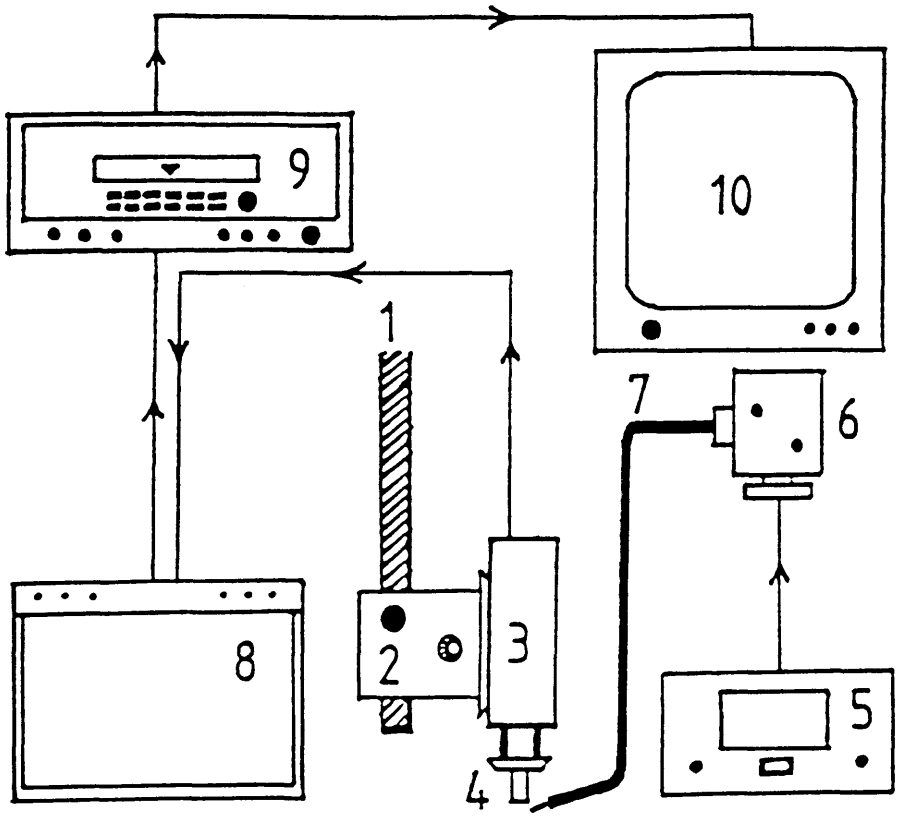


Figure 8: Schematic diagram of the television microscopy system:

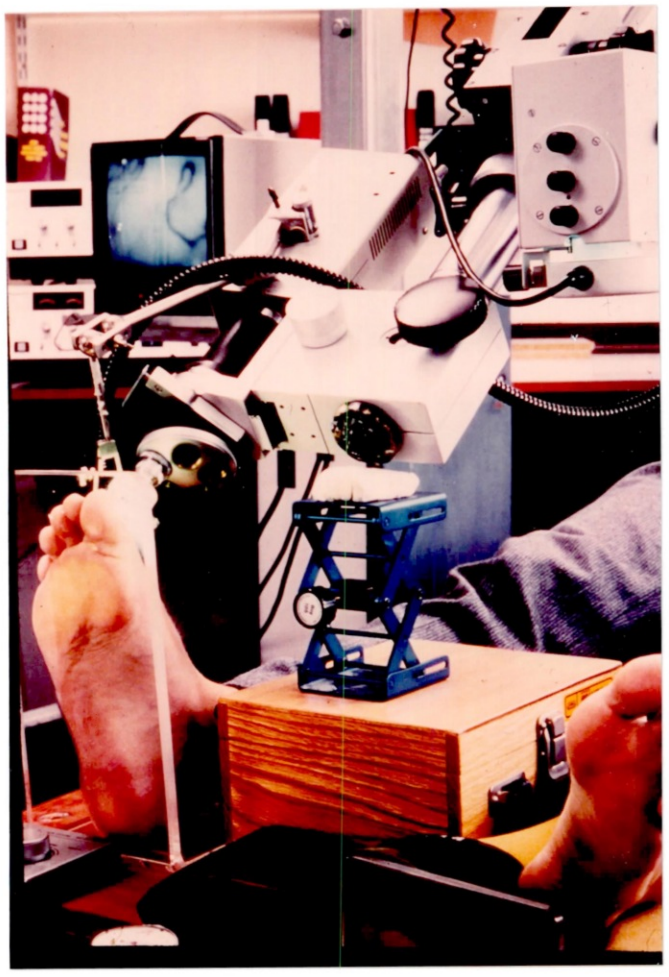
1. Part of the microscope stand
2. Focusing system
3. TV camera
4. Objective lens (X20)
5. Light source (Hg vapour lamp)
6. Lamp house and filter
7. Fibreoptic light guide
8. Time-date generator
9. Video-tape recorder
10. TV monitor.

visualisation of capillaries. The visualised image was continuously recorded on a video tape (Panasonic, Model AG 6200-B, Matsushita Electric Industrial Co Ltd, Japan) and simultaneously displayed on a television monitor (Model VM-906 AE/KS2, Hitachi Denshi Ltd, Japan) with a final screen magnification of 480 (as calculated from the screen image of a calliper scale). To minimize toe movements, the pulp of the toe was surrounded by plasticine. A metal bracket attached to the microscope objective and resting gently on the nail was also used to obtain a stable image. A time-date generator was incorporated in the system for the simultaneous recording of the elapsed time in 1/100th sec on the video tape.

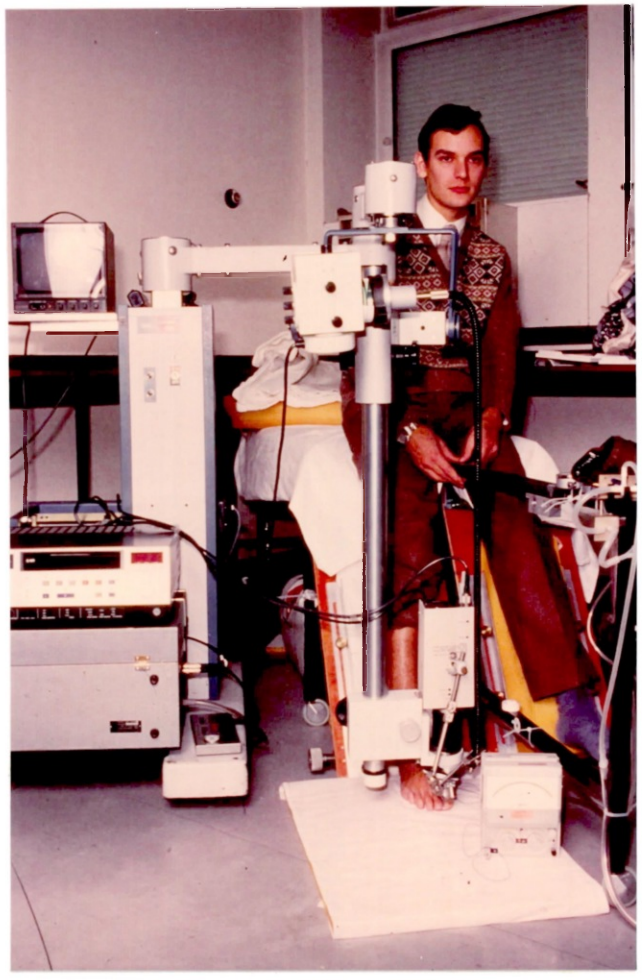
Recording of the Postural Changes in Capillary Blood Flow

In both the horizontal and dependent positions, video recordings of a minimum of 10 capillaries were made for a minimum period of one minute for each vessel. Recordings were made first with the subject lying down with both feet at heart level, then with the subject sitting on the edge of the couch with the feet at a mean distance of 97 cm below the heart (Figure 9a & b).

The microscope stand has a remote control operated multi-jointed arm carrying the camera and lens. This versatile arrangement made it possible to adjust the camera and lens in a nearly horizontal angle so as to measure nailfold capillary



(a)



(b)

Figure 9: The experimental set-up of the television microscopy technique for measurement of capillary blood flow velocity in the nailfold of the right big toe with the subject supine (a) and sitting (b).

blood flow velocity with the subject in the horizontal position and the leg flat thereby avoiding the possible influence of the hydrostatic column of blood on capillary blood flow if it had been measured with the knee flexed and foot and toes flat. In the sitting position, the camera and lens were adjusted vertically. Recordings in the horizontal position started after at least 20 minutes of acclimatization in the constant-temperature room, and in the semi-erect position at least 5 minutes after the subject sat up.

Analysis Procedure

At the end of each experiment, the video tape was played back and capillary blood flow velocity was measured in each individual capillary recorded, in both the horizontal and dependent positions, using "frame to frame" analysis of the prerecorded video images.

Analysis included all the capillaries recorded in focus, in which the arterial limb was visible for at least 3 cm on the screen. The continuous red cell column flowing in the capillary was occasionally interrupted by plasma gaps which were used as markers of blood flow. The analysis proceeded as follows:

1. The prerecorded capillaries were displayed on the television screen. With the video tape stopped, the outline of each individual capillary was drawn on a

transparent acetate sheet.

2. On playing the tape again, once a plasma gap was seen, the tape was stopped and the position of the gap (the leading edge of the red cell column) was marked on the transparent sheet. The tape was then advanced by one or more frames (each frame = 0.04 second) and the new position of the gap was marked on the sheet (Figure 10).
3. The distance between the 2 marks on the sheet was measured by a divider and ruler in mm and was then divided by the elapsed time (number of frames advanced x 0.04 second) and also by the magnification factor (480) to obtain capillary flow velocity in mm/second.
4. All measurements were performed on the arterial limb of the capillary. In each individual capillary, when the erythrocytes were moving, the velocity was measured at 5 second intervals for at least one minute, and the mean velocity for each capillary was calculated. If the erythrocytes were not moving at any time point, a zero velocity was not included in the calculation of the mean velocity for that capillary. If the velocity was not assessable at any time point due to, for example, lack of plasma gaps, the tape was advanced to a maximum of 2.5 seconds to derive a representative value. No assumptions were made about the flow velocity during the intervening periods other than at the 5 second interval points.
5. The overall mean capillary blood flow velocity in both the horizontal and dependent positions was calculated

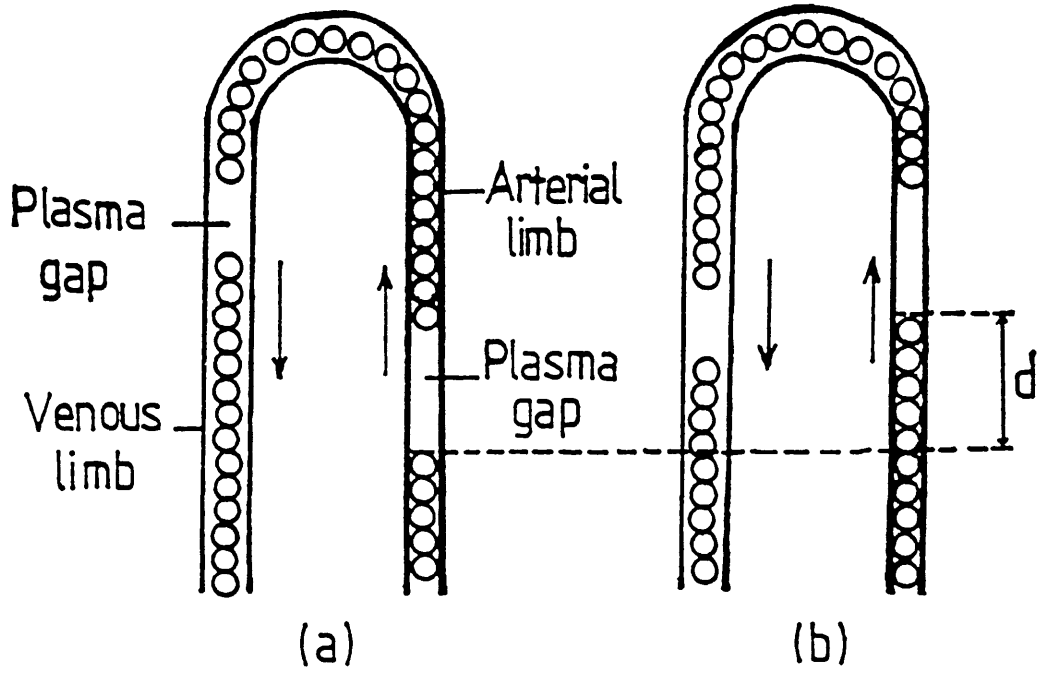


Figure 10: Schematic diagram of a blood capillary illustrating the method by which capillary blood flow velocity was measured by "frame to frame" technique. In (a) a plasma gap was identified and the leading edge of the red cell column marked on a transparent acetate sheet. The video tape was then advanced in (b) by one or more frames with a known time interval (t) and the new position of the column marked. The distance (d) between the 2 marks was measured in mm and divided by the time (t) to derive the velocity. Arrows indicate the direction of red cell flow.

for any particular subject from the individual mean velocities obtained from all capillaries analysed.

6. The diameter of the erythrocyte column in the arterial limb of the capillary was also measured using a special calliper at each 5 second point and at the same site where capillary blood velocity was measured. Capillary volume flow was then calculated as the product of capillary blood velocity and the cross sectional area of the erythrocyte column.
7. Periods during which capillary flow completely stopped were calculated in relation to the recorded time for each capillary. The overall percentage of time that stop flow was observed was then calculated for the capillary population studied, in both the horizontal and dependent positions.

Repeated analysis of the recordings made in 3 subjects using the "frame-to-frame" method produced coefficients of variation of 4.3% when capillary blood flow velocity was measured in the horizontal position and 6.9% when the flow velocity was measured in the sitting position (Table 5). To check for the reproducibility of the postural changes in toe nailfold capillary blood flow velocity, repeat tests were performed on 2 subjects with coefficients of variation of 7.6% when the flow velocity was measured at heart level, 9.3% when it was measured 97 cm below the heart and 2.0% for the percentage fall in flow velocity in the dependent position (Table 6).

Table 5: Repeated analysis of capillary blood flow velocity (CBV) measured in the horizontal and sitting positions using the "frame to frame" method. C.V., coefficient of variation.

Subject	Horizontal CBV (mm/sec)			Sitting CBV (mm/sec)		
	First Analysis	Second Analysis	C.V.	First Analysis	Second Anaylsis	C.V.
1	0.109	0.116	4.4%	0.012	0.014	10.9%
2	0.650	0.621	3.2%	0.051	0.047	5.8%
3	0.244	0.263	5.3%	0.018	0.017	4.0%
Mean	0.334	0.333	4.3%	0.027	0.026	6.9%

Table 6: Reproducibility of the postural changes in capillary blood flow velocity (CBV) measured in mm/sec using television microscopy in 2 male subjects. C.V., coeficient of variation.

Subject	Horizontal CBV			Dependent CBV			Postural fall in CBV (%)		
	1st Test	2nd Test	C.V.	1st Test	2nd Test	C.V.	1st Test	2nd Test	C.V.
1	0.109	0.127	10.8%	0.012	0.011	6.1%	89.0%	91.3%	1.8%
2	0.249	0.234	4.4%	0.056	0.047	12.4%	77.5%	79.9%	2.2%
Mean C.V.			7.6%			9.3%			2.0%

D. STRAIN GAUGE PLETHYSMOGRAPHY

As pointed out earlier in the "INTRODUCTION", the postural increase in the ratio of precapillary to postcapillary resistance and the associated fall in blood flow could act to reduce oedema formation in the dependent position (Henriksen et al, 1983a). Accordingly, one would anticipate that with greater degrees of postural vasoconstriction, the risk of oedema development in the dependent position would be greatly reduced.

In order to explore the possible relationship between the magnitude of the postural vasoconstrictor response and the rate of fluid filtration into the foot, in two of the studies to be described, strain gauge plethysmography was used to measure foot swelling rate in the dependent position at the same time as laser Doppler flowmetry was used to measure the postural changes in foot skin blood flow.

Principle of the Method

When a limb changes in volume, it is assumed that the length of the limb remains constant and the whole of the volume change occurs in the transverse sectional area of the limb, so that the percentage change in the area of the section will be twice the percentage change in circumference of the section, for small changes in area (Whitney, 1953), thus:

$$\frac{\text{Change in volume}}{\text{Initial volume}} = 2 \times \frac{\text{Change in circumference}}{\text{Initial circumference}}$$

This rule applies for circular sections as well as sections of other shapes (e.g. foot) so long as the shape of the section remains unaltered during changes in volume (Whitney, 1953).

The strain gauge plethysmograph consists of a thin elastic silicon tube filled with mercury whose resistance varies with the length of the tube. The gauge is designed to encircle the limb segment with the rubber tube in a slightly stretched position, so that when the limb changes in volume, the resulting changes in cross sectional area will then lead to changes in the length of the tube and hence to changes in mercury resistance. This change in resistance is then transformed into a voltage output by an amplifier circuit connected with the strain gauge. By calibrating the instrument, the percentage change in limb volume could be calculated from the percentage change in limb circumference (output voltage).

A double-stranded gauge was used (Janssen Scientific Instruments, Beerse, Belgium). The linearity of the gauge output was checked by stretching it by known distances using a special calliper and recording the voltage output on a chart recorder (Figure 11).

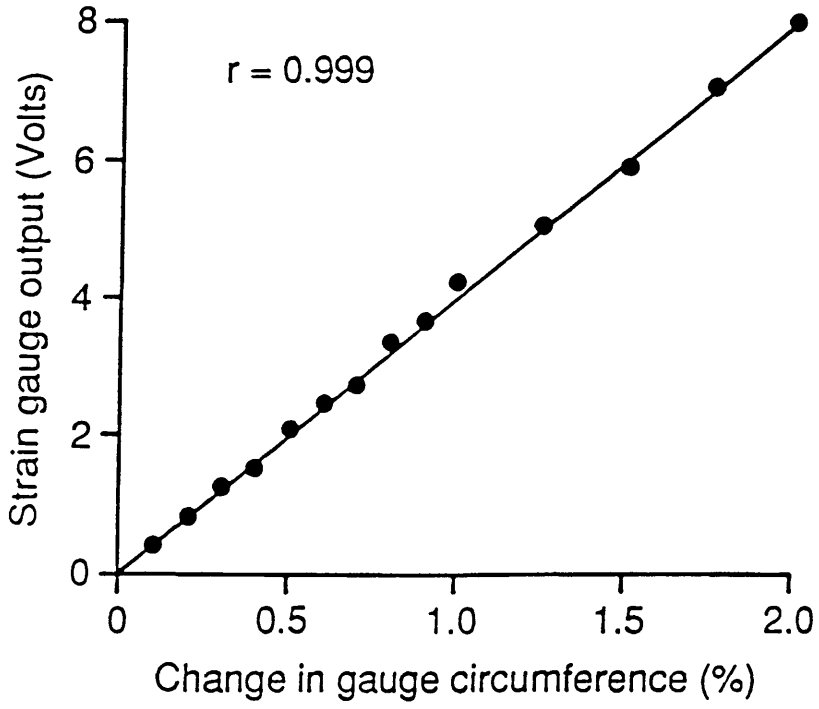


Figure 11: The linear relationship between the percentage change in the circumference of the mercury-in-rubber strain gauge and its voltage output.

Procedure for Measuring Foot Swelling Rate Using Strain Gauge Plethysmography

1. Experiments were performed with the subject lying in the supine position, after at least 20 minutes of acclimatization in the constant temperature room (22°C).
2. The gauge encircled the foot midway between the ankle and toes, and was kept in position by narrow strips of adhesive tape.
3. Before each measurement, the volume trace was restored to a zero baseline and calibration of the gauge was made in situ by producing a known autocalibration signal (Figure 12) on the chart recorder. By setting up a new resistance in the gauge circuit, taken as a reference for the initial foot volume, any changes in gauge resistance produced by changes in foot volume will be displayed as a percentage relative to the initial resistance, thus obviating the need for the absolute foot volume.
4. Changes in foot volume were recorded first for at least 5 minutes with the foot maintained at heart level, and then for at least 20 minutes with the foot placed passively 50 cm below the heart, and finally for 5 minutes with the foot returned to heart level again (Figure 12). The percentage change in foot volume was virtually zero when the foot was kept at heart level. On lowering the foot in the dependent position there was an initial rapid increase in foot volume for about 2-4

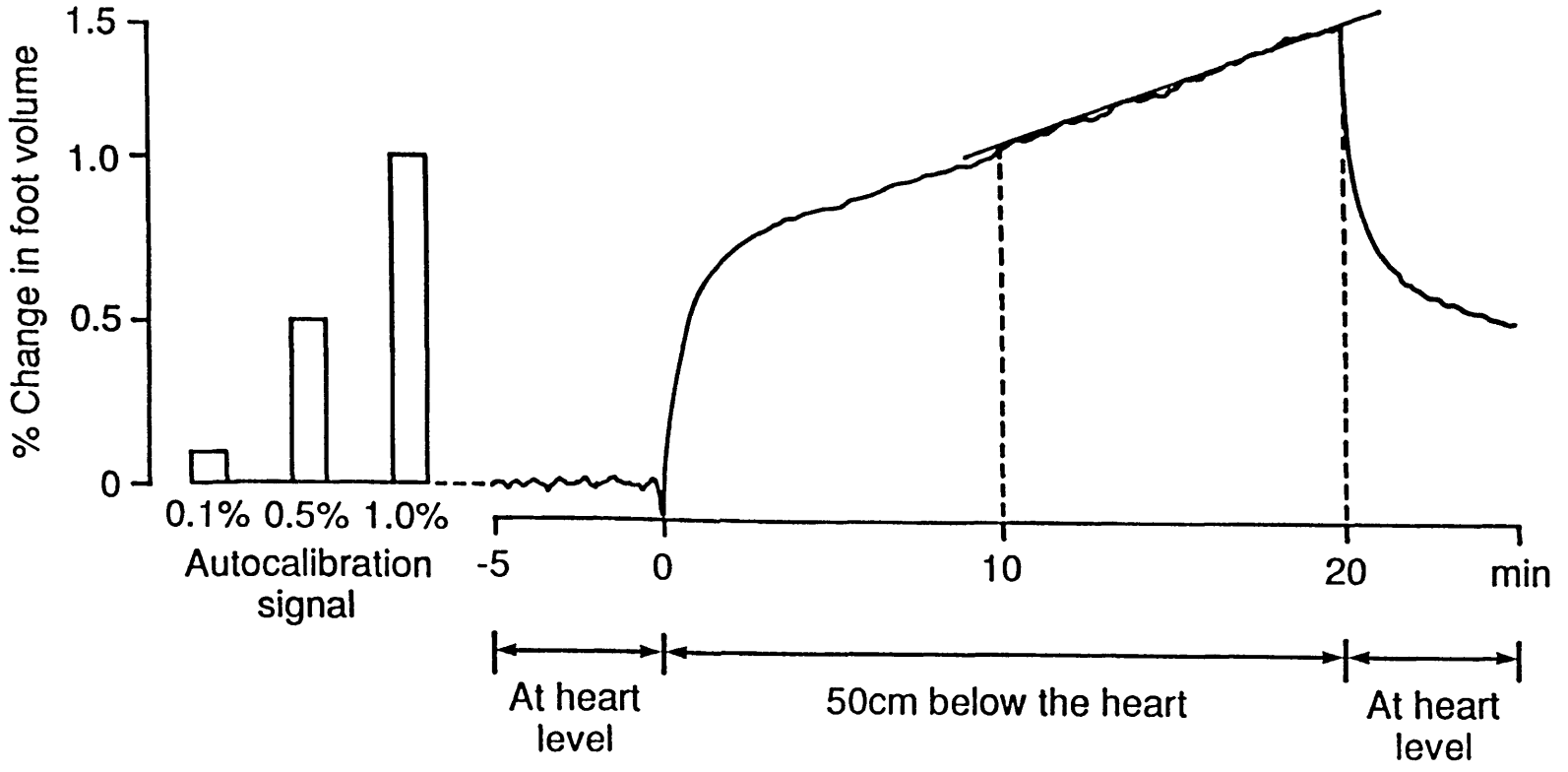


Figure 12: An example of the tracings obtained when a mercury-in-rubber strain gauge plethysmograph was used to measure changes in foot volume (foot swelling rate) from changes in foot circumference when the foot was lowered 50 cm below the heart. Foot swelling rate was calculated from the slope of the curve between 10 and 20 minutes. At time zero, the foot was placed in the dependent position.

minutes representing largely the increase in vascular volume (Sejrsen et al, 1981b) after which there was a steady increase in foot volume with time (Figure 12) representing mainly fluid filtration into the foot or "foot swelling rate" (Michel & Moyses, 1987).

5. Foot swelling rate was measured from the slope of the foot swelling curve (Figure 12) recorded between 10 and 20 minutes with the foot in the dependent position and expressed as $ml.min^{-1}.100ml^{-1}foot$.

Effect of Temperature on Strain Gauge Output

Since the mercury filling the gauge has a high thermal coefficient, the gauge is considered to be sensitive to variations in external temperature (Whitney, 1953). Indeed, it was found that when the temperature of air surrounding the unstretched strain gauge was increased by 1°C, the gauge output increased by approximately 0.05%, but since room temperature was kept constant during the course of each experiment, it was considered to have a negligible influence on the gauge output.

Also, variations in skin temperature during measurements might influence the strain gauge output. This possibility was tested in a male subject aged 36 years. Foot swelling rate was measured with the foot 50 cm below the heart, first at a resting skin temperature of 29°C, then at temperatures of 26, 32 and 36°C induced by local cooling or heating of the

skin proximal to the gauge using cold or hot air from a hair dryer. Care was taken to isolate the air currents from the strain gauge by a piece of cardboard encircling the foot proximal to the gauge and held vertical without touching the skin. After at least 15 minutes of equilibration to the new skin temperature, with the foot in the horizontal position, the gauge resistance was reset to a new reference level using the autozeroing system. This nullified the changes in gauge resistance induced by changes in gauge temperature and foot vascular tone as a result of the alteration in local temperature. On lowering the foot, the relative changes in gauge output in the steady state would, therefore, reflect mainly the changes in foot swelling rate at the new skin temperature.

As shown in Figure 13, there was a positive relationship between skin temperature and foot swelling rate; increasing the temperature by 10°C (from 26 to 36) led to increased foot swelling rate from 0.04 to 0.08 $ml.min^{-1}.100ml^{-1}$ foot, with a regression coefficient of 0.0041 $ml.min^{-1}.100ml^{-1}.°C^{-1}$. Similar observations have been previously found in the forearm during venous occlusion (Landis & Gibbon, 1933).

However, in 6 preliminary experiments performed at resting skin temperature on 3 male subjects aged 21-37 years, each subject being studied on 2 separate occasions, when the foot was lowered from the horizontal to the dependent position there was a reduction in skin temperature of 0.4 - 1.6°C with

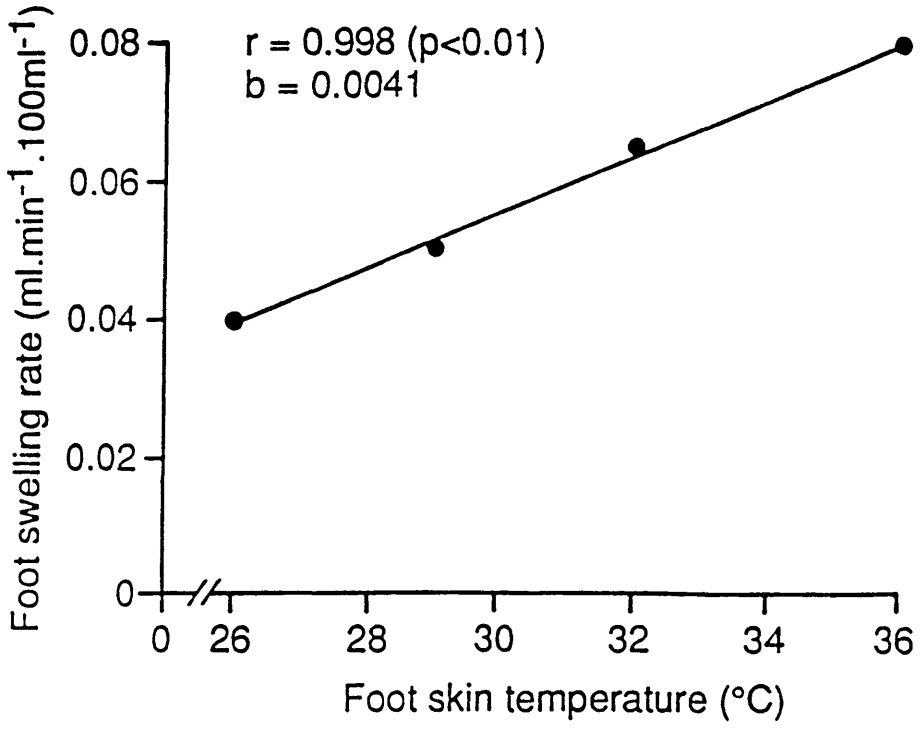


Figure 13: The effect of changes in local skin temperature on foot swelling rate in a male subject aged 36 years. The plotted local temperatures were the average temperatures measured using 2 thermocouples attached to the skin between the 2 strands of the gauge on the dorsal and plantar surface of the foot. r, correlation coefficient, b, regression coefficient.

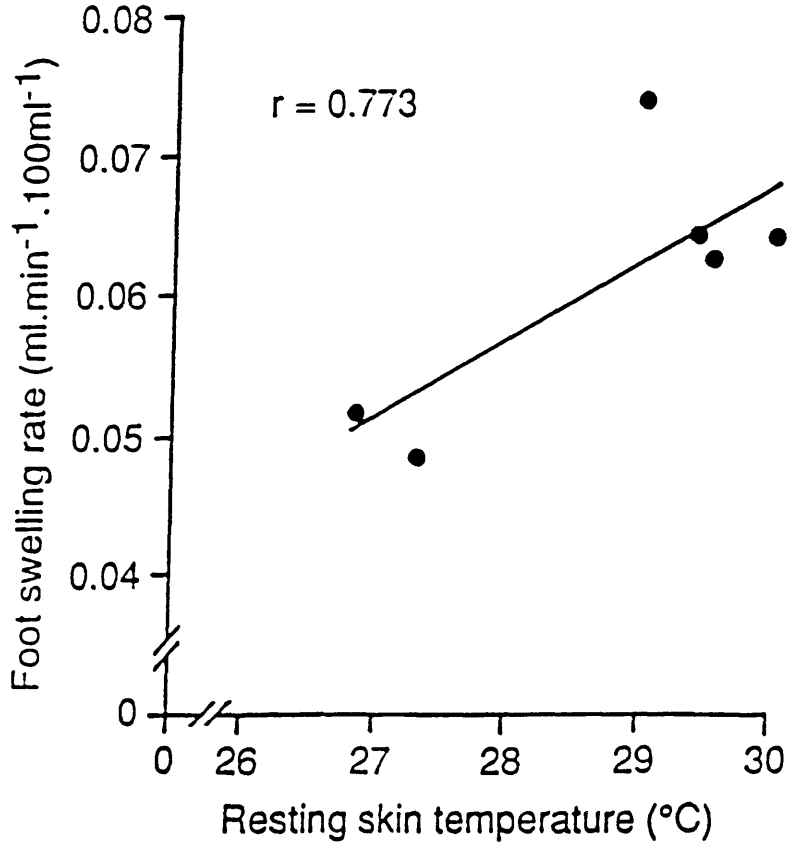


Figure 14: The relationship between resting foot skin temperature and foot swelling rate obtained from 6 experiments performed on 3 normal male subjects.

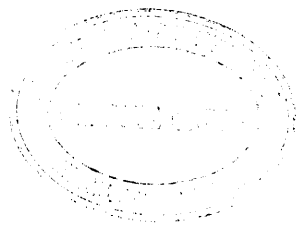
a mean reduction of 0.8°C during the first 10 minutes of dependency, but the temperature remained nearly constant from 10 to 20 minutes (not varying by more than ± 0.2°C); the period during which foot swelling rate was actually measured.

On the other hand, when foot swelling rate was plotted against resting foot skin temperature in these preliminary experiments, there was a tendency for a positive correlation ($r = 0.773$, Figure 14) though it was not statistically significant probably because of the small number of experiments. Thus, resting foot skin temperature appears to have a significant influence on the swelling rate of the dependent foot. However, during the 10th to 20th min of dependency, the changes in foot skin temperature were too small to have a significant effect on foot swelling rate.

To test for the reproducibility of foot swelling measurements, repeat tests were performed on 3 male subjects. The average foot swelling rates were 0.062 and 0.059 $ml.min^{-1}.100ml^{-1}foot$ from the 2 test series, with a mean coefficient of variation of 5.4%.

E. STATISTICAL ANALYSIS

Since all the studies performed in this thesis are of comparative nature, comparisons between groups of data were made using the following tests:



1. Parametric tests

These tests were performed on either paired or independent groups of observations, when the data were found to be normally distributed. Normal distribution was judged when each observation was not different from the group mean by more than 2 standard deviations (Swinscow, 1978) or by using the F-test (Wardlaw, 1985). The paired t-test was used to compare the means of paired samples, and the unpaired t-test to compare the means of 2 independent samples.

2. Non-parametric tests

These tests were used to compare data from 2 paired or independent groups when the data were found not conforming with a normal distribution, or to compare 2 groups of percentages. Wilcoxon's signed rank test was used to compare data from 2 paired samples or percentages, and Wilcoxon's rank sum test was used to compare data from 2 independent samples or percentages (Swinscow, 1978).

3. Analysis of variance

This type of analysis was carried out to check the statistical significance of the differences between more than 2 groups of data conforming with a normal distribution (Wardlaw, 1985). The one-way analysis of variance was used when one variable was involved, and the two-way analysis of

variance was used to test for the effect of 2 independent variables as well as their interaction in the different groups. For the latter test, analysis was performed on a microcomputer using s special programmed disk. For data not conforming with a normal distribution, a non-parametric analysis of variance was used (Friedman's test) (Wardlaw, 1985).

4. Dunnett's test

This test was used when the mean of each of 2 or more experimental groups had to be compared with a control group (Dunnett, 1955).

5. Correlation and Regression

This type of analysis was used when a positive or a negative relationship was found between 2 related groups of data plotted in a scatter diagram. The relation is expressed by the correlation coefficient "r" and the line of best fit was obtained by regression analysis (Swinscow, 1978). In a few instances, Spearman's rank correlation was used (Snedecor & Cochran, 1978).

Throughout the text, the results will be expressed as means ± standard error of the mean.

RESULTS

Chapter III

EFFECT OF INTERACTION OF POSTURAL CHANGE AND THERMOREGULATORY STRESS ON FOOT SKIN BLOOD FLOW

Chapter III

EFFECT OF INTERACTION OF POSTURAL CHANGE AND THERMOREGULATORY STRESS ON SKIN BLOOD FLOW

Section 1 EFFECT OF INDIRECT HEATING ON THE POSTURAL CHANGES IN FOOT SKIN BLOOD FLOW

Introduction

As has been pointed out in Chapter I, a change in skin temperature, body temperature or a change in the height of an extremity in relation to the heart are among the several factors that can alter the local cutaneous vascular tone. Thus, foot blood flow has been shown to decrease when the limb is lowered below heart level (Gaskell & Burton, 1953; Beaconsfield & Ginsburg, 1955b), a response which has been suggested to be due mainly to an increase in precapillary resistance thought to be largely mediated by local mechanisms (Mellander et al, 1964; Henriksen, 1977; Levick & Michel, 1978; Henriksen & Paaske, 1980).

It has also been shown that one of the primary mechanisms by which humans adjust to a thermal stress is to change the resting vasomotor tone of the cutaneous vessels particularly in the hands and feet (Rowell, 1983). This thermoregulatory function is thought to be largely facilitated by the presence of a large number of arteriovenous anastomoses in these areas

(Grant & Bland, 1931). Thus, the release of the central sympathetic tone during body heating causes vasodilatation in the hands and feet (Gibbon & Landis, 1932; Pickering & Hess, 1933).

However, the interaction between the centrally-elicited thermoregulatory vasodilator reflexes and the locally-produced postural vasoconstrictor responses is still unclear. Therefore, the aim of the present study was to investigate the effect of indirect heating on the postural changes in skin blood flow in the human foot.

Methods

Experiments were performed on 12 healthy male subjects aged 19-83 years (mean age 38 years) under carefully controlled conditions as described in Chapter II.

Indirect Heating

Body heating was achieved with an electric blanket (CFP 8185, Bioscience, U.K.) wrapped around the trunk, with its temperature maintained at 44°C for a period of 45 minutes. The influence of indirect heating on the postural changes in skin blood flow was examined during the last 15 minutes of this period.

The Study Protocol

The experimental design consisted of measuring skin blood flow using laser Doppler flowmetry (as described in Chapter II) first with the foot kept at heart level, then with the foot placed passively 50cm below the heart. These measurements were performed before and during indirect heating in 2 skin areas: (i) the dorsum of the foot (where arteriovenous anastomoses are absent), and (ii) the plantar surface of the big toe (of the same foot), where these shunt vessels are relatively numerous (Grant & Bland, 1931).

Skin temperature was measured continuously with an adherent thermocouple (as described in Chapter II) in both skin areas, both before and during heating. Sublingual temperature was also measured before and 30 minutes after the beginning of indirect heating using a standard mercury clinical thermometer.

Measurement of Arterial Blood Pressure and Heart Rate

Arterial blood pressure (using ordinary sphygmomanometry) and heart rate (radial pulse) were measured first with the subject in the supine position and both feet kept at heart level just before recording the horizontal skin blood flow, and then during the 4th-5th minute after the foot was lowered in the dependent position just after recording the 4th minute dependent flow. Measurements were taken both before and during indirect heating in each subject.

In order to test for the reproducibility of the experimental results, the same protocol was carried out on 3 of the 12 subjects on a second occasion.

RESULTS

Dorsum of the Foot (Figures 15A & 16, Table 7)

Before indirect heating, mean skin blood flow measured during the 4th minute with the foot in the dependent position was significantly reduced compared with the preceding mean rest flow measured at heart level (Figure 16). There was also a small but significant fall in mean skin temperature in the dependent position (Table 7). During the last 15 minutes of indirect heating, mean skin blood flow and skin temperature measured at heart level were significantly elevated (Table 7). When the foot was lowered 50 cm below the heart, mean skin blood flow again fell significantly to a value which was insignificantly different from that obtained before heating (Figure 16). However, mean skin temperature measured in the dependent position during heating fell to a value which was still significantly higher than the corresponding value measured in the same position before heating (Table 7).

Plantar Surface of the Big Toe (Figures 15B & 17, Table 7)

Before heating, as in the dorsum of the foot, mean skin blood flow and skin temperature measured in the toe pulp fell

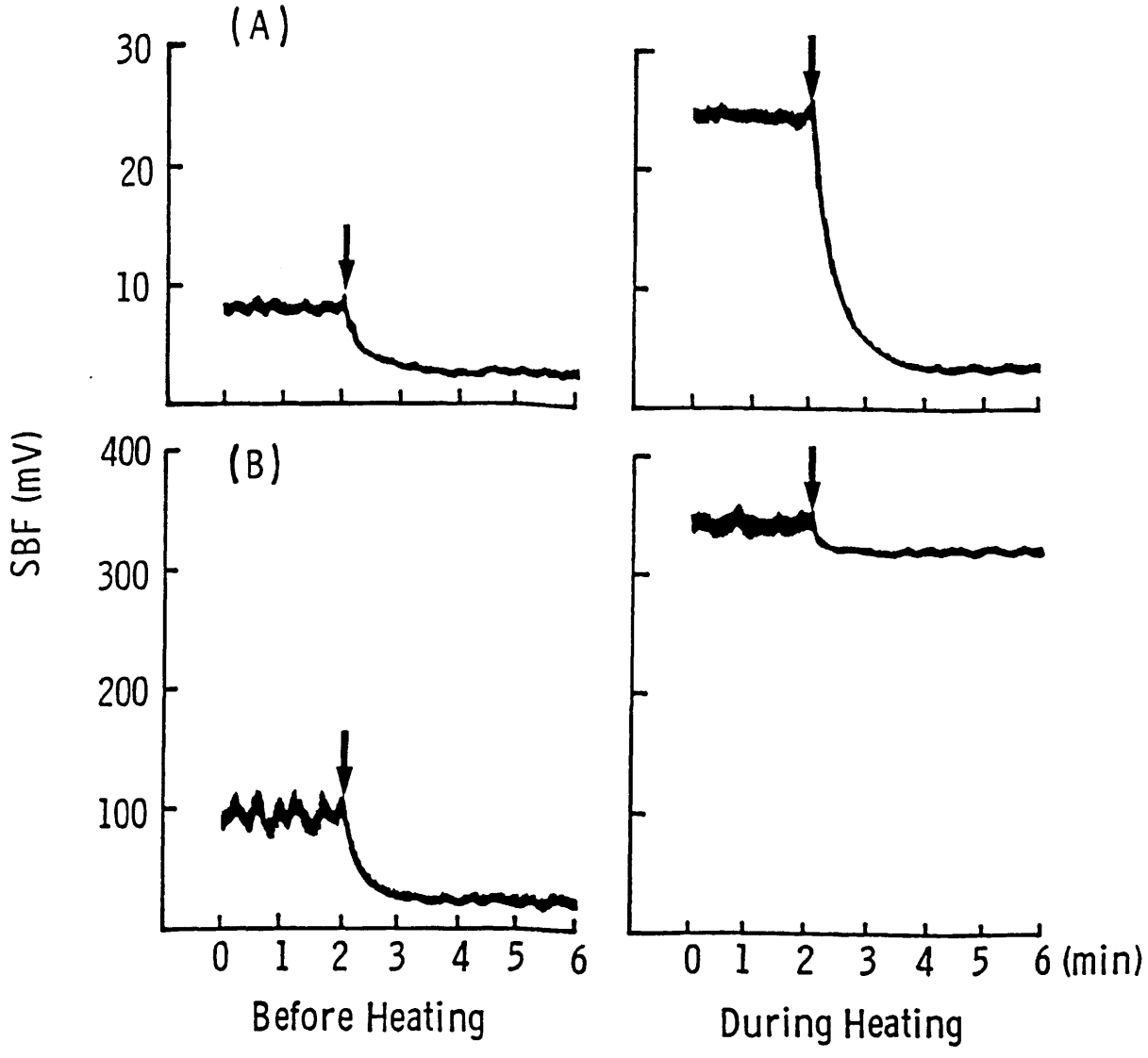


Figure 15: An example of the tracings obtained using laser Doppler flowmetry showing the postural changes in skin blood flow (SBF) measured on the dorsum of the foot (A) and on the plantar surface of the big toe (B), both before and during indirect heating. Arrows indicate the times when one foot was lowered passively to 50 cm below heart level, with the rest of the body remaining in the horizontal position.

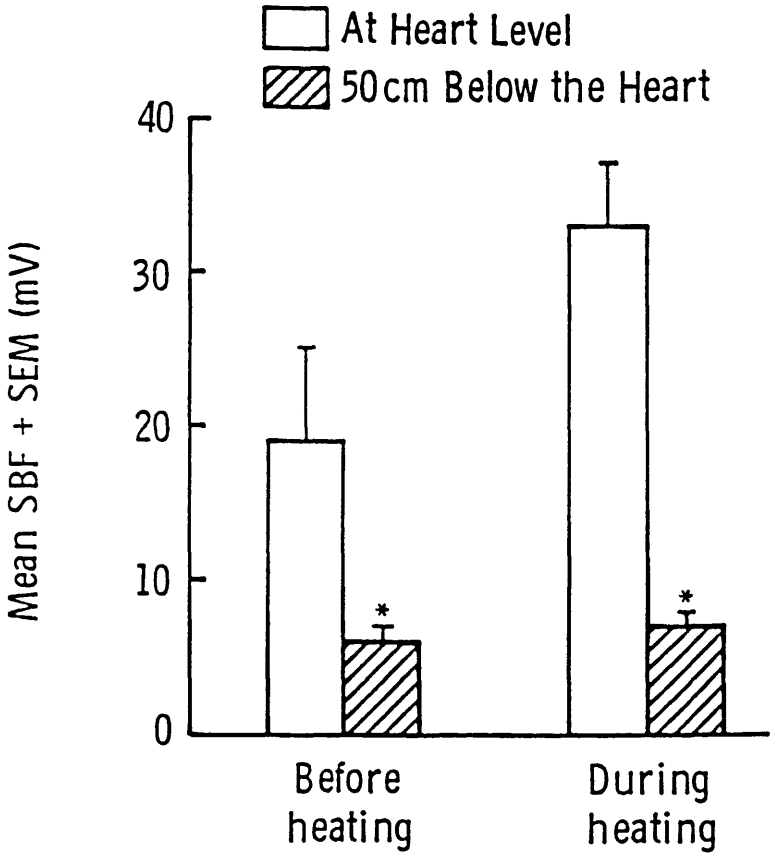


Figure 16: Effect of the dependent position on skin blood flow (SBF) measured on the dorsum of the foot before and during indirect heating in 12 healthy male subjects. *P<0.01, as compared with mean flow values measured at heart level.

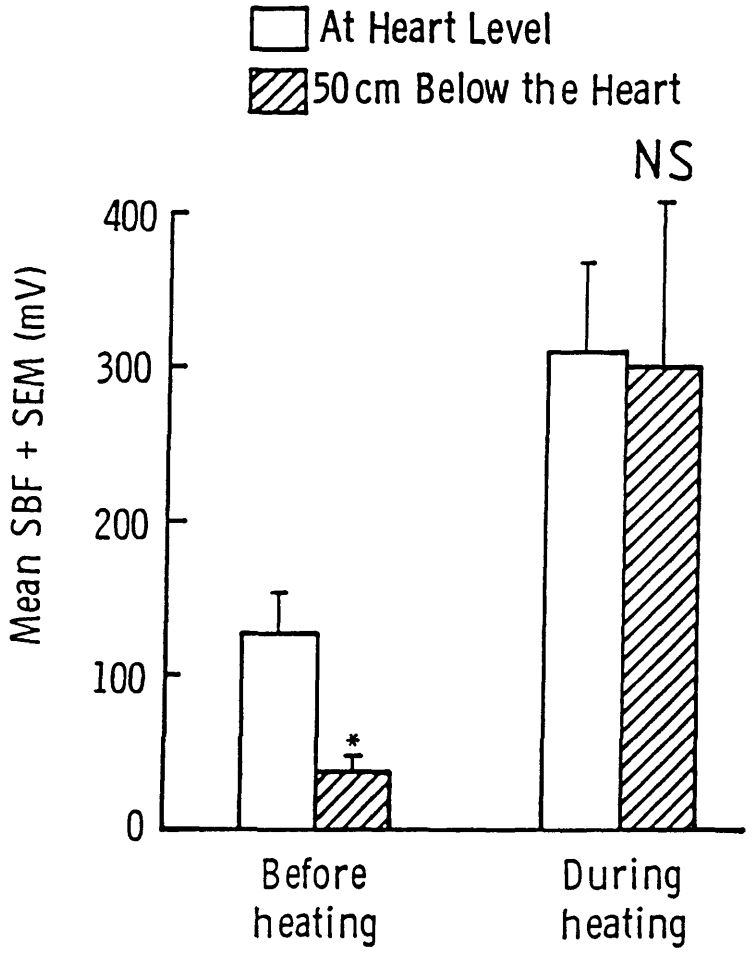


Figure 17: Effect of the dependent position on skin blood flow (SBF) measured on the plantar surface of the big toe before and during indirect heating in 12 healthy male subjects. *P<0.01, NS, nonsignificant, as compared with mean flow values measured at heart level.

Table 7: The postural changes in skin blood flow (as mV) measured using laser Doppler flowmetry on the dorsum of the foot and on the plantar surface of the big toe in 12 healthy male subjects, before and during indirect heating. The changes in foot skin temperature and body temperature are also shown. H, with the foot horizontal, D, during the 4th minute with the foot dependent. The postural fall in flow was calculated as (H - D)/H%. Data are given as mean ± SEM. NS, nonsignificant, *P<0.01, **P<0.001 (as compared with the mean values measured with the foot horizontal), +P<0.02, ++P<0.01 (as compared with the corresponding values measured on the dorsum of the foot).

Dorsum of the Foot	Before Heating	During Heating	Statistical Significance
Skin Blood Flow (H)	18.6 ± 4.6	33.4 ± 3.7	P<0.001
Skin Blood Flow (D)	6.1 ± 1.1*	7.4 ± 1.4*	NS
Postural Fall in Flow	59.5 ± 7.1%	77.7 ± 3.1%	P=0.01
Skin Temperature, °C (H)	30.2 ± 0.2	33.2 ± 0.4	P<0.01
Skin Temperature, °C (D)	29.9 ± 0.2**	32.7 ± 0.4**	P<0.01
Pulp of the Big Toe			
Skin Blood Flow (H)	127.1 ± 25.2++	310.1 ± 58.2++	P<0.01
Skin Blood Flow (D)	37.6 ± 9.6*++	298.1 ± 109.7++	P<0.01
Postural Fall in Flow	66.6 ± 8.3%	23.4 ± 15.9%++	P<0.01
Skin Temperature, °C (H)	28.1 ± 0.8+	33.1 ± 0.5	P<0.001
Skin Temperature, °C (D)	27.7 ± 0.8*++	33.2 ± 0.6	P<0.001
Body Temperature (°C)	37.0 ± 0.1	38.1 ± 0.1	P<0.001

significantly in the dependent position compared with the preceding mean values recorded at heart level (Figure 17 & Table 7). During body heating, mean skin blood flow and skin temperature measured at heart level were significantly elevated (Table 7). In contrast to the dorsum of the foot, when the foot was placed in the dependent position there was a minor nonsignificant reduction in flow to a mean value which was significantly higher than the corresponding value measured in the same position before heating (Figure 17) i.e. the postural fall in flow normally seen before heating was almost abolished during heating. This finding was paralleled by the absence of any significant change in mean skin temperature in the dependent position during heating (Table 7).

Body temperature was also significantly increased by an average of 1.1°C during body heating (Table 7). Repeat tests performed on 3 of the subjects indicated that the postural changes in skin blood flow in both skin areas, and the changes in skin and body temperatures before and during indirect heating were quite reproducible (Table 8).

Comparison of skin blood flow and skin temperature data obtained from the dorsum of the foot and the pulp of the big toe:

Before indirect heating, mean skin blood flow measured in the horizontal and dependent positions in the toe pulp was significantly (6-7 times) higher than that measured on the

Table 8: Reproducibility of the postural changes in skin blood flow (as mV), and the changes in skin and body temperatures before and during indirect heating in 3 male subjects. H, with the foot horizontal, D, during the 4th minute with the foot dependent. The postural fall in flow was calculated as H-D/H%. C.V., coefficient of variation.

	Before Heating			During Heating		
	1st Test	2nd Test	C.V.	1st Test	2nd Test	C.V.
Dorsum of the Foot						
Skin Blood Flow (H)	12.3 ± 3.7	14.4 ± 3.7	11.1%	23.6 ± 4.3	21.7 ± 0.8	5.9%
Skin Blood Flow (D)	3.2 ± 1.2	4.2 ± 1.3	19.1%	4.6 ± 0.9	6.3 ± 3.3	22.1%
Postural Fall in Flow	73.9 ± 2.9%	72.0 ± 3.9%	1.8%	79.3 ± 5.8%	71.1 ± 15.4%	7.7%
Skin Temperature, °C (H)	29.5 ± 0.6	30.5 ± 0.4	2.4%	32.7 ± 0.5	33.3 ± 0.3	1.3%
Skin Temperature, °C (D)	29.3 ± 0.6	30.2 ± 0.4	2.1%	32.3 ± 0.5	33.0 ± 0.2	1.5%
Pulp of the Big Toe						
Skin Blood Flow (H)	154.3 ± 61.8	170.3 ± 24.7	7.0%	226.7 ± 84.9	313.0 ± 57.9	22.6%
Skin Blood Flow (D)	25.9 ± 18.3	16.6 ± 2.3	30.9%	179.2 ± 116.4	249.0 ± 90.5	23.1%
Postural Fall in Flow	86.3 ± 6.3%	89.8 ± 2.2%	2.8%	34.8 ± 31.0%	16.9 ± 35.5%	49.0%
Skin Temperature, °C (H)	27.6 ± 2.3	27.9 ± 0.9	0.8%	32.0 ± 1.2	32.7 ± 0.4	1.5%
Skin Temperature, °C (D)	27.2 ± 2.2	27.5 ± 0.9	0.8%	32.0 ± 1.3	32.8 ± 0.2	1.7%
Body Temperature (°C)	36.97 ± 0.03	36.8 ± 0.03	0.3%	38.0 ± 0.3	37.7 ± 0.1	0.6%

dorsum of the foot, whereas mean skin temperature in the toe pulp was significantly lower than that recorded on the dorsum of the foot (Table 7). During heating the mean horizontal flow in the dorsum of the foot increased by 79.6%, and mean dependent flow by 21.3%, whereas in the toe pulp, mean horizontal flow increased by 144.0% and mean dependent flow by 692.8%. Also, mean horizontal flow in the toe pulp during heating was about 10 times, and mean dependent flow 42 times higher than the corresponding mean flow values measured on the dorsum of the foot (Table 7).

Changes in Arterial Blood Pressure and Heart Rate:

Before indirect heating, brachial systolic blood pressure measured during the 4th-5th minute after the foot was lowered in the dependent position was slightly (average of 1.5 mmHg) but not significantly lower than the mean value measured when both feet were maintained at heart level. However, there was a small but significant increase in diastolic blood pressure (2.6 mmHg) and heart rate (2.5 beats/min), a small but significant reduction in pulse pressure (4.1 mmHg), but the mean arterial pressure was not significantly changed (Table 9)

During indirect heating, systolic blood pressure measured with both feet kept at heart level was slightly (3 mmHg) but not significantly increased, whereas there was a small significant reduction in diastolic blood pressure (3.3mmHg), a significant increase in pulse pressure (6.3 mmHg) and heart rate (8.8

Table 9: The changes in arterial blood pressure (brachial artery) and heart rate measured during the 4th -5th min after one foot was passively lowered from the horizontal position to a dependent position 50 cm below heart level, both before and during indirect heating in 12 healthy male subjects. *P<0.05, **P<0.01, ***P<0.001, as compared with the corresponding values measured before heating. NS, nonsignificant. Mean arterial pressure was calculated as diastolic pressure plus 1/3 pulse pressure.

	Before Indirect Heating			During Indirect Heating		
	Both Feet Horizontal (H)	One Foot Dependent (D)	Statistical Significance (H vs D)	Both Feet Horizontal (H)	One Foot Dependent (D)	Statistical Significance (H vs D)
Arterial Blood Pressure (mm Hg)						
Systolic Blood Pressure	121.3 ± 2.1	119.8 ± 1.3	NS	124.3 ± 2.6	121.8 ± 2.1	P<0.05
Diastolic Blood Pressure	78.8 ± 1.1	81.4 ± 1.2	P<0.001	75.5 ± 1.1***	77.6 ± 1.5**	NS
Arterial Pulse Pressure	42.5 ± 1.8	38.4 ± 1.2	P<0.02	48.8 ± 2.8*	44.3 ± 2.9	P<0.001
Mean Arterial Pressure	92.9 ± 1.2	94.2 ± 1.1	NS	91.8 ± 1.1	92.3 ± 1.1	NS
Heart Rate (beats/min)	67.0 ± 1.6	69.5 ± 1.4	P<0.001	75.8 ± 1.7***	79.3 ± 1.6***	P<0.001

beats/min) but the mean arterial pressure was maintained. When the foot was passively lowered in the dependent position during heating, systolic blood pressure measured during the 4th-5th minute was significantly reduced (2.5 mmHg), diastolic pressure showed a nonsignificant increase (2.1 mmHg), pulse pressure was significantly reduced (4.5 mmHg), heart rate was significantly increased (3.5 beats/min), but the mean arterial pressure was again not significantly altered (Table 9).

Section 2 EFFECT OF INDIRECT HEATING ON THE POSTURAL CHANGES IN TOE NAILFOLD CAPILLARY BLOOD FLOW

Introduction

Using laser Doppler flowmetry, it has been shown in the preceding study that during body heating the postural control of foot skin blood flow was impaired in areas rich in arteriovenous anastomoses, whereas it was maintained in areas lacking these anastomoses. However, it is not known whether such impairment involves the capillary flow component of the vascular bed under study. Since the laser Doppler flowmeter has been suggested to record flow from capillaries as well as other superficial vessels (Tooke et al, 1983), it cannot distinguish between nutritive (capillary) and non-nutritive (shunt) components of skin blood flow. Therefore, the aim of the present study was to compare the postural changes in capillary blood flow of the toe nailfold as measured by the direct technique of television microscopy with the skin blood flow changes recorded from the same skin area by laser Doppler flowmetry, both before and during indirect heating.

Methods

Experiments were performed on seven healthy male subjects aged 22-57 years (mean age 35 years).

Experimental Procedure

Capillary blood flow velocity, the stop flow time, erythrocyte column diameter and capillary volume flow were measured in the nailfold of the right big toe using video-television microscopy as described in the "METHODOLOGY". The flow signal from the same site was then recorded using laser Doppler flowmetry. Skin temperature was also continuously measured using a sensitive thermocouple attached to the skin (by adhesive tape) about 2mm proximal to the nailfold. Recordings were made first with the subject lying down and both feet at heart level (H), then with the subject sitting (quietly) and the feet at a mean distance of 97cm below the heart (D). The postural fall in flow was calculated as (H-D) x 100/H. Sublingual temperature was measured using a mercury clinical thermometer. Brachial arterial blood pressure (using sphygmomanometry) and heart rate (radial pulse) were also measured in the horizontal and, after at least 5 minutes, in the sitting position. The whole procedure was performed before and 30 minutes after heating the trunk by an electric blanket (Easipower Ltd, Hythe, U.K.) with its temperature maintained at 44°C for at least one hour.

RESULTS

a. Postural changes in toe nailfold blood flow before indirect heating

Arterial limb capillary blood flow velocity and capillary

volume flow measured in the nailfold of the great toe fell significantly to very low values in the sitting position as compared with values obtained in the supine position. Periods of complete cessation of flow became more common and the percentage of stop flow time increased significantly in the dependent position (Table 10). The erythrocyte column width (which might be taken as an approximate estimate of internal capillary diameter) in the arterial limb of the capillaries increased significantly in the dependent position by an average of 2.4 μm (Table 10). All the recorded capillaries remained filled with blood, even during the periods of stop flow, and the capillary density (number of capillaries per microscope field) in the sitting position remained unchanged from that observed in the supine position.

The blood flow signal recorded from the toe nailfold using laser Doppler flowmetry also showed a significant reduction in the sitting as compared with the supine position (Figure 18, Table 10). Moreover, the values of the postural fall in capillary blood flow and laser Doppler flow expressed as a percentage relative to supine rest flow were quite similar. There was also a significant reduction in toe nailfold skin temperature on dependency (Table 10).

b. <u>The postural changes in toe nailfold blood flow during indirect heating</u>

After 30 minutes of body heating, oral temperature was

Table 10: Effect of indirect heating on the postural changes in capillary blood flow and laser Doppler flow recorded from the nailfold of the right big toe in 7 healthy male subjects. H, with the subject in the horizontal position and the toe at heart level, D, after at least 5 min with the subject sitting quietly and the toe 97 cm below the heart. Results are means ± SEM, *P<0.05, **P<0.01, ***P<0.001 as compared with (H), NS, nonsignificant. +Uncorrected for periods of stop flow.

		Before Heating	During Heating	Statistical Significance
Capillary Blood Velocity (CBV)+,mm/sec	(H)	0.479 ± 0.142	1.147 ± 0.262	P<0.01
	(D)	0.036 ± 0.008**	0.082 ± 0.019**	P<0.05
Postural Fall in CBV		90.6 ± 2.3%	91.6 ± 2.4%	NS
Erythrocyte Column Diameter (μm)	(H)	7.634 ± 0.575	7.93 ± 0.23	NS
	(D)	10.04 ± 0.818**	9.33 ± 0.35***	NS
Capillary Blood Flow (CBF)+, pl/sec	(H)	26.76 ± 11.08	58.79 ± 14.06	P<0.01
	(D)	2.94 ± 0.78**	5.89 ± 1.6**	NS
Postural Fall in CBF		82.5 ± 5.0%	88.2 ± 3.5%	NS
Percentage of Stop Flow Time	(H)	6.7 ± 5.0%	0.0	P<0.05
	(D)	44.7 ± 11.9%*	4.9 ± 1.6%**	P=0.05
Laser Doppler Flow (LDF), mV	(H)	59.3 ± 16.5	210.7 ± 48.5	P<0.01
	(D)	6.9 ± 1.6**	22.3 ± 3.4**	P<0.01
Postural Fall in LDF		85.1 ± 3.3%	86.2 ± 2.9%	NS
Toe Nailfold Temperature, °C	(H)	29.2 ± 1.2	33.1 ± 0.7	P<0.01
	(D)	28.3 ± 1.2***	32.9 ± 0.5	P<0.01
Body Temperature, °C		36.53 ± 0.05	37.14 ± 0.05	P<0.001

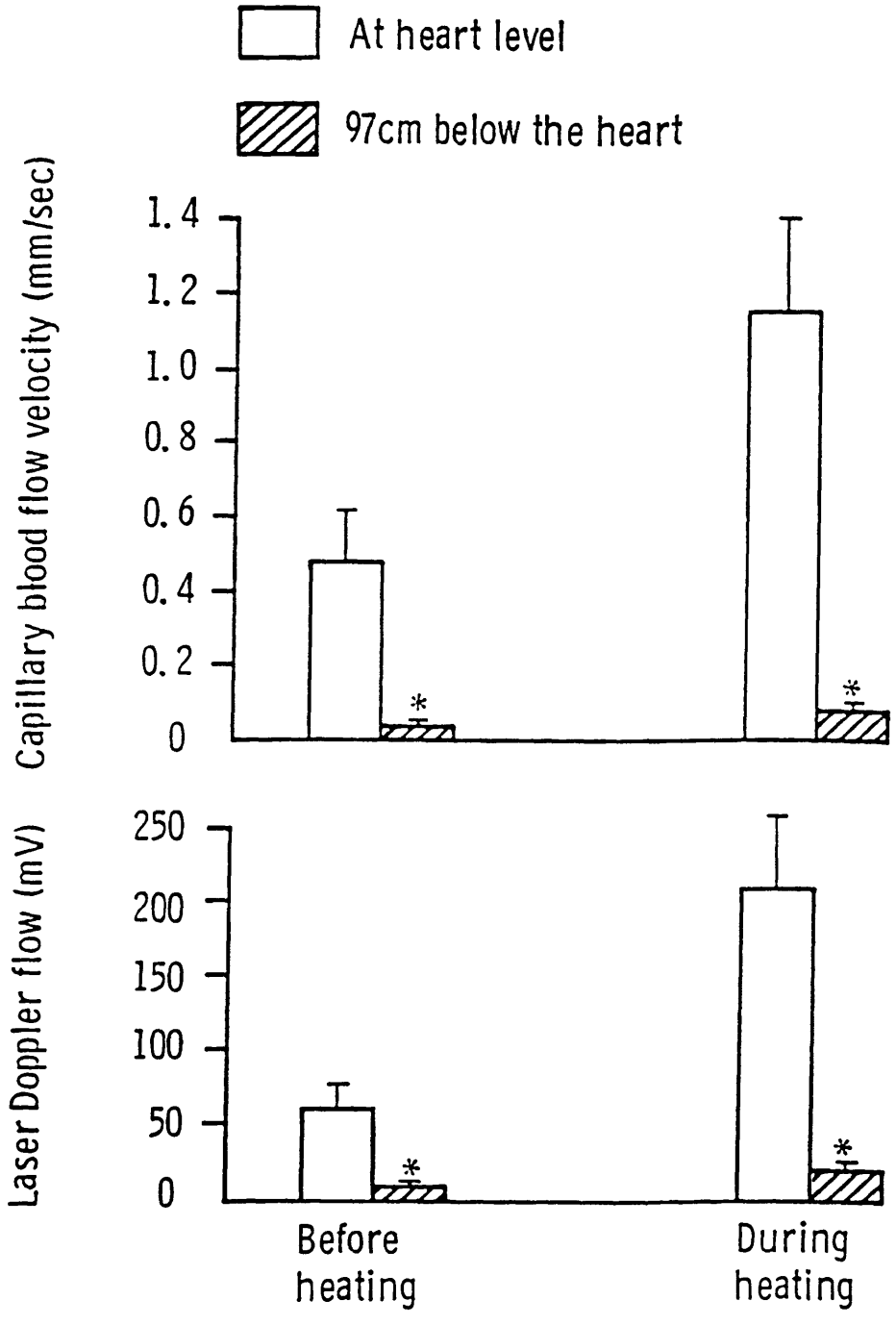


Figure 18: The postural changes in capillary blood flow velocity (uncorrected for periods of stop flow) and laser Doppler flow measured in the nailfold of the right big toe in 7 healthy male subjects before and during indirect heating. *P<0.01, as compared with mean values measured at heart level. Values are given as means with bars indicating SEM.

significantly elevated by an average of 0.6°C, and toe nailfold skin temperature measured at heart level increased significantly by about 4°C (Table 10). Despite the significant increase in toe nailfold capillary blood velocity, capillary blood flow and laser Doppler flow measured at heart level during indirect heating, all values fell significantly in the sitting position, and the percentage fall in flow on dependency remained unchanged (Table 10, Figure 18). There were virtually no periods of stop flow in the capillaries recorded in the supine position during heating, though the percentage of stop flow time increased significantly in the sitting position but only to a mean value which was significantly lower than that obtained before heating (Table 10). The erythrocyte column width showed only a slight nonsignificant increase (0.3μm) when measured at heart level during heating, but was significantly increased in the dependent position by an average of 1.4μm (Table 10).

Capillary blood flow corrected for periods of stop flow

The capillary blood flow values given in Table 10 were calculated from erythrocyte column width and capillary blood velocity uncorrected for periods of stop flow. When the mean capillary blood velocity measured for each subject was corrected for the percentage of the time of stop flow, the capillary volume flow values were 26.42 ± 11.15 and 1.67 ± 0.51 pl/sec in the supine and sitting positions before heating, and 58.79 ± 14.06 and 5.63 ± 1.52 pl/sec during heating

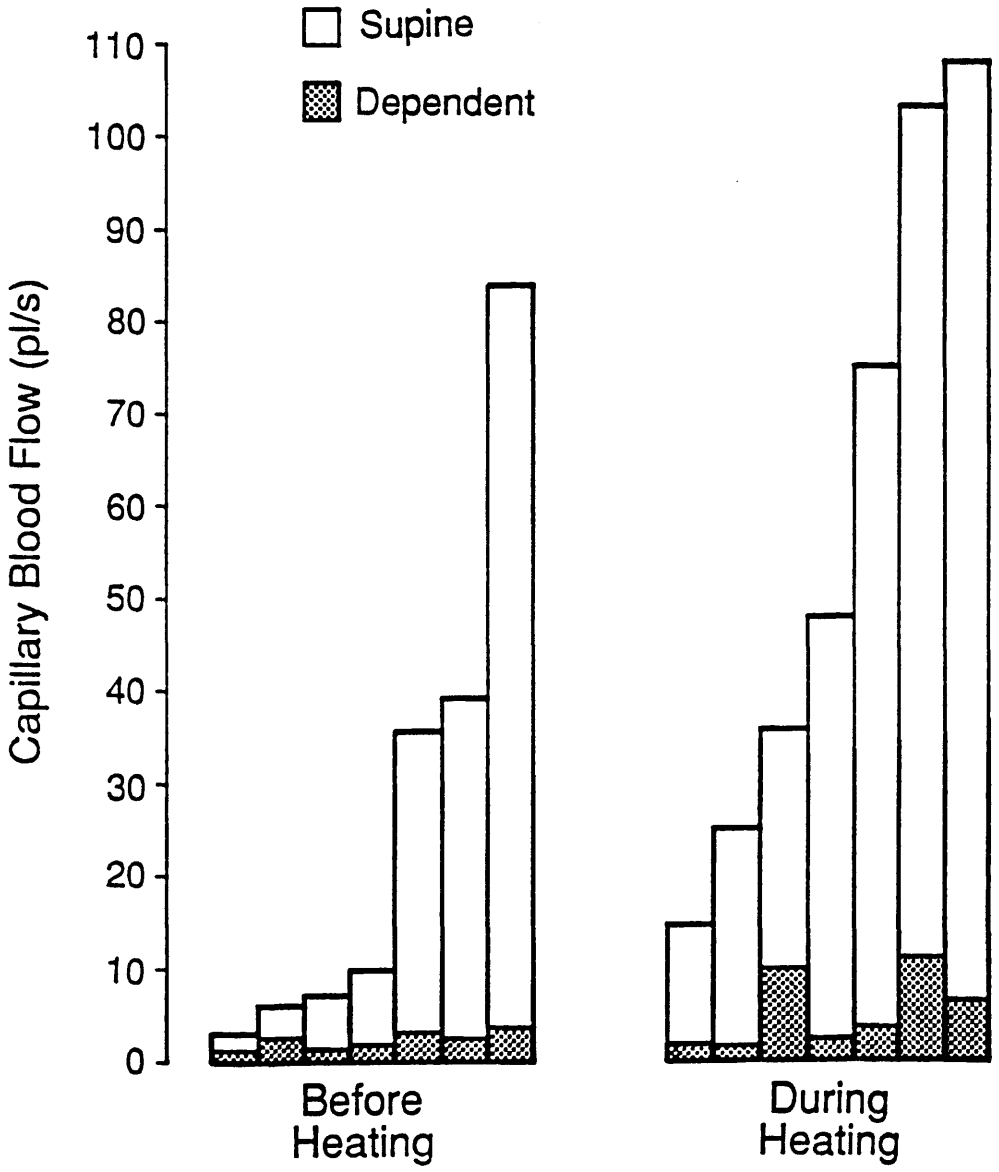


Figure 19: The postural changes in capillary blood flow of the toe nailfold (corrected for periods of stop flow) measured before and during indirect heating in 7 healthy male subjects. Individual values are shown.

respectively. Although 3 of these corrected mean values were not significantly different from the uncorrected mean values given in Table 10, the corrected mean capillary blood flow measured in the dependent position before heating was significantly lower than the uncorrected mean shown in Table 10 (1.67 ± 0.51 vs 2.94 ± 0.78 pl/sec, $P<0.01$). Figure 19 presents the individual capillary blood flow values calculated in the supine and dependent positions before and during indirect heating as corrected for the percentage of time of stop flow.

c. Correlation between toe nailfold capillary blood velocity and laser Doppler flow

Strong positive correlations were found between toe nailfold capillary blood flow velocity as measured by television microscopy and the flow values obtained from the same skin area using laser Doppler flowmetry in the supine and dependent positions both before and during indirect heating (Figures 20-23). However, there was often a small positive intercept at the laser Doppler flow axis at zero capillary flow velocity in all correlations.

d. The postural changes in arterial blood pressure and heart rate before and during indirect heating

Before indirect heating and at least 5 minutes after the

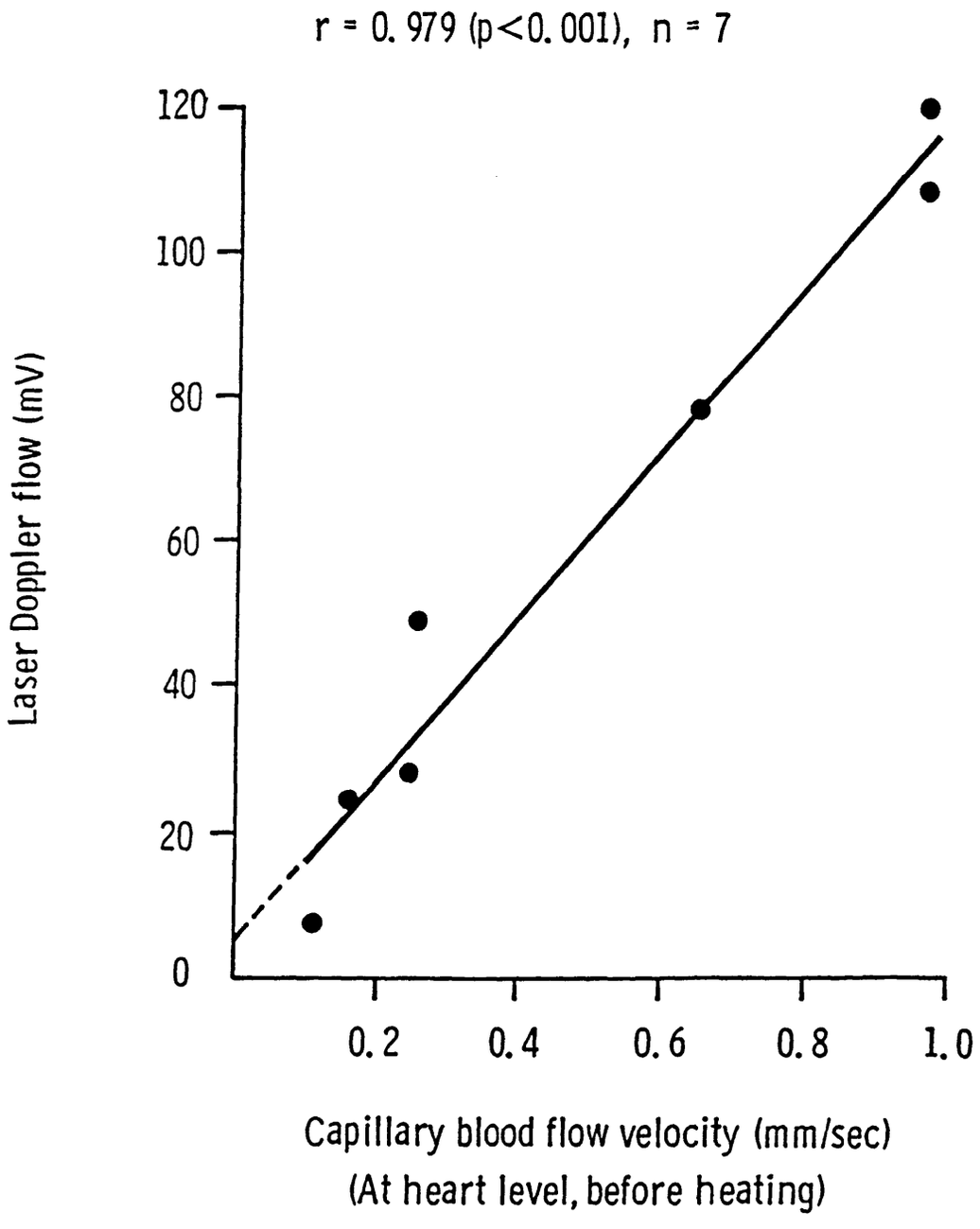


Figure 20: Correlation between capillary blood flow velocity measured by television microscopy and the flow signal measured by laser Doppler flowmetry, when recorded at heart level from the nailfold of the right big toe, before indirect heating.

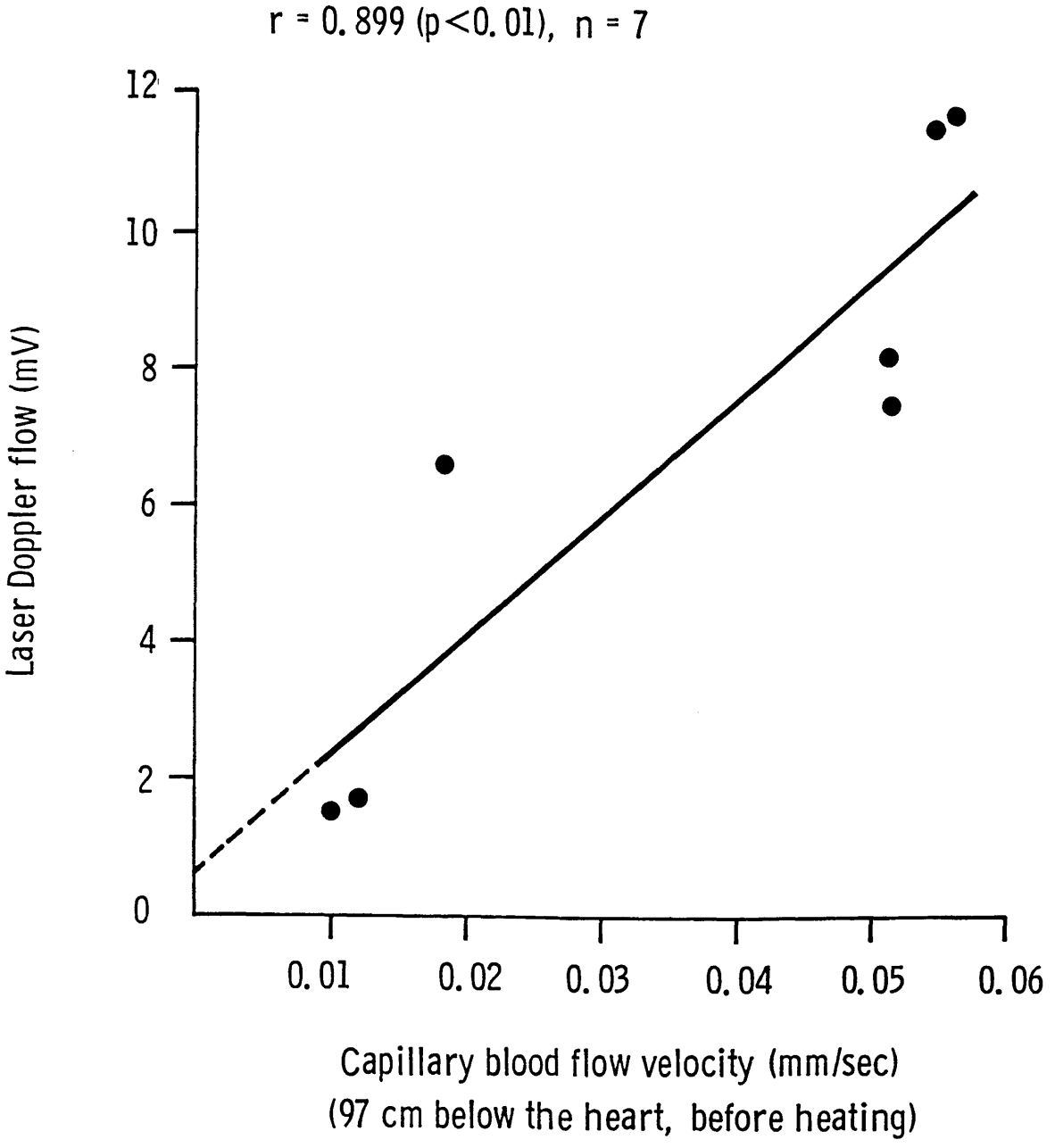


Figure 21: Correlation between toe nailfold capillary blood flow velocity and laser Doppler flow when measured 97 cm below the heart before indirect heating.

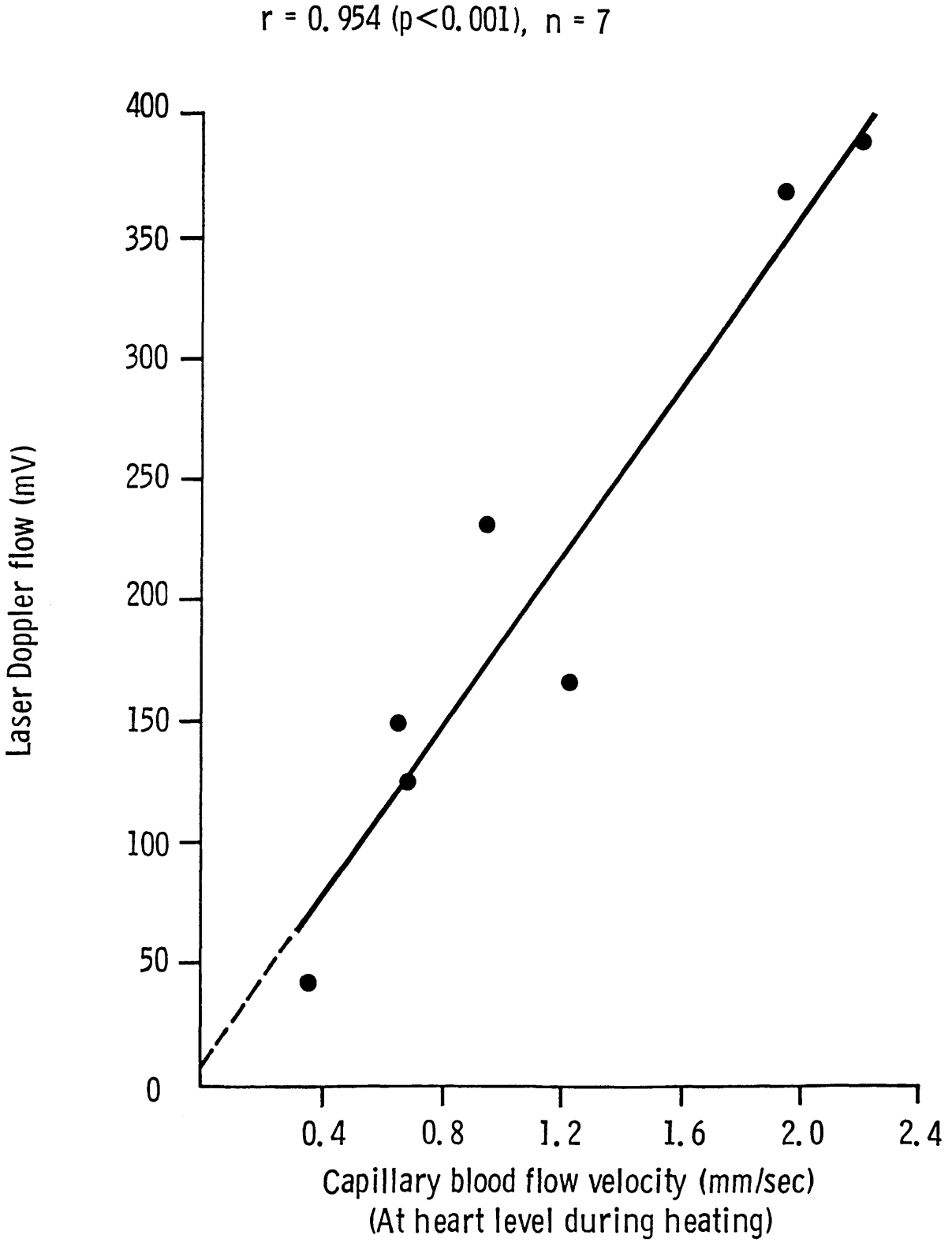


Figure 22: Correlation between toe nailfold capillary blood flow velocity and laser Doppler flow measured at heart level during indirect heating.

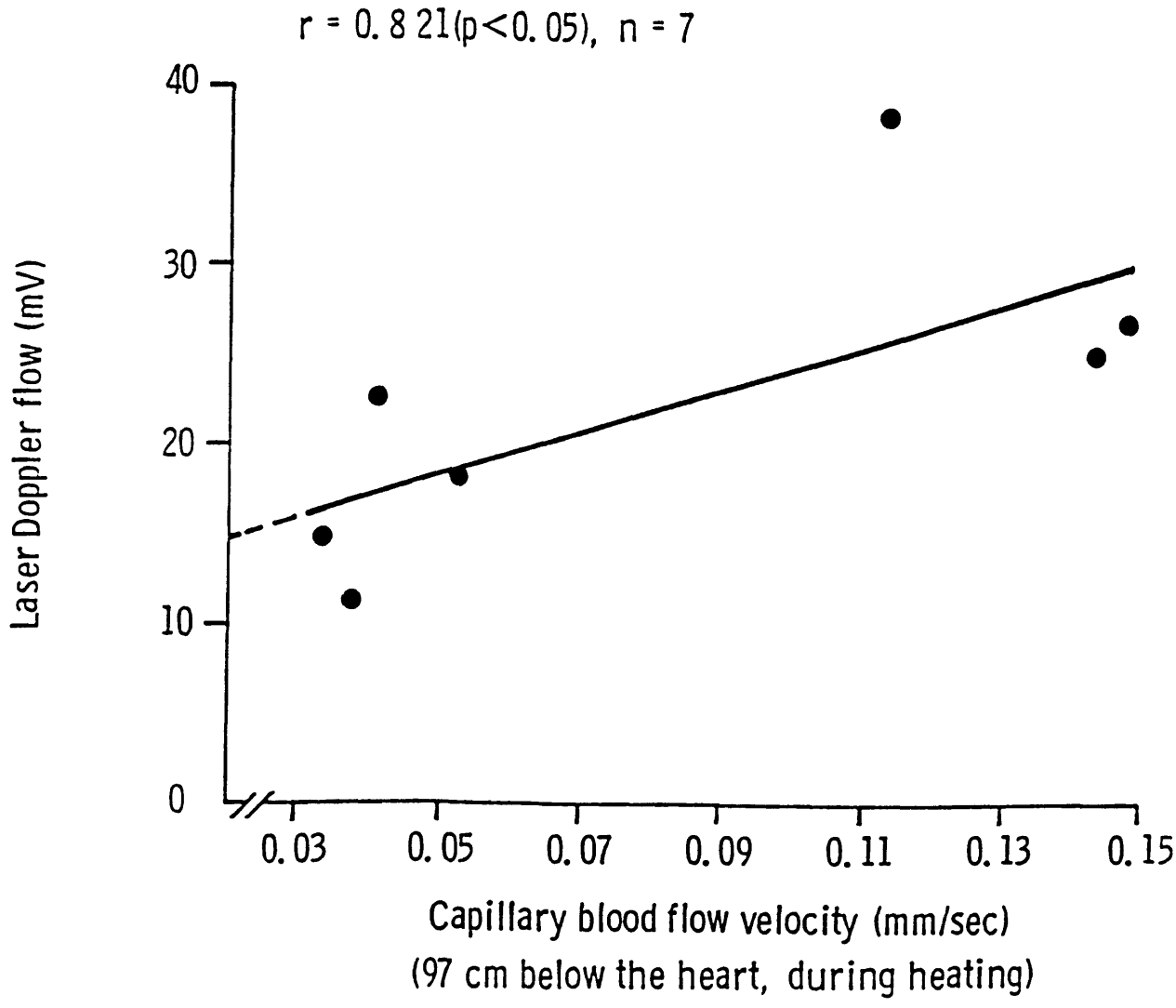


Figure 23: Correlation between toe nailfold capillary blood flow velocity and laser Doppler flow measured 97 cm below the heart during indirect heating (using Spearman's rank correlation).

subject changed from the supine to the semierect position, systolic blood pressure was unchanged but diastolic blood pressure, mean arterial pressure and heart rate were increased and pulse pressure was reduced (Table 11).

During indirect heating, with the subject in the supine position, there was a small nonsignificant increase in systolic pressure, a reduction in diastolic pressure and an increase in pulse pressure and heart rate but the mean arterial pressure remained essentially unchanged. In the semierect position during heating, systolic and pulse pressures were significantly reduced, diastolic pressure and heart rate were significantly increased but the mean arterial pressure was not significantly changed (Table 11).

Table 11: The changes in arterial blood pressure and heart rate measured in the supine and semierect position in 7 healthy male subjects before and after indirect heating. Mean arterial pressure was calculated as diastolic pressure plus 1/3 pulse pressure. *P<0.01, ** P<0.001 as compared with the corresponding mean values before heating. NS, nonsignificant.

	Before Indirect Heating			During Indirect Heating		
	Supine	Semi erect	P	Supine	Semi erect	P
Arterial Blood Pressure (mm Hg)						
Systolic Blood Pressure	117.6 ± 2.3	117.9 ± 3.0	NS	119.7 ± 2.5	114.6 ± 1.6	<0.05
Diastolic Blood Pressure	69.9 ± 3.0	80.6 ± 3.2	<0.001	66.7 ± 2.5*	73.6 ± 2.7*	<0.01
Arterial Pulse Pressure	47.7 ± 1.7	37.4 ± 1.3	<0.01	53.0 ± 2.0*	41.0 ± 1.7	<0.01
Mean Arterial Pressure	85.8 ± 2.7	93.0 ± 3.1	<0.01	84.4 ± 2.3	87.2 ± 2.3*	NS
Heart Rate (beats/min)	61.4 ± 1.5	67.9 ± 1.7	<0.001	68.0 ± 1.6**	75.7 ± 2.0*	<0.01

Section 3 EFFECT OF CHANGES IN LOCAL SKIN TEMPERATURE ON THE POSTURAL CHANGES IN FOOT SKIN BLOOD FLOW

Introduction

As has been pointed out earlier in the main INTRODUCTION, the thermoregulatory function of the cutaneous circulation is largely mediated by changes in skin blood flow not only in response to changes in environmental and core temperatures but also in response to changes in local skin temperature (Spealman, 1945).

In the 2 preceding sections of this chapter, it has been shown that foot blood flow measured under resting conditions is reduced on standing or even when the extremity is passively lowered below heart level in supine subjects, a response thought to be mediated mainly by local control mechanisms (Gaskell & Burton, 1953; Mellander et al, 1964; Henriksen & Sejrsen, 1976).

On the other hand, changes in local skin temperature (e.g. by moderate local cooling or warming) not only result in vasoconstriction or vasodilatation (Allwood & Burry, 1954; Hellon, 1963) but can also produce variations in capillary hydrostatic pressure (Levick & Michel, 1978), capillary filtration rate (Landis & Gibbon, 1933) and vascular smooth muscle reactivity (Keatinge & Harman, 1980; Vanhoutte, 1980). However, the influence of changes in local skin temperature on

the posturally induced vasoconstriction is still unclear. Since it has been demonstrated in "Section 1" of this chapter that, during indirect heating in man (resulting mainly in centrally elicited thermoregulatory vasodilatation), the postural vasoconstrictor response was markedly attenuated in skin areas rich in arteriovenous anastomoses but preserved in areas lacking these anastomoses, the aim of the present study was to investigate the effect of locally induced alterations in skin temperature on the postural changes in skin blood flow in the human foot.

Methods

Experiments were performed on 38 healthy adult subjects (31 males and 7 females), aged 23-70 years (mean age 37 years).

Induction of Changes in local Skin Temperature

Protocol 1: In 12 of the subjects, the postural changes in foot skin blood flow were recorded first at a skin temperature of 30°C, then during moderate local cooling and warming induced by blowing cold or hot air currents from a hair dryer to achieve skin temperatures of 28, 26 then 32, 34 and 36°C. By adjusting the distance between the hair dryer and the skin, it was possible to maintain a skin temperature within 0.1°C from the required level.

Protocol 2: In 26 subjects, higher ranges of skin

temperature (38-44°C) were achieved using a thermostatically-controlled disc-shaped heater (3 cm in diameter) supplied by the manufacturers with the laser Doppler flowmeter. However, in preliminary experiments, this heater was inadequate to achieve skin temperatures above 42°C, and for this purpose a specially constructed heater was used (Figure 24).

Measurement of Skin and Body Temperatures:

Skin temperature was measured continuously on the recording site using a sensitive thermocouple, and oral temperature was measured before and during each change in local skin temperature using a standard mercury clinical thermometer.

Measurement of the Postural Changes in Skin Blood Flow

The postural changes in foot skin blood flow were measured (by lowering the foot 50 cm below the heart in supine subjects) using laser Doppler flowmetry as described earlier (METHODOLOGY). Recordings were made during each change in local skin temperature from 2 skin areas: (i) the dorsum of the foot; an area where arteriovenous anastomoses have been shown to be absent, and (ii) the plantar surface of the big toe, where these shunt vessels are relatively numerous (Grant & Bland, 1931). After each change in local skin temperature, 10-15 minutes were allowed for the flow to attain a new steady level before the postural changes in flow were assessed.

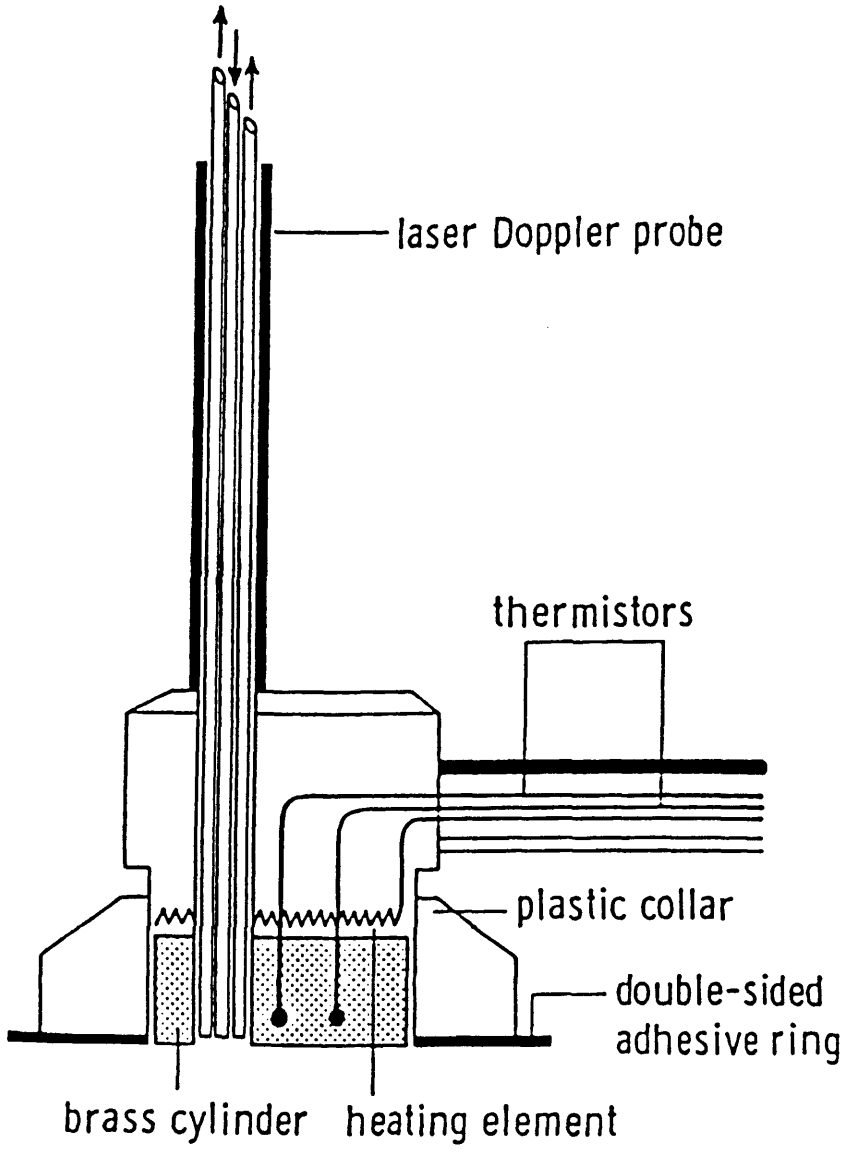


Figure 24: Diagram of the specially constructed heater & probe holder used for induction of local skin heating at 42-44°C. The heater consists of a cylindrical brass core heated by a small heating element. Two thermistors are inserted into the brass cylinder close to the face in contact with the skin, which feedback to a thermostat control unit. The circular face of the brass cylinder (0.9 cm in diameter) is held in direct contact with the skin to be heated when placed in a plastic collar attached to the skin by a double-sided adhesive ring. The heater has a small eccentric channel (2.4 mm in diameter) through which the laser Doppler probe tip carrying the optical fibres can be inserted. Rotation of the heater within the plastic collar allows blood flow to be measured from an area which has been directly heated immediately prior to rotation (Rayman, 1987).

Repeat tests were also performed on 6 of the subjects to check for the reproducibility of the experimental results at each change in local skin temperature. In 6 subjects (in protocol 2), the postural changes in flow were recorded again after spontaneous cooling of the heated skin area. In order to show whether central reflex effects are involved in the changes in flow measured at heart level after changes in the local temperature of one foot, in 12 experiments (in protocol 1) skin blood flow was simultaneously measured on anatomically identical skin sites in the contralateral foot using another laser Doppler flowmeter.

RESULTS

Two-way analysis of variance revealed a significant effect of both posture and skin temperature on skin blood flow ($P = 1.8 \times 10^{-37}$ & $P = 2.8 \times 10^{-7}$ for the dorsum of the foot, $P = 2.5 \times 10^{-37}$ & $P = 4.6 \times 10^{-8}$ for the pulp of the big toe respectively) as well as a significant interaction between posture and temperature on flow ($P = 0.00025$, & $P = 0.005$, for the 2 skin areas respectively).

Dorsum of the Foot

At skin temperatures of 26-36°C, despite a gradual increase in flow measured at heart level with increasing temperature, when the foot was lowered 50cm below the heart the flow fell significantly at each temperature tested (Table 12, Figure 25).

This maintenance of postural vasoconstriction despite rising skin temperature (within the physiological range) is emphasized by the relative constancy of the percentage fall in flow on dependency (Table 12). Moreover, moderate local warming (from 30 to 34 and 36°C) was even associated with greater postural fall in flow ($P<0.0009$, Friedman's test).

With further skin heating to 38-40°C, the flow measured at heart level rose significantly. Although there was still a significant fall in flow on dependency (Table 13, Figure 25), the marked drop in the postural fall in flow (%) indicated partial attenuation of the normal postural response. At higher skin temperatures (40-44°C), the horizontal flow was greatly increased but the postural fall in flow was completely abolished, as indicated by a significantly higher flow in the dependent position and a negative percentage fall in flow (Table 13). It was also found, using Friedman's test, that local heating above 38°C had a significant effect on the % fall in flow on dependency ($P<0.0008$).

Plantar surface of the Big Toe

An almost similar pattern of response was found (Tables 12 & 13, Figure 26). Thus the postural vasoconstrictor response was maintained at 26-36°C, partially attenuated at 38-42°C and totally abolished at 42-44°C. However, the mean flow values measured in the toe pulp both in the horizontal and dependent positions were about 3-12 times higher than the corresponding

Table 12: The postural changes in foot skin blood flow measured during moderate local cooling and warming (Protocol 1) in 12 healthy male subjects. The postural fall in flow was calculated as (H-D) x 100/H. Values are given as means ± SEM. n, number of experiments. [a]P<0.05, [b]P<0.02, [c]P<0.01, [d]P<0.001, as compared with the mean values obtained at 30°C; the starting temperature.

	Dorsum of the Foot (n=6)				Plantar Surface of the Big Toe (n=6)			
	Skin Blood Flow (mV)				Skin Blood Flow (mV)			
Skin Temp. (°C)	At Heart Level (H)	50cm Below the Heart (D)	Postural Fall in Flow	Statistical Significance of (H) vs (D)	At Heart Level (H)	50cm Below the Heart (D)	Postural Fall in Flow	Statistical Significance of (H) vs (D)
26	6 ± 2[c]	3 ± 1[c]	61 ± 5%	P<0.01	51 ± 7[c]	9 ± 2[a]	82 ± 4%[a]	P<0.01
28	10 ± 3[c]	4 ± 1	60 ± 5%	P<0.02	76 ± 12[c]	17 ± 4[a]	75 ± 5%	P<0.01
30	14 ± 3	5 ± 1	64 ± 6%	P<0.01	132 ± 23	36 ± 10	72 ± 5%	P<0.01
32	19 ± 4	7 ± 2	64 ± 6%	P<0.01	225 ± 49[b]	65 ± 14[a]	71 ± 3%	P<0.01
34	27 ± 5[a]	6 ± 1	75 ± 4%[a]	P<0.01	268 ± 55[c]	73 ± 13[c]	71 ± 4%	P<0.01
36	36 ± 7[c]	8 ± 2	77 ± 4%[a]	P<0.01	300 ± 61[c]	75 ± 11[d]	73 ± 4%	P<0.01

Table 13: The postural changes in foot skin blood flow measured before and at different stages of local heating (Protocol 2) in 26 normal subjects. The postural fall in flow was calculated as (H-D) x 100/H. Values are given as means ± SEM, n, number of experiments. $^{a}P<0.05$, $^{b}P<0.02$, $^{c}P<0.01$, $^{d}P<0.001$, as compared with the mean values obtained at resting skin temperature (*). The minus sign of the postural fall in flow at mean skin temperatures of 41.9 & 42.6°C indicates increased flow in the dependent position above that in the horizontal position.

Dorsum of the Foot (n=19)					Plantar Surface of the Big Toe (n=19)				
	Skin Blood Flow (mV)					Skin Blood Flow (mV)			
Skin Temp. (°C)	At Heart Level (H)	50cm Below the Heart (D)	Postural Fall in Flow	Statistical Significance of (H)vs(D)	Skin Temp. (°C)	At Heart Level (H)	50cm Below the Heart (D)	Postural Fall in Flow	Statistical Significance of (H)vs(D)
30.1 ± 0.2*	21 ± 3	6 ± 1	68 ± 4%	P<0.001	28.9 ± 0.3*	201 ± 35	61 ± 13	70 ± 4%	P<0.01
33.3 ± 0.2	42 ± 7^{c}	15 ± 4^{a}	63 ± 7%	P<0.01	33.1 ± 0.2	309 ± 49^{d}	145 ± 27^{d}	55 ± 5%	P<0.001
38.9 ± 0.2	158 ± 27^{d}	100 ± 22^{b}	43 ± 8%^{c}	P<0.001	38.6 ± 0.1	458 ± 51^{d}	326 ± 45^{d}	28 ± 7%^{c}	P<0.01
41.9 ± 0.1	390 ± 41^{d}	476 ± 47^{d}	-26 ± 8%^{c}	P<0.01	40.9 ± 0.2	638 ± 54^{d}	522 ± 57^{d}	17 ± 7%^{c}	P<0.01
42.6 ± 0.1	613 ± 72^{d}	728 ± 62^{d}	-22 ± 8%^{c}	P<0.01	42.4 ± 0.1	790 ± 89^{d}	1096 ± 131^{d}	-40 ± 12%^{c}	P<0.02

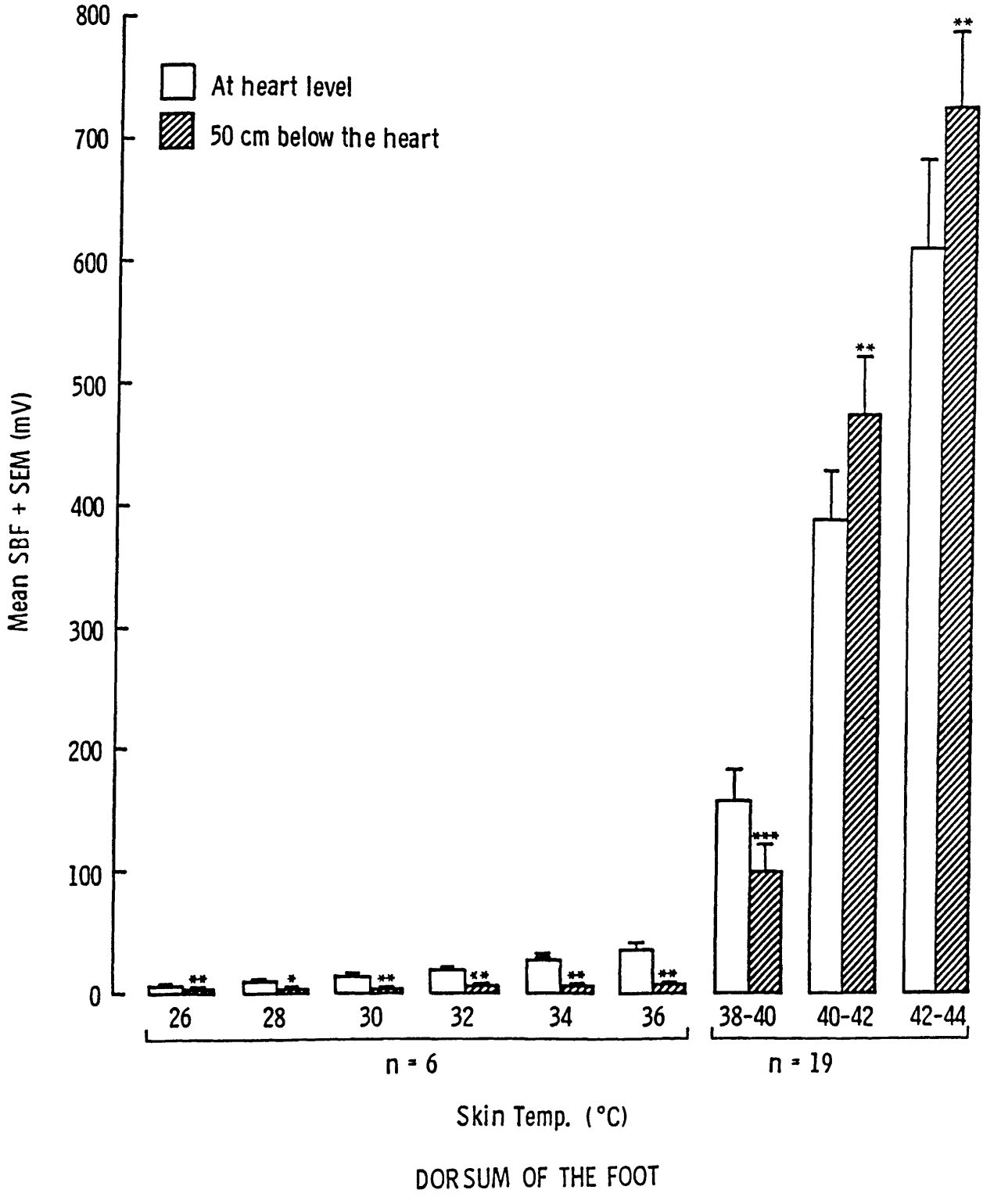


Figure 25: Effect of changes in local skin temperature on the postural changes in skin blood flow (SBF) measured on the dorsum of the foot. Results are given as means with bars indicating SEM. n, number of experiments. *P<0.02, **P<0.01, ***P<0.001 as compared with mean flow values measured at heart level.

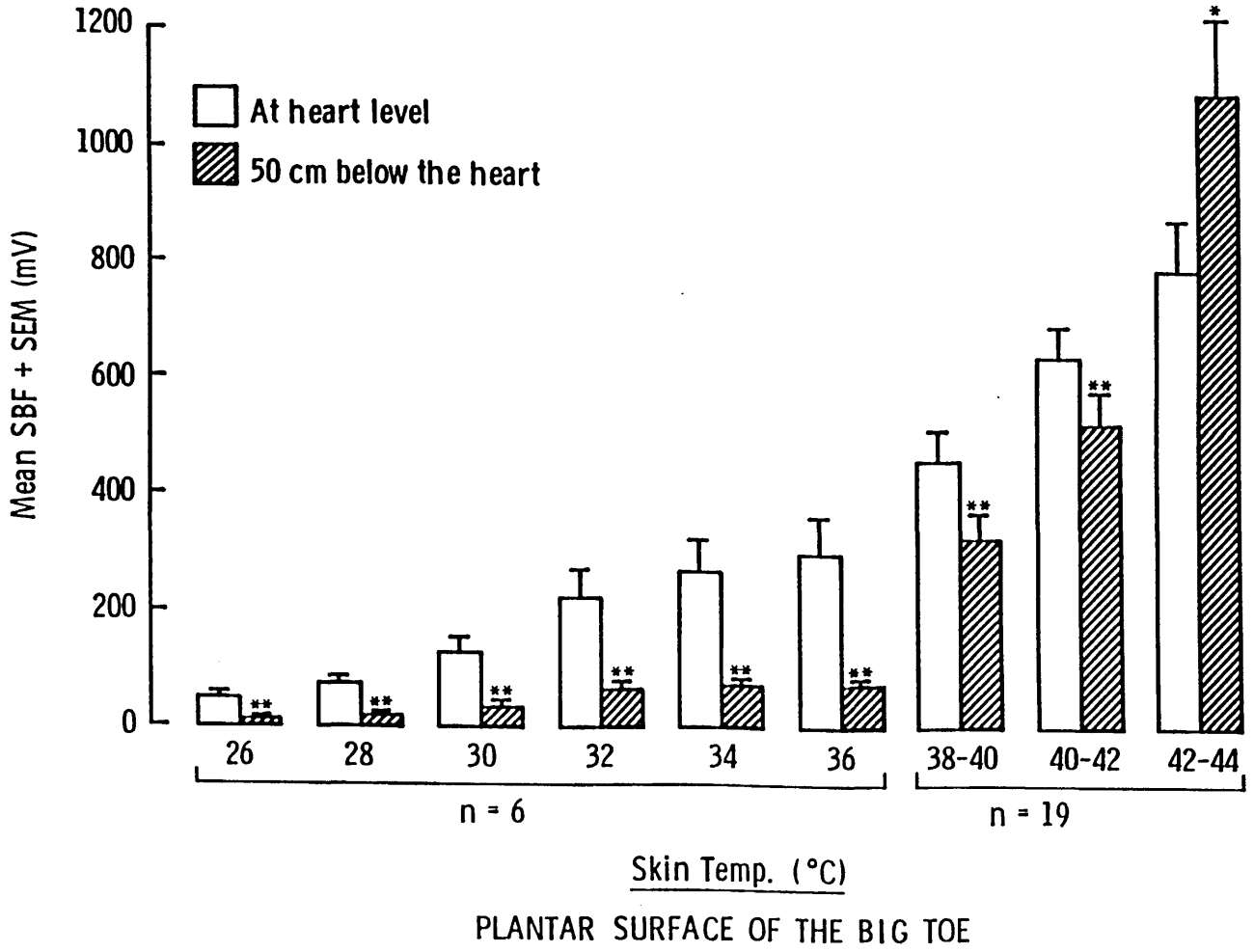


Figure 26: Effect of changes in local skin temperature on the postural changes in skin blood flow (SBF) measured on the plantar surface of the big toe. Results are means with bars indicating SEM. n, number of experiments, *P<0.02, **P<0.01, as compared with mean flow values measured at heart level.

values measured on the dorsum of the foot. Nonparametric analysis of variance (Friedman's test) revealed that both moderate local cooling (from 30 to 26°C) and local heating above 38°C produced significant changes in the % fall in flow on dependency ($P<0.0081$, $P<0.0075$, respectively). Repeat tests showed quite reproducible % fall in flow on dependency at any given local skin temperature as indicated by the low coefficients of variation (Table 14).

There was no significant change in body temperature during the course of any of the experiments performed.

After return of the heated area to resting skin temperature, the postural vasoconstrictor response was restored. On the dorsum of the foot, the flow fell by 79.5 ± 4.2% after spontaneous cooling as compared with 77.5 ± 3.8% before heating. On the plantar surface of the big toe the corresponding values were 81.8 ± 3.2% and 80.2 ± 3.5 % respectively.

It was also observed that the increased flow measured at heart level after local heating (30→36°C) in one foot (by 159 ± 23%, n = 4) was not accompanied by any significant change in flow in the contralateral foot (4.7 ± 0.2 %), whereas the reduction in flow after moderate local cooling (30→26°C) in one foot (by 55 ± 4%, n = 8) was almost always accompanied by a concomitant, although less marked, fall in flow (by 27 ± 2%) in the contralateral foot, indicating the involvement of a central

Table 14: Reproducibility of the postural changes in foot skin blood flow, expressed as the percentage ratio of the difference between flow measured in the horizontal (H) and dependent (D) positions to flow measured in the horizontal position (H-D/H%), both before (*) and at different stages of local heating in 6 healthy male subjects. n, number of experiments. The minus sign at temperatures above 40°C indicates increased flow in the dependent position above that in the horizontal position.

	Dorsum of the Foot (n=6)			Pulp of the Big Toe (n=6)		
Skin Temp (°C)	Postural Fall in Flow 1st Test	2nd Test	Mean Coefficient of variation	Postural Fall in Flow 1st Test	2nd Test	Mean Coefficient of variation
27 - 31*	72 ± 5%	71 ± 4%	8.0%	76 ± 4%	78 ± 3%	6.8%
32 - 36	69 ± 6%	70 ± 5%	5.1%	62 ± 5%	64 ± 5%	4.5%
38 - 40	48 ± 7%	46 ± 7%	3.8%	31 ± 7%	30 ± 6%	3.3%
40 - 42	-19 ± 14%	-17 ± 12%	2.7%	17 ± 5%	18 ± 4%	2.3%
42 - 43	-22 ± 8%	-20 ± 6%	1.6%	-40 ± 12%	-36 ± 11%	1.9%

reflex effect.

Comparison of the postural changes in flow in women and men before and during local heating

As shown in Table 15, the postural changes in foot skin blood flow obtained in women before and at different stages of local heating were qualitatively similar to those obtained in men. However, despite the presence of similar skin temperatures (at rest or induced by local heating), and despite recording flow from anatomically identical skin areas in both men and women, the mean flow values measured in women in both the horizontal and dependent positions at different temperatures were generally lower than the corresponding values measured in the men. Indeed, in the dorsum of the foot at a mean skin temperature of 33.4°C, the women had significantly lower mean flow recorded at heart level than that obtained in the men (26.5 ± 3.6 vs 51.6 ± 9.2 mV, $P<0.05$), and in the pulp of the toe, the mean dependent flow measured at 40.7°C in women was also significantly lower than the corresponding value obtained at a similar temperature in the men (397.2 ± 82.8 vs 629.7 ± 54.3 mV, $P<0.05$).

Table 15: The postural changes in foot skin blood flow (SBF, as mV) measured in 2 skin areas before (+) and during different stages of local heating. A comparison of data obtained in men and women. H, with the foot at heart level, D, during the 4th min with the foot dependent (50cm below the heart). The postural fall in flow was calculated as (H-D) x 100/H. The minus sign at the higher temperature indicates increased dependent flow above horizontal flow. *P<0.05, as compared with the corresponding mean value in the men. n, number of subjects.

	Dorsum of the Foot		Pulp of the Big Toe	
	Men (n = 8)	Women (n = 5)	Men (n = 7)	Women (n = 6)
Skin Temp+ (°C)	30.0 ± 0.2	29.9 ± 0.4	29.3 ± 0.5	27.9 ± 0.5
SBF (H)	19.2 ± 4.0	14.3 ± 2.2	225.0 ± 63.2	77.9 ± 27.6
SBF (D)	6.3 ± 1.1	4.6 ± 1.1	87.5 ± 28.5	32.7 ± 19.5
(H-D) x 100/H	61.1 ± 8.6%	67.0 ± 6.3%	64.2 ± 7.5%	67.8 ± 7.0%
Skin Temp (°C)	33.3 ± 0.2	33.4 ± 0.2	33.3 ± 0.2	32.9 ± 0.3
SBF (H)	51.6 ± 9.2	26.5 ± 3.6*	359.9 ± 69.5	249.6 ± 64.7
SBF (D)	17.7 ± 5.9	9.5 ± 2.5	162.6 ± 40.1	125.0 ± 39.1
(H-D) x 100/H	61.4 ± 10.7%	66.0 ± 4.2%	57.0 ± 6.3%	51.8 ± 6.9%
Skin Temp (°C)	39.1 ± 0.2	38.7 ± 0.3	38.7 ± 0.1	38.5 ± 0.2
SBF (H)	176.9 ± 34.0	128.2 ± 43.2	533.3 ± 67.5	369.6 ± 64.7
SBF (D)	102.8 ± 21.3	96.7 ± 51.2	382.7 ± 54.4	259.5 ± 69.3
(H-D) x 100/H	40.1 ± 5.8%	47.1 ± 18.9%	23.3 ± 10.2%	34.4 ± 9.9%
Skin Temp (°C)	42.0 ± 0.1	41.7 ± 0.1	41.0 ± 0.2	40.7 ± 0.2
SBF (H)	375.5 ± 57.6	413.2 ± 62.1	703.2 ± 82.5	561.2 ± 57.0
SBF (D)	459.1 ± 61.1	502.0 ± 80.5	629.7 ± 54.3	397.2 ± 82.8*
(H-D) x 100/H	-28.3 ± 11.9%	-21.4 ± 9.6%	4.7 ± 9.6%	31.4 ± 8.6%

DISCUSSION

1. The Postural Changes in Foot Skin Blood Flow Before and During Indirect Heating

The indirect heating experiments were performed at a constant and comfortable environmental temperature (22 ± 0.5°C) since local cooling has been shown to delay the onset and markedly reduce the vasodilatation associated with body heating (Allen et al, 1984).

The reduction in skin blood flow when the extremity is lowered below heart level, which has been previously described using venous occlusion plethysmography (Gaskell & Burton, 1953; Beaconsfield & Ginsburg, 1955b) and ^{133}Xenon clearance (Henriksen & Sejrsen, 1976), has been confirmed in this study using laser Doppler flowmetry. Although the exact mechanism underlying this response is still unclear, it has been suggested that the postural increase in cutaneous vascular resistance in response to the increase in local transmural pressure is largely mediated by local neurogenic (Henriksen & Sejrsen, 1976) or myogenic (Mellander et al, 1964) mechanisms. However, the relative role played by either or both of these mechanisms in the orthostatic increase in precapillary resistance remains to be elucidated.

The postural changes in skin blood flow observed before and during indirect heating cannot be ascribed to changes in

systemic blood pressure following the postural change, since mean arterial pressure remained essentially unchanged during the 4th to 5th minute of dependency both before and during heating. Because the perfusion pressure also remains unchanged when the limb is lowered below heart level, the observed changes in flow in the dependent foot can be ascribed mainly to changes in the local cutaneous vascular resistance. Indirect heating was accompanied by a significant increase in foot skin blood flow when measured at heart level. That this reflex thermoregulatory vasodilatation is due mainly to the release of sympathetic vasoconstrictor tone induced by the elevated core temperature (Gibbon & Landis, 1932; Pickering & Hess, 1933), gains much support from the results of nerve block experiments (Arnott & Macfie, 1948; Roddie et al, 1957b), and from the recording of decreased sympathetic efferent activity in human skin nerves during moderate body warming (Delius et al, 1972).

Arteriovenous Shunt Flow During Indirect Heating

It has been shown by using radioactive microspheres (Hales & Iriki, 1977) that warming the hypothalamus or the spinal cord in sheep is associated with a marked increase in flow through arteriovenous anastomoses, while capillary flow remains essentially unchanged. Moreover, elimination of the adrenergic supply to the hindlimb circulation in dogs (Cronewett et al, 1983) appears to cause a marked dilatation of arteriovenous anastomoses with little effect on capillaries. These findings

could explain the significant increase in skin blood flow seen in the pulp of the big toe during indirect heating, on the basis that this skin area contains a relatively large number of arteriovenous anastomoses (Grant & Bland, 1931; Nelms, 1963), which dilate after the release of sympathetic vasoconstrictor tone by body heating. On the other hand, the data cannot offer a reasonable explanation for the significant increase in skin blood flow in the dorsum of the foot where these shunt vessels are relatively few or even absent (Grant & Bland, 1931). However, if the significant increase in skin and presumably blood temperatures during body heating are taken into account, the increased flow measured at heart level in the dorsum of the foot and to a less extent also in the toe pulp, could be attributed to altered blood viscosity (Virgilio et al, 1964) or to the effects of heat on vascular smooth muscle (Vanhoutte, 1980) either directly or via alterations in the local concentrations of metabolites (Hales & Iriki, 1977).

The observations that (i) the mean rest flow was higher in the toe pulp, (ii) the increase in mean skin temperature during indirect heating was greater in the toe pulp (5°C pulp, 3°C dorsum), and (iii) the percentage increase in mean skin blood flow in the toe pulp measured at heart level in response to body heating was much greater (144% pulp, 79.6% dorsum) and the absolute increase in mean flow was significantly higher in the toe pulp (183 ± 60.4 mV pulp, 14.8 ± 3.1 mV dorsum, $P<0.01$), could all be accounted for by the relatively higher prevalence of arteriovenous anastomoses in the toe pulp. Similar findings

were observed in the terminal circulation of the fingers by laser Doppler flowmetry (Svensson et al, 1983). These observations add much support to the assumption that laser Doppler flowmetry not only measures flow in superficial capillaries and subpapillary vessels but can also pick up at least part of the slightly deeper shunt flow element (Svensson et al, 1983; Tooke et al, 1983).

Postural Vasoconstriction During Indirect Heating

The most striking observation in the present study was the marked difference between the two skin sites when the postural vasoconstrictor response was challenged by thermoregulatory vasodilatation. In the dorsum of the foot, the postural response was still preserved whereas in the toe pulp it was markedly attenuated. This finding became more evident when the postural fall in mean flow before and during indirect heating was calculated as a percentage relative to the preceding mean rest flow. In the dorsum of the foot, there was a marked postural fall in mean skin blood flow both before and during indirect heating (67.2% vs 77.8%). In contrast, in the plantar surface of the big toe, the postural fall in mean flow was only significant before heating (70.4%) compared with a non-significant fall during heating (3.9%) i.e. the interaction or competition between postural vasoconstriction and thermoregulatory vasodilatation was in favour of vasodilatation in skin areas with relatively high proportion of arteriovenous anastomoses, presumably to serve heat dissipation (Love, 1948).

The marked attenuation of the postural vasoconstrictor response in the toe pulp during body heating, in the present study, could explain the rise in toe temperature on standing during indirect heating (Youmans et al, 1935) and on upright tilting at high environmental temperature (Nielsen et al, 1939). Moreover, it has been shown that the vasoconstriction induced in the finger by lower body negative pressure was abolished by contralateral heating, suggesting that thermal stimuli could modify the nonthermoregulatory vasoconstrictor reflexes (Heistad et al, 1973).

These results suggest that the thermoregulatory function may override the local postural control of cutaneous vascular tone in areas rich in arteriovenous anastomoses, whereas the postural regulation of flow takes precedence over thermoregulation in areas without anastomoses.

2. The Postural Changes in Nailfold Capillary Blood Flow Before and During Indirect Heating

Since the laser Doppler flowmeter cannot measure the capillary component of the vascular bed separately, the technique of direct television microscopy was used to assess the postural changes in the capillary circulation of the toe nailfold, both at rest and during exposure to a thermal stress.

Capillary blood flow measured at rest in supine subjects exhibited cyclical variations with time, and in some

capillaries flow may stop and restart periodically. This intermittent flow is a manifestation of vasomotion (Davis et al, 1964; Fagrell et al, 1977). In the present study vasomotion was observed in nearly all capillaries with periods of cessation of flow in 6 of the 7 subjects studied. Synchronous variations in flow were sometimes observed in adjacent capillaries. Vasomotion appeared to be strongly influenced by local skin temperature. Indeed, there was a positive correlation between resting toe nailfold skin temperature and capillary blood flow velocity, whereas the percentage of stop flow time was negatively correlated with skin temperature (Figure 27).

On quiet standing, capillary blood flow measured in the arterial limb of the capillary before indirect heating was decreased to very low values. This reduction occurred despite a significant increase in arterial limb capillary diameter in the dependent position, and was achieved by a reduction in both capillary blood velocity and the duration of flow. During indirect heating, despite the significant increase in capillary blood flow measured in both the horizontal and dependent positions, the percentage of the postural fall in capillary blood flow was unchanged. Thus, it appears that the postural control mechanisms take precedence over thermoregulation in foot skin capillaries. This observation provides further support to the previous finding of preservation of the postural vasoconstrictor response in the dorsum of the foot where arteriovenous anastomoses are absent (Grant & Bland, 1931).

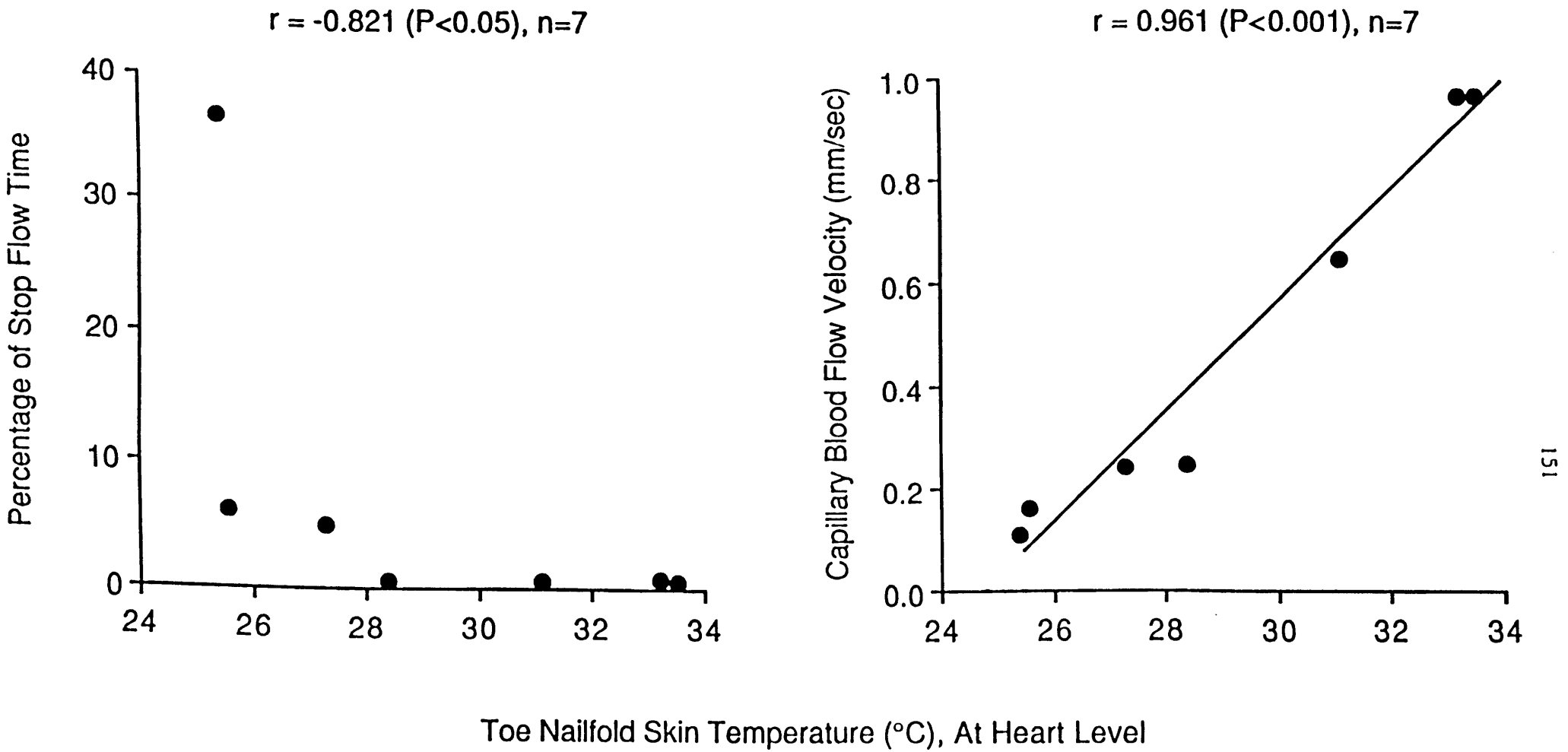


Figure 27: The relationship between resting toe nailfold skin temperature and both capillary blood flow velocity and the percentage of stop flow time measured at heart level before indirect heating in 7 healthy male subjects.

However, the increase in capillary flow during heating suggests that nutritive capillaries may also subserve a thermoregulatory function, but to a much smaller extent than venous plexuses and arteriovenous shunts (Conrad, 1971; Hales & Iriki, 1977).

The mechanism underlying the postural fall in capillary blood flow is not yet completely understood, but the postural increase in precapillary resistance is thought to be largely mediated by local mechanisms (Mellander et al, 1964; Henriksen, 1977; Levick & Michel, 1978). The preservation of the postural fall in capillary blood flow during the release of the central sympathetic vasoconstrictor tone by indirect heating adds much support to this view, and further suggests that the mechanisms involved in the capillary response to posture are probably independent of those controlling flow through arteriovenous shunts. These mechanisms might involve vascular resistance elements in close proximity to the capillary bed, presumably distal to the shunts. Although the results seem to support this assumption indirectly, this study, however, provides no direct evidence regarding the location of such mechanisms.

The increased capillary hydrostatic pressure during standing (Levick & Michel, 1978) is expected to result in increased capillary filtration. However, the increase in precapillary resistance on standing not only results in a reduction in capillary blood flow, which (with continued filtration) permits a build-up of capillary oncotic pressure (Moyses & Michel, 1984), but also in a reduction in the expected rise in

capillary hydrostatic pressure (Levick & Michel, 1978). These findings suggest that the postural vasoconstrictor response might be considered as one of the physiological mechanisms which limit the rate of oedema formation on standing (Mellander et al, 1964; Levick & Michel, 1978; Sejrsen et al, 1981a).

The significant correlations found between toe nailfold capillary blood flow velocity as measured by television microscopy and the flow signals measured from the same site using a laser Doppler flowmeter, both in the supine and standing positions before and during indirect heating, confirm the value of laser Doppler flowmetry as a reliable non-invasive technique capable of assessing reflex changes in superficial skin microcirculation. However, the small positive intercept on the laser Doppler flow axis at zero capillary blood flow velocity (Figures 20-23) might be taken as another indication that laser Doppler flowmetry records flow from other superficial skin blood vessels in addition to the capillaries.

3. The Postural Changes in Foot Skin Blood Flow During Alterations in Local Skin Temperature

The relationship between local skin temperature and skin blood flow previously reported using venous occlusion plethysmography (Allwood & Burry, 1954), has been confirmed in this study using laser Doppler flowmetry. More importantly, it has been demonstrated that the postural reduction in foot skin blood flow was maintained throughout a physiological range of skin

temperature of 26-36°C, whereas it was markedly attenuated at 38-40°C and totally abolished at or above 42°C. Indeed, at this temperature skin blood flow was higher in the dependent than in the horizontal position.

Mechanisms of Changes in Flow After Changes in Local Temperature:

Because room and body temperatures did not show any significant change throughout the experiments, the observed changes in skin blood flow after local cooling or heating are likely to be attributable to locally-induced changes in skin temperature.

The changes in flow after local cooling or heating are believed to be mainly mediated by local mechanisms (Crockford et al, 1962; Hellon, 1963) as they are unaffected by sympathetic (Freeman, 1935) and somatic (Doupe, 1943) denervation. However, in the present study local cooling of one foot resulted almost instantaneously in a concomitant, but relatively less marked reduction in flow in the contralateral foot, indicating that the blood flow response to cold represents a summation of local effects and a centrally elicited reflex effect (Hertzman & Roth, 1942).

Although the nature of the local mechanisms underlying the changes in flow produced by alterations in local skin temperature remains uncertain, the direct effects of heat on vascular smooth muscle (Keatinge & Harman, 1980; Vanhoutte,

1980) and changes in blood viscosity (Virgilio et al, 1964) are likely to be involved.

Postural Vasoconstriction During Changes in Local Skin Temperature:

The mechanisms underlying the reduction in skin blood flow when the foot is placed in the dependent position appear to be fully operative over a wide range of skin temperature (26-36°C). In contrast, the marked attenuation or abolition of postural vasoconstriction at higher skin temperatures suggests an impairment of these mechanisms and might be explained by a heat-induced inhibition of the response of vascular smooth muscle to sympathetic nerve impulses or to circulating catecholamines (Vanhoutte et al, 1981). The increased flow measured in the dependent position during local heating at 42°C could be ascribed to a passive distension of the cutaneous vascular bed in response to the postural increase in the local transmural pressure. It is unlikely that the failure of postural vasoconstriction during local heating at 42-44°C is related to a thermal injury of the vascular bed as the postural response was completely restored after spontaneous cooling. It is likely that the high blood flow associated with local heating has a useful protective effect: by carrying heat away from the heated area, it reduces the temperature below the skin surface and the likelihood of thermal damage (Greenfield, 1963).

The pattern of the postural vasoconstrictor response at any given skin temperature during local heating was quite similar in skin areas with and without arteriovenous anastomoses (Table 13), even at temperatures similar to those induced by indirect heating (33.1-33.3°C). This is in contrast to the findings obtained during indirect heating where the partial release of the sympathetic vasoconstrictor tone was accompanied by a marked attenuation of postural vasoconstrictor response in the toe pulp (with arteriovenous shunts) yet preservation of the response in the dorsum of the foot (without shunts). This discrepancy between the effects of direct and indirect heating is best explained by the observations that arteriovenous anastomoses are more sensitive to central thermoregulatory reflexes than to influences of local skin temperature (Zanick & Delaney, 1973; Hales & Iriki, 1977).

The differences in flow values between men and women (Table 15) are interesting. Recordings of flow were made at similar skin temperatures and from identical anatomical sites in both groups, but there was a significant difference in flow at 2 of the 4 temperatures tested. These differences, therefore, must have been due to other factors in addition to the effect of local temperature. One possible explanation is that female sex hormones are thought to have a modulating influence on peripheral blood flow and vascular tone (Altura, & Altura, 1977).

Furthermore, when the postural fall in flow (%) at resting skin

temperature was calculated in these women according to the menstrual cycle phase at the time of the experiment, obvious differences were found. The flow fell by 68.0 ± 0.8%, 81.9 ± 3.1% and 52.4 ± 5.7% during the menstrual (days 2-5, n = 3), follicular (days 10-13, n = 4) and luteal (days 19-26, n = 4) phases of the cycle respectively. The difference in the postural fall in flow between the follicular and luteal phases was statistically significant ($P<0.05$).

These preliminary observations made it necessary to study the postural vasoconstrictor response in women in more detail, which will be discussed in the next chapter.

Chapter IV

POSTURAL VASOCONSTRICTION IN WOMEN

Chapter IV

POSTURAL VASOCONSTRICTION IN WOMEN

Section 1 POSTURAL VASOCONSTRICTION IN WOMEN DURING THE NORMAL MENSTRUAL CYCLE

Introduction

As has been pointed out earlier in the main "INTRODUCTION", skin blood flow varies at the different phases of the normal menstrual cycle (Edwards & Duntley, 1949), and female sex steroids can modify peripheral blood flow and vascular tone (Altura & Altura, 1977). However, few data are available concerning the reaction of the peripheral vasculature in women to changes in limb posture at the different stages of the reproductive cycle.

Cyclical oedema (Thorn, 1957) is one of the characteristic features of the premenstrual syndrome (Reid & Yen, 1981). Although it has been suggested that premenstrual oedema is related to fluctuations in the female sex hormones (Thorn et al, 1938), or an abnormality in the vasopressin and/or the renin-angiotensin-aldosterone system (Reid & Yen, 1981), the exact mechanism behind the excessive tissue fluid accumulation remains unclear. Since the postural increase in precapillary resistance has been suggested to act as an oedema preventing mechanism (Sejrsen et al, 1981a), failure of such a

vasoconstrictor response might partly predispose to the incidence of dependent oedema in the premenstrual period. Indeed, the observations that oedema was precipitated by upright posture (Kuchel et al, 1970) and improved by the use of vasoconstrictor drugs (Streeten & Conn, 1959) lend much support to this hypothesis and further suggest that some form of a postural defect might exist in these women resulting in an undue pooling of blood in the dependent extremities.

It has also been shown in preliminary experiments (Chapter III, Section 3) that women had a different flow response from that of men at similar skin temperatures. Furthermore, the women showed a variable postural response under resting conditions at different stages of their cycles. Therefore, the aim of the present study was to investigate the postural vasoconstrictor response in women in more detail during the different phases of the normal menstrual cycle, and to relate the postural changes in flow to the swelling rate of the dependent foot and to the blood levels of female sex hormones.

Methods

Fifteen healthy women aged 20-39 years were studied during the menstrual (days 1-2), follicular (days 12-15) and luteal (days 21-26) phases of the menstrual cycle. Three of the women were studied during 2 consecutive cycles. All cycles (n = 24), except 2, were ovulatory cycles as judged by a characteristic biphasic basal body temperature (Martin, 1943) and/or changes

in serum progesterone concentration. Thirteen men aged 21-37 years were also studied during the same period on 2 separate occasions at least one week apart. Each subject wore similar clothing on repeat visits.

Menstrual History

None of the women was taking oral contraceptive pills or any medication throughout the cycle under study. All cycles were regular ranging in length between 28 and 34 days, and each cycle ended by normal menstruation. Observations were made from one menstrual period to the next, thus excluding the possibility of pregnancy or amenorrhoea. Most women experienced one or more of the common premenstrual symptoms e.g. abdominal distension, irritability, low backache etc. Four of the women reported observing mild ankle swelling and feeling tight shoes or tight rings recurring 4-7 days before the onset of menstruation. Physical examination was carried out in all women on each visit, and bilateral pitting oedema of the ankles was confirmed during the luteal phase in those subjects who complained of premenstrual oedema, but there was no clinical evidence of any cardiac, renal or hepatic disease. The women were asked about their premenstrual symptoms during the menstrual phase of the next cycle i.e. after the study was completed.

Measurement of the Postural Changes in Foot Skin Blood Flow

The postural vasoconstrictor response in the foot was assessed by measuring the postural changes in foot skin blood flow using laser Doppler flowmetry as described in Chapter II. On each visit the flow was measured with the subject lying down, first with the foot maintained at heart level for 2 minutes(H), then with the foot lowered passively 50cm below the heart for 4 minutes (D). The postural fall in flow was calculated as (H-D) x 100/H. Recordings were made on 2 skin areas: (i) the dorsum of the foot (without arteriovenous anastomoses), and (ii) the pulp of the big toe (with anastomoses) (Grant & Bland, 1931).

During each experiment, arterial blood pressure (by auscultation) and heart rate (radial pulse) were measured after at least 10 minutes of supine rest. Sublingual temperature was measured using a standard mercury clinical thermometer. Foot skin temperature was continuously measured from the recording site using a sensitive thermocouple. Body weight was also measured using an ordinary scale. Subjects were studied in 3 protocols:

In protocol 1, ten women (10 cycles) were studied during the follicular and luteal phases of the cycle, and the measurements were compared with data obtained form 10 age-matched men studied at 2 separate occasions.

In the 2nd protocol, 6 women (7 cycles) were studied during the

3 phases of their cycles, and in addition to the measurements described above, basal body temperature was recorded daily throughout the cycle and serum oestradiol and progesterone concentrations were determined at each visit. Venous blood samples were withdrawn from an arm vein just before the start of the experiment. Serum oestradiol concentration was measured by a direct radioimmunoassay method (Steranti Research Ltd, St. Albans, U.K.). Serum progesterone concentration was measured by a direct radioimmunoassay using an antiserum raised against progesterone-11-alpha-hemisuccinate (Guildhay Antisera, Guildford, U.K.).

In protocol 3, four women (4 cycles) were studied on the experimental days described above at the 3 phases of the cycle, and 3 other men were studied at 2 separate visits. In this protocol, the changes in foot circumference (foot swelling rate) were measured as described in Chapter II using a strain gauge plethysmograph (Whitney, 1953) at the time of measurement of the postural changes in foot skin blood flow (on the dorsum of the foot).

RESULTS

Protocol 1: Postural vasoconstriction during the follicular vs luteal phase

The results of protocol 1 are shown in Table 16. In the men, there was no significant difference in any of the variables

Table 16: The postural changes in foot skin blood flow (mV) measured in 10 women during the follicular and luteal phases of the menstrual cycle as compared with 10 age-matched men studied at 2 different visits at least one week apart. The changes in arterial blood pressure, heart rate, body temperature and body weight are also shown. H, with the foot horizontal, D, during the 4th min with the foot dependent. The postural fall in flow was calculated as (H-D)/H%. *P<0.05, **P<0.01, ***P<0.001 as compared with the corresponding mean values measured in men. NS, nonsignificant.

	Women (n = 10)			Men (n = 10)		
	Follicular Phase	Luteal Phase	P	First Visit	Second Visit	P
Dorsum of the Foot						
Skin Temperature (°C)	28.5 ± 0.4**	29.7 ± 0.3	<0.001	30.0 ± 0.2	29.9 ± 0.4	NS
Skin Blood Flow (H)	9.9 ± 1.4	13.4 ± 1.9	NS	12.3 ± 0.9	13.8 ± 1.5	NS
Skin Blood Flow (D)	2.7 ± 0.6	4.9 ± 0.6**	<0.01	2.6 ± 0.2	2.7 ± 0.3	NS
Postural Fall in Flow	72.7 ± 3.6%	60.6 ± 4.8%**	<0.05	78.1 ± 1.9%	78.9 ± 2.2%	NS
Pulp of the Big Toe						
Skin Temperature (°C)	25.2 ± 0.6	27.2 ± 0.7	<0.01	25.8 ± 0.7	26.2 ± 0.8	NS
Skin Blood Flow (H)	31.6 ± 7.4	88.8 ± 40.6	NS	55.5 ± 15.9	57.2 ± 17.5	NS
Skin Blood Flow (D)	3.4 ± 0.8*	36.2 ± 18.3	<0.01	7.2 ± 1.4	7.7 ± 1.6	NS
Postural Fall in Flow	87.2 ± 2.7%	62.3 ± 5.4%**	<0.01	85.0 ± 2.6%	84.7 ± 2.8%	NS
Arterial Blood Pressure (mmHg)						
Systolic	108.6 ± 1.5**	109.7 ± 1.0***	NS	116.7 ± 1.7	117.3 ± 1.5	NS
Diastolic	69.8 ± 1.0*	68.4 ± 1.5**	NS	74.0 ± 1.7	75.1 ± 1.3	NS
Mean	82.7 ± 1.1**	82.2 ± 1.3**	NS	88.2 ± 1.4	88.5 ± 1.2	NS
Heart Rate (beats/min)	68.8 ± 2.4	70.2 ± 1.8	NS	67.8 ± 1.4	66.5 ± 1.1	NS
Body Temperature (°C)	36.57 ± 0.04**	36.97 ± 0.04**	<0.001	36.81 ± 0.05	36.74 ± 0.06	NS
Body Weight (Kg)	59.4 ± 3.0**	60.1 ± 3.1**	<0.001	70.05 ± 1.42	70.0 ± 1.38	NS

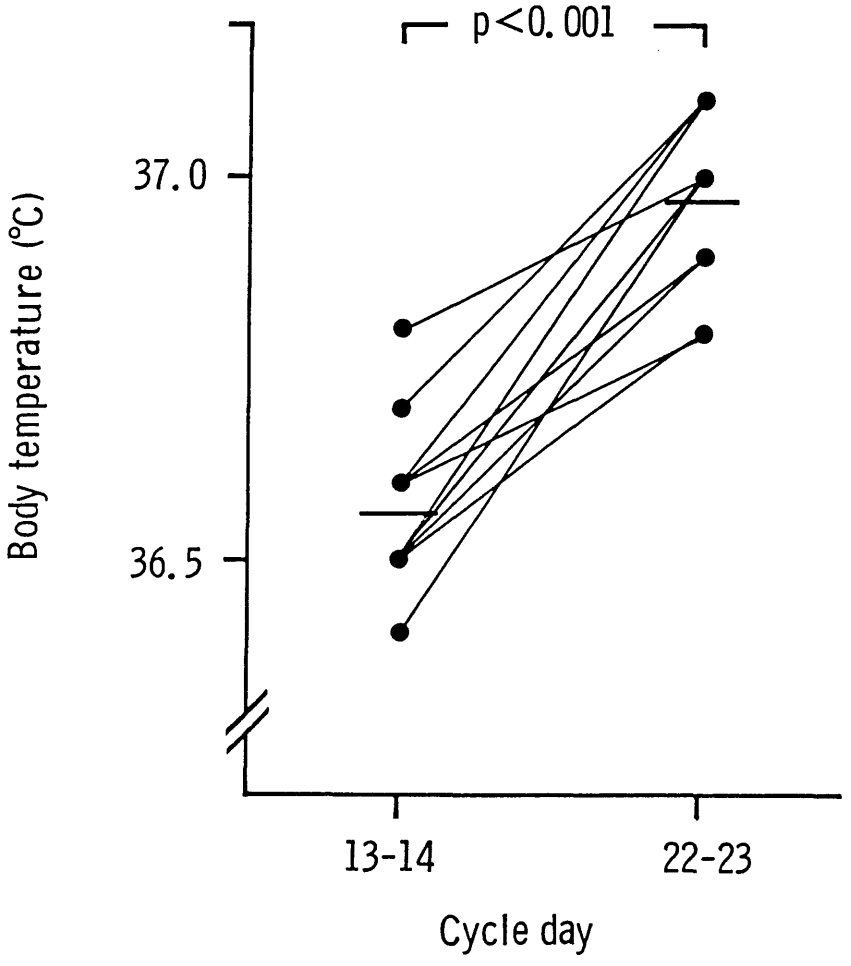


Figure 28: Body temperature measured in 10 women during the follicular and luteal phases of 10 ovulatory cycles. Horizontal bars represent mean values.

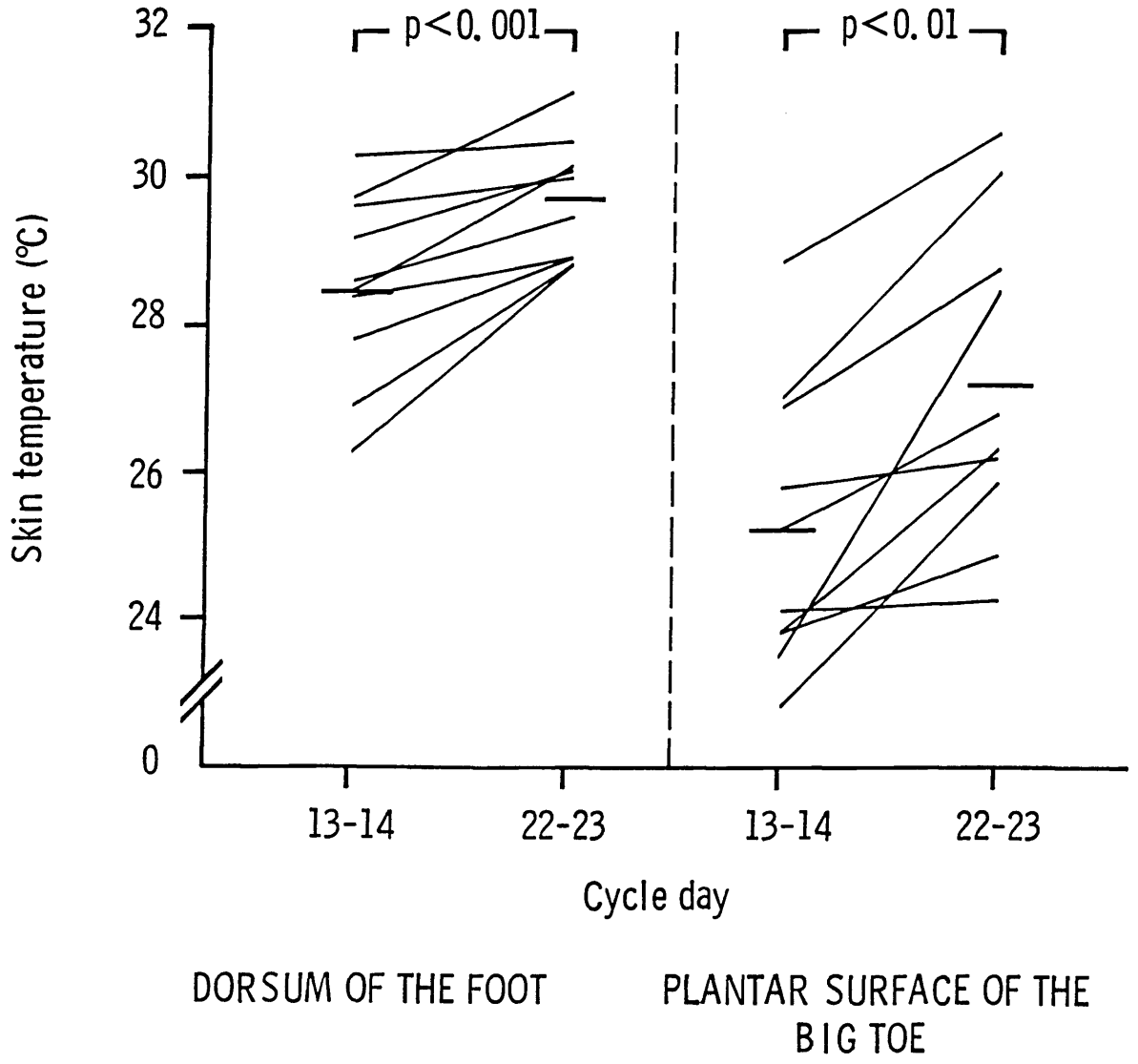


Figure 29: Foot skin temperature measured in 10 women during the follicular and luteal phases of 10 ovulatory cycles. Horizontal bars represent mean values.

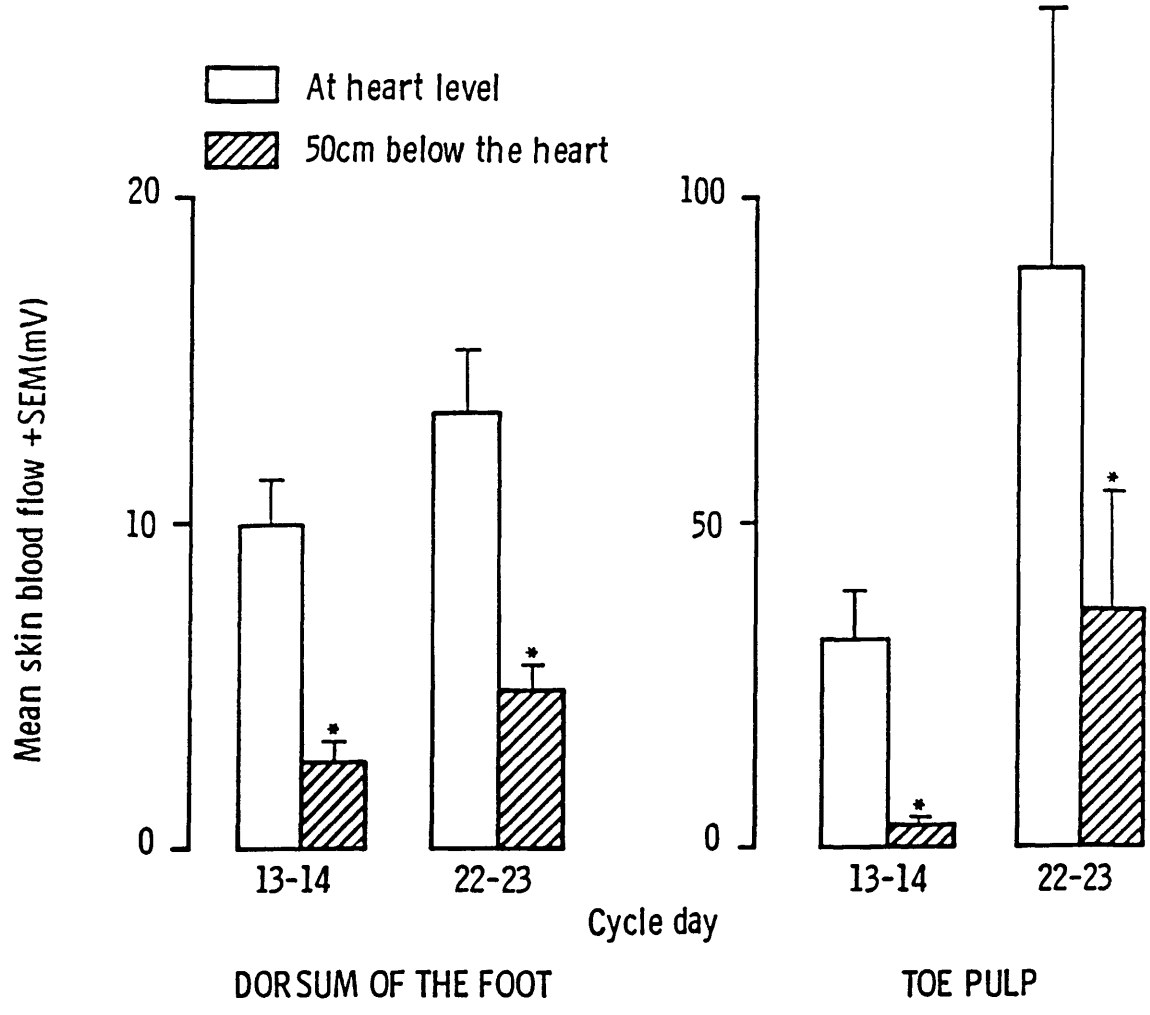


Figure 30: The postural changes in foot skin blood flow measured on 2 skin sites in 10 women during the follicular and luteal phases of 10 ovulatory cycles. *P<0.01 compared with mean flow values measured at heart level.

assessed on the 2 visits. In contrast, during the luteal phase in women, skin and body temperatures were significantly increased (Figures 28 & 29), skin blood flow measured at heart level showed a nonsignificant increase, but the flow measured in the dependent position was significantly elevated in both skin areas, as compared with the corresponding values measured during the follicular phase of the cycle (Figure 30, Table 16). Moreover, the % fall in flow on dependency was significantly attenuated during the luteal phase compared with the follicular phase, together with a significant increase in body weight (Table 16).

When data obtained in men and women were compared, arterial blood pressure, body temperature and body weight were significantly different in both groups. More importantly, the postural fall in flow (%) obtained in women during the follicular phase was similar to that obtained in the men, but values obtained during the luteal phase were significantly lower than those obtained in the men (Table 16).

Protocol 2: <u>Postural vasoconstriction at different stages of the cycle as related to female sex hormones</u>

The results of protocol 2 are shown in Figures 31-35 and Table 17. Five of the 7 cycles studied in this protocol were ovulatory as revealed by a characteristic biphasic basal body temperature curve (Figure 31) and increased serum progesterone concentration during the luteal phase (Figure 32).

Female Sex Hormones

In 5 ovulatory cycles, oestradiol concentrations were 130.8 ± 28.3, 684.3 ± 350.9 and 569.9 ± 80.9 pmol/l, and progesterone concentrations were <5, <5 and 35.5 ± 8.6 nmol/l during the menstrual, follicular and luteal phases of the cycle respectively (Figure 32). In the 2 anovulatory cycles, during the late phase, there was no change in basal body temperature (Table 17) or serum progesterone concentration which remained at <5 nmol/l throughout each of the 2 cycles, whereas serum oestradiol concentrations were 231, 264, 2101 and 377, 567 and 611 pmol/l at the 3 phases of the 2 cycles respectively.

The Postural Changes in Foot Skin Blood Flow

There was a positive correlation between serum oestradiol concentrations and skin blood flow measured at heart level on the dorsum of the foot at different stages of the cycle (Figure 33). Skin temperature dropped during the follicular phase but rose again during the luteal phase (Figure 34).

During the follicular phase, when oestradiol concentrations were high, there was a significantly greater reduction in skin blood flow measured in the dependent foot (Figure 35) associated with a significant increase in the postural fall in flow (%) as compared with the menstrual phase (Dorsum: 73.0 ± 8.6% vs 60.8 ± 7.5%, $P<0.05$, Toe pulp: 94.3 ± 1.3% vs 75.9 ± 3.8%, $P<0.01$). During the luteal phase, however, when both

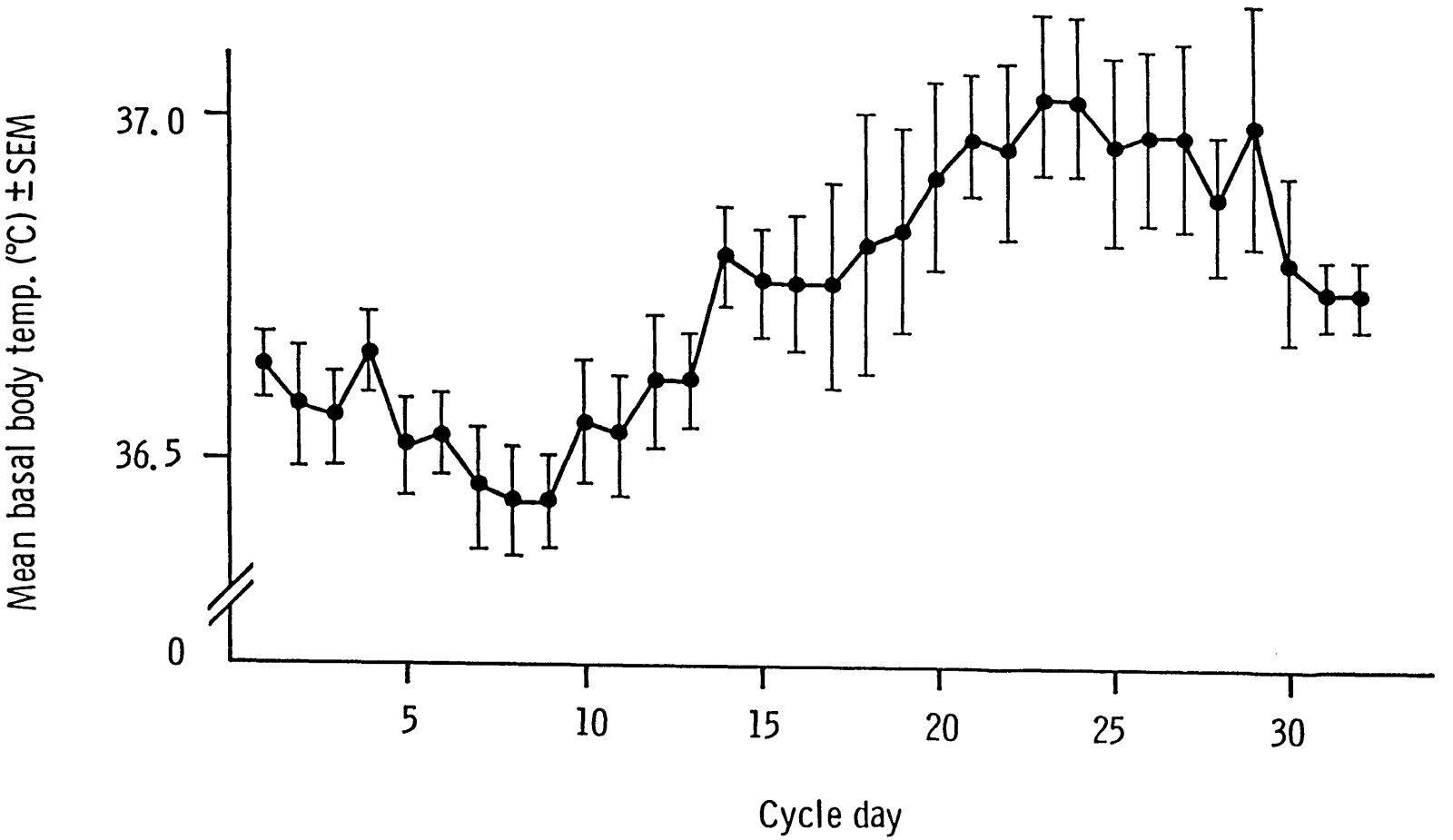


Figure 31: Basal body temperature (recorded sublingually every morning) in 5 women with ovulatory menstrual cycles.

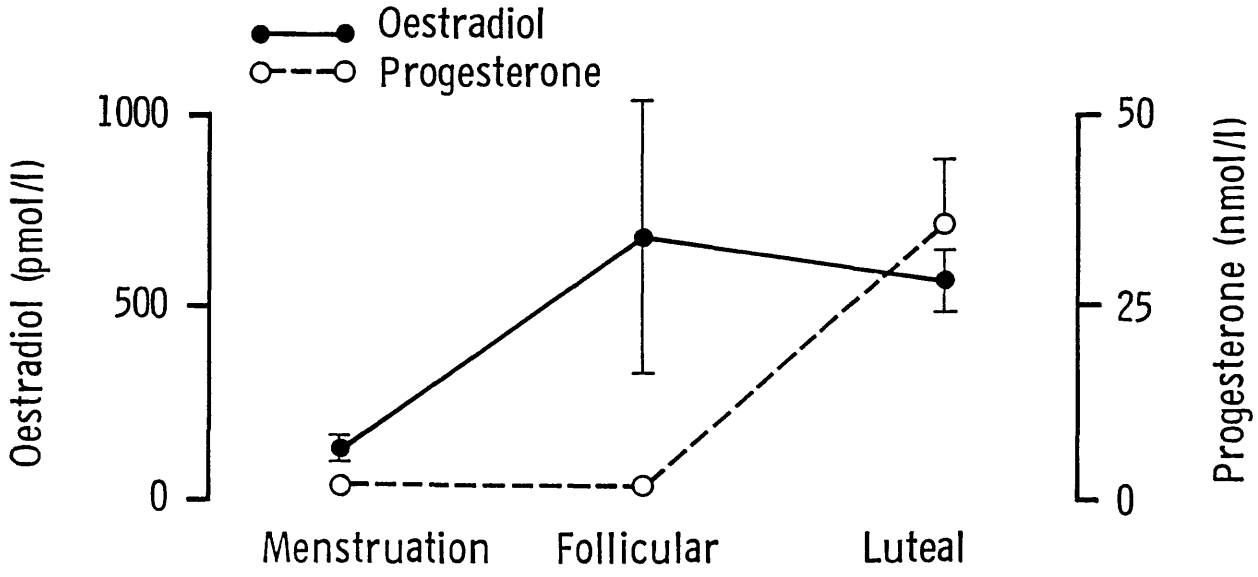


Figure 32: Serum oestradiol and progesterone concentrations measured during the menstrual, follicular and luteal phases in 5 women with ovulatory cycles.

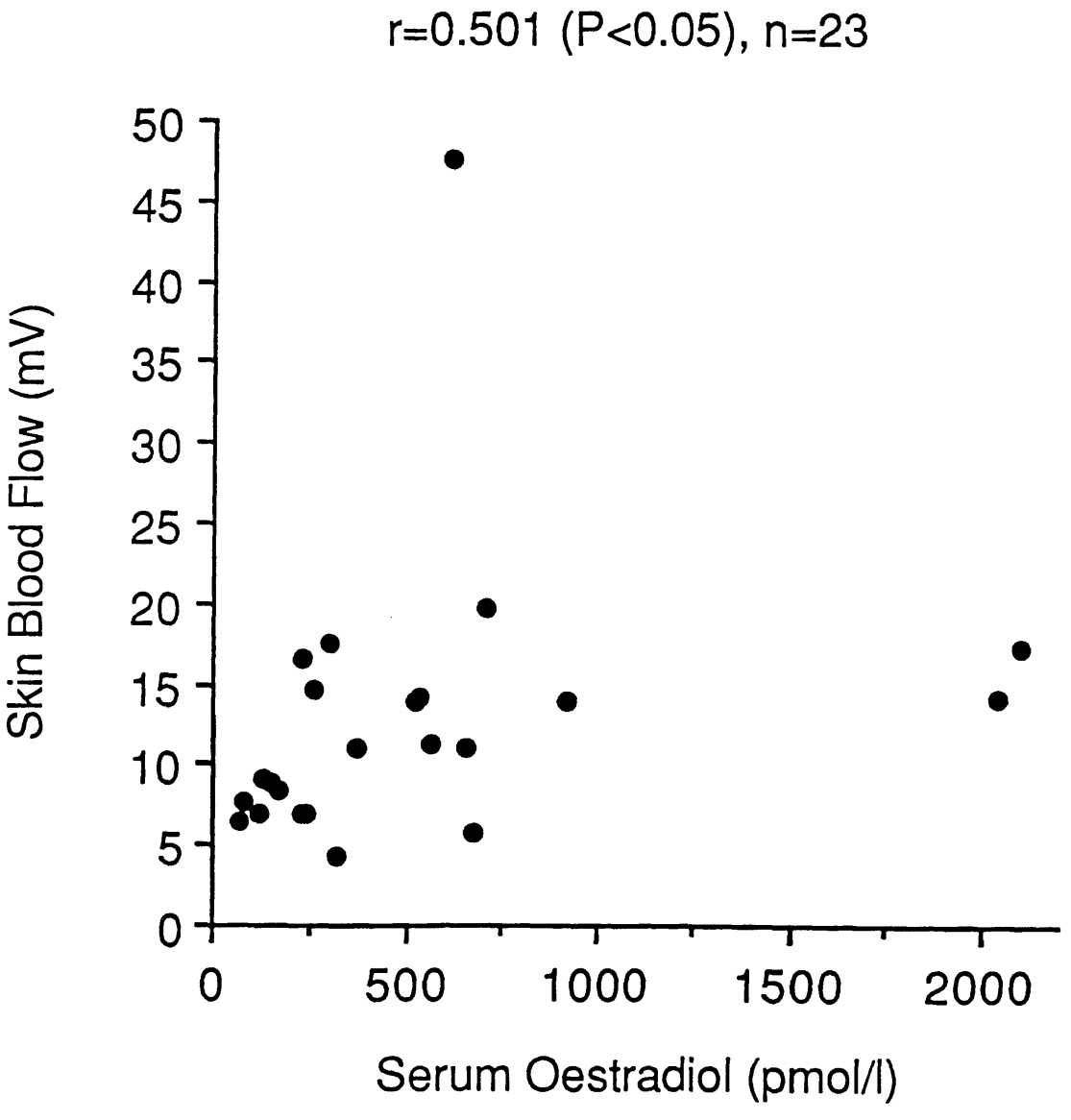


Figure 33: Correlation between serum oestradiol concentrations measured at different stages of the menstrual cycle and skin blood flow measured at heart level on the dorsum of the foot in 6 women (7 cycles). n, number of flow measurements and hormone determinations.

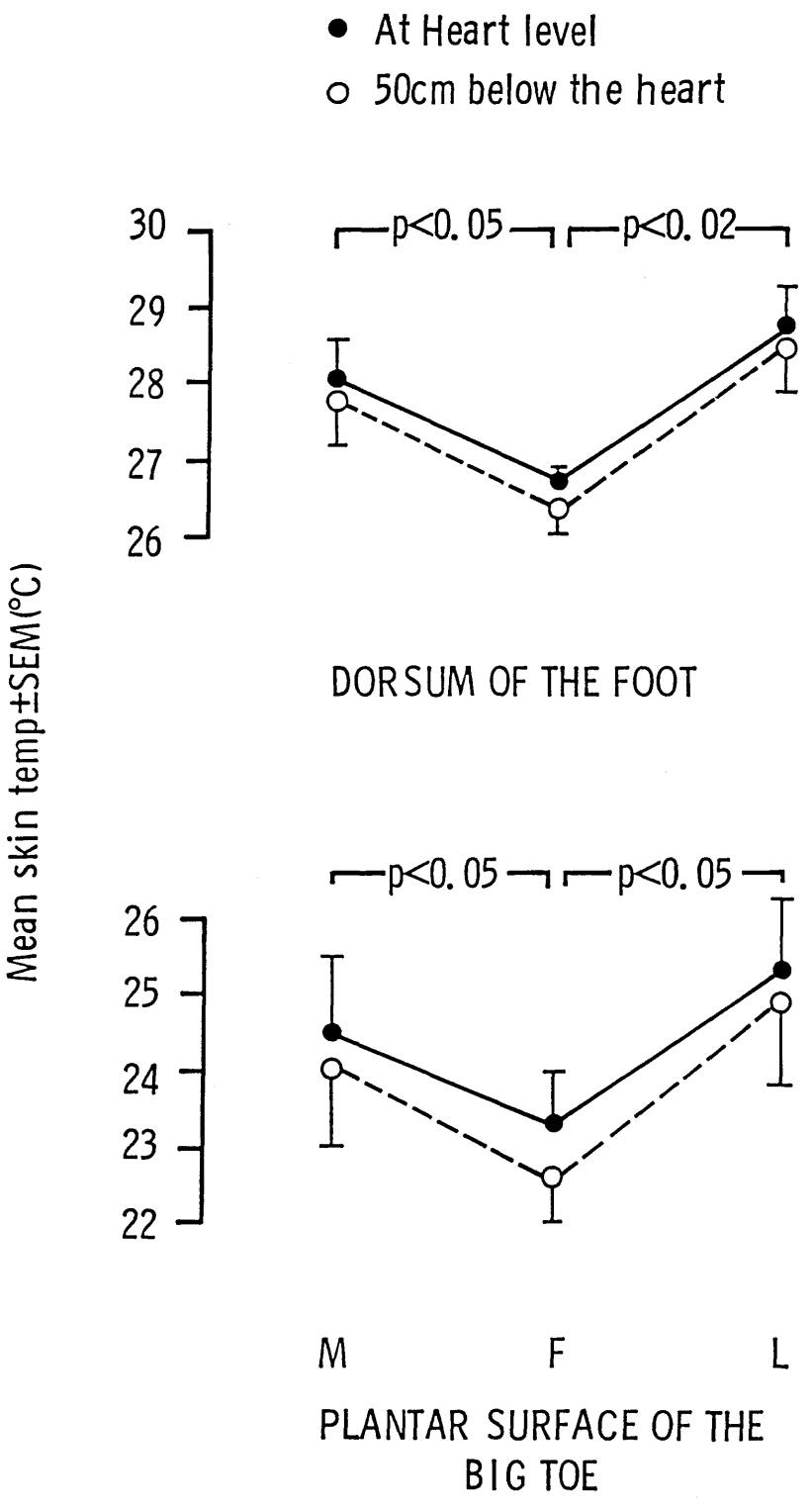


Figure 34: Foot skin temperature recorded from 2 skin areas during the menstrual (M), follicular (F) and luteal (L) phases in 5 women with ovulatory cycles.

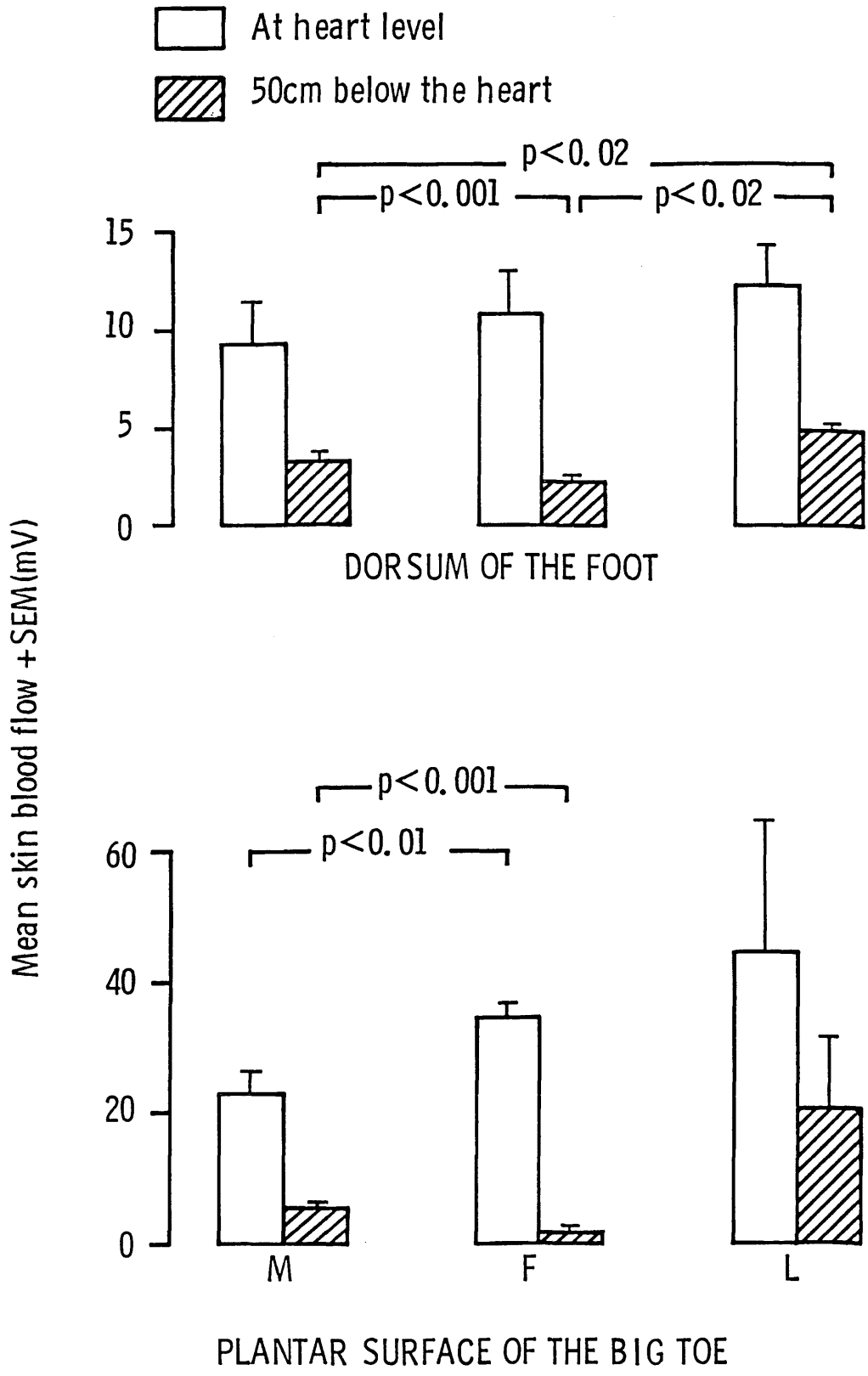


Figure 35: The postural changes in skin blood flow measured on 2 skin areas during the menstrual (M), follicular (F) and luteal (L) phases in 5 women with ovulatory cycles.

oestradiol and progesterone levels were high, there was a significantly attenuated postural fall in flow as compared with both the follicular and menstrual phases (Dorsum: 54.5 ± 11.5% vs 73.0 ± 8.6 & 60.8 ± 7.5%, $P<0.05$, Toe pulp: 55.4 ± 7.8% vs 94.3 ± 1.3 & 75.9 ± 3.8%, $P<0.01$) i.e. the postural vasoconstrictor response was preserved and even partially augmented during the follicular phase but markedly impaired during the luteal phase. The pattern of the response was quite similar in areas with (dorsum of the foot) and without (toe pulp) arteriovenous anastomoses, but the flow values were much greater in the toe pulp than in the dorsum of the foot.

Unlike ovulatory cycles, the postural vasoconstrictor response was maintained and even partially augmented during both the follicular and late phases as compared with the menstrual phase in the 2 subjects with anovulatory cycles (Table 17).

Protocol 3: <u>Foot Swelling Rate as Related to Postural Vasoconstriction</u>

In 3 men, the simultaneously measured postural fall in flow (%) and foot swelling rate were very consistent on the 2 visits (78.7 ± 1.0% vs 79.6 ± 1.0%, 0.062 ±0.007 vs 0.059 ± 0.004 $ml.min^{-1}$. 100 ml^{-1} foot, Figure 36). In contrast, 4 women with ovulatory cycles (one with premenstrual oedema) showed an inverse relationship between the postural fall in flow and foot swelling rate. Thus, as compared with the values obtained during menstruation (71.4 ± 2.4%, 0.057 ± 0.015

Table 17: The postural changes in foot skin blood flow (mV) measured during the menstrual (M), follicular (F) and late (L) phases in 2 women with anovulatory cycles. Individual values are presented. H, with the foot horizontal, D, during the 4th min with the foot dependent. The postural fall in flow = (H-D) x 100/H. The changes in body temperature, foot skin temperature and body weight are also shown.

	SUBJECT 1			SUBJECT 2		
	M	F	L	M	F	L
Body Temperature (°C)	36.5	36.6	36.5	36.5	36.6	36.5
Body Weight (Kg)	46.0	46.5	46.0	46.0	47.0	46.5
Dorsum of the Foot						
Skin Temperature (°C)	27.7	23.4	25.8	30.2	29.8	29.7
Skin Blood Flow (H)	6.9	14.6	17.2	11.0	11.2	47.6
Skin Blood Flow (D)	3.1	2.6	2.7	3.9	1.8	3.6
Postural Fall in Flow	55.1%	82.2%	84.3%	64.5%	83.9%	92.4%
Pulp of the Big Toe						
Skin Temperature (°C)	24.3	21.1	22.9	25.3	24.8	25.2
Skin Blood Flow (H)	23.6	8.6	27.8	27.0	37.3	85.8
Skin Blood Flow (D)	4.8	1.7	0.7	5.8	1.7	3.3
Postural Fall in Flow	79.7%	80.2%	97.5%	78.5%	95.4%	96.2%

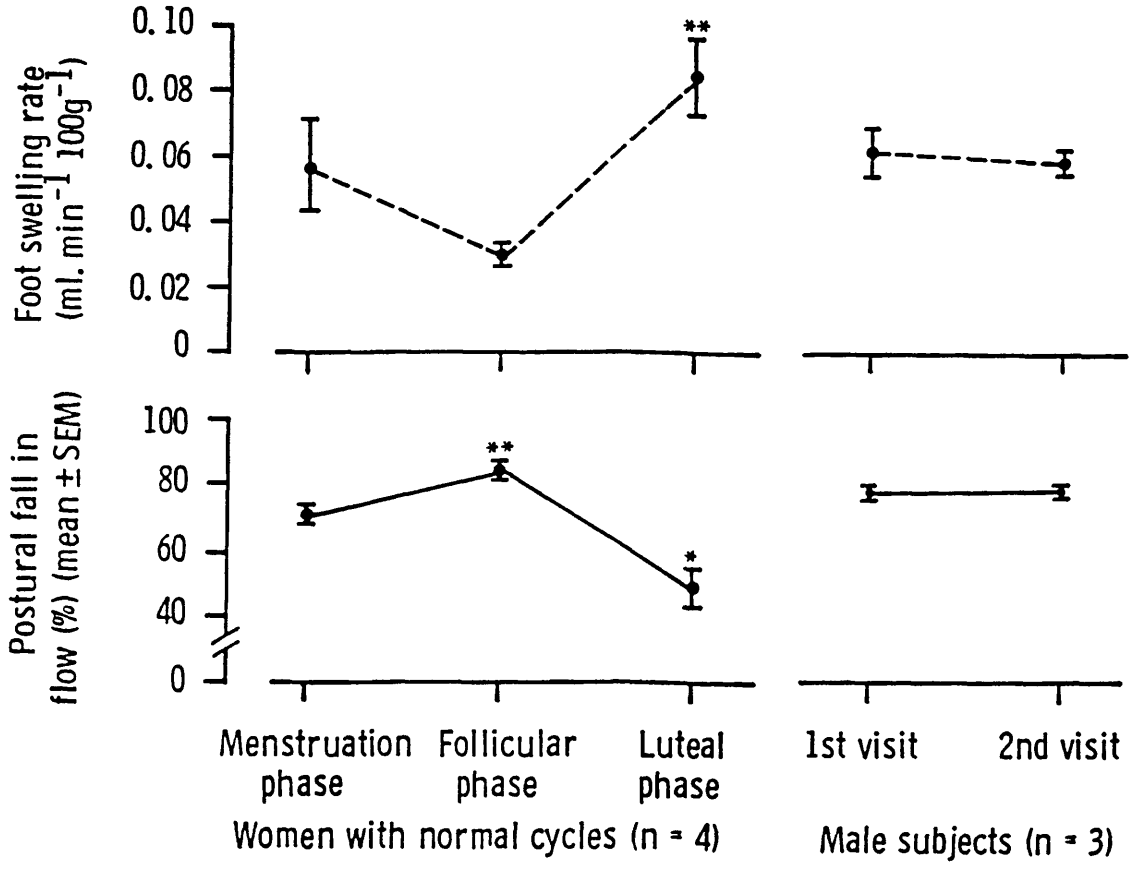


Figure 36: The postural fall in skin blood flow measured on the dorsum of the foot in 4 women at different stages of their ovulatory cycles, and in 3 men at 2 separate visits, as related to foot swelling rate measured between the 10th and 20th min with the foot in the dependent position. *P<0.05, *P<0.02 (compared with the menstrual phase).

$ml.min^{-1}.100ml^{-1}$), there was a greater postural fall in flow during the follicular phase associated with a reduction in foot swelling rate (85.2 ± 1.9%, 0.030 ± 0.004 $ml.min^{-1}$. 100 ml^{-1}), whereas during the luteal phase, there was a significantly impaired postural fall in flow associated with a greater foot swelling rate (49.0 ± 5.9%, 0.085 ± 0.012 $ml.min^{-1}.100ml^{-1}$) (Figure 36).

Collective Data and Analysis of Variance

All data obtained in 22 ovulatory cycles were grouped together and are presented in Table 18. Analysis of variance performed on collective data showed no significant differences in systolic blood pressure, heart rate or skin blood flow measured at heart level on the dorsum of the foot at different stages of the cycle, but the analysis revealed significant differences in diastolic and mean arterial pressures ($P<0.01$, $P<0.05$), body temperature ($P<0.01$), body weight ($P<0.05$), foot skin temperature (Dorsum: $P<0.01$, Toe pulp: $P = 0.0009$), blood flow measured at heart level in the toe pulp ($P = 0.0099$), blood flow measured in the dependent position (Dorsum: $P<0.01$, Toe pulp: $P = 0.0009$) and the postural fall in flow (Dorsum: $P = 0.0008$, Toe pulp: $P = 0.0009$).

The collective data on the postural changes in foot skin blood flow shown in Table 18 were consistent with the characteristic pattern of the postural response obtained at different stages of the cycle in each study protocol. In addition, during the

Table 18: The postural changes in skin blood flow (mV) measured on the dorsum of the foot and the pulp of the big toe in 15 women at different phases of 22 ovulatory menstrual cycles. The changes in arterial blood pressure, heart rate, body temperature, foot skin temperature and body weight are also shown. H, with the foot horizontal, D, during the 4th min with the foot dependent. The postural fall in flow was calculated as (H-D) x 100/H. Values are given as mean ± SEM. *P<0.05, **P<0.02, ***P<0.01, ****P<0.001 (as compared with M), +P<0.01, ++P<0.001 (as compared with F).

	Menstruation (M)	Follicular (F)	Luteal (L)
Arterial Blood Pressure (mm Hg)			
Systolic	109.6 ± 0.9	108.3 ± 1.1	108.0 ± 1.2
Diastolic	70.2 ± 1.1	71.4 ± 0.8	66.6 ± 0.9***++
Mean	83.3 ± 1.0	83.7 ± 0.8	80.4 ± 0.8**+
Heart Rate (beats/min)	67.4 ± 1.5	66.5 ± 1.3	70.3 ± 1.3+
Body Temperature (°C)	36.62 ± 0.04	36.59 ± 0.03	37.0 ± 0.03****++
Body Weight (Kg)	55.29 ± 3.37	55.54 ± 3.38	56.0 ± 3.42****++
Dorsum of the Foot			
Skin Temperature (°C)	27.7 ± 0.4	27.4 ± 0.3	29.0 ± 0.3***++
Skin Blood Flow (H)	11.8 ± 1.6	11.9 ± 1.1	14.9 ± 1.5
Skin Blood Flow (D)	3.5 ± 0.3	2.5 ± 0.2***	6.4 ± 0.6****++
Postural Fall in Flow	66.1 ± 3.9%	75.8 ± 2.9%	55.7 ± 3.9%*+
Pulp of the Big Toe			
Skin Temperature (°C)	24.2 ± 0.8	24.1 ± 0.5	26.2 ± 0.5***+
Skin Blood Flow (H)	25.4 ± 2.4	34.0 ± 4.0	68.4 ± 22.2
Skin Blood Flow (D)	5.8 ± 0.6	3.0 ± 0.5***	29.3 ± 10.2**+
Postural Fall in Flow	75.8 ± 3.0%	90.1 ± 1.6%***	59.1 ± 3.9*+

luteal phase, diastolic and mean arterial pressures were significantly reduced, whereas heart rate, body temperature, foot skin temperature and body weight were significantly increased as compared with both the follicular and menstrual phases of the cycle (Table 18). Moreover, the changes in all variables assessed at different stages of the cycle were quite reproducible when studied in 3 women during 2 consecutive cycles (Table 19).

Women with Premenstrual Oedema

In the 4 women who reported premenstrual ankle swelling, the changes in most of the parameters assessed were generally similar to those obtained in the rest of women at different stages of the cycle including the characteristic fluctuations in the postural fall in flow. However, during the luteal phase, women with premenstrual oedema showed significantly higher flow values particularly when measured in the dependent position, as well as greater increments in body weight, than the rest of women (Table 20). Foot swelling rate was measured in one of the 4 women with oedema, and this subject showed a higher foot swelling rate during the luteal phase than the average rate obtained in the other 3 subjects (0.107 vs 0.077 $ml.min^{-1}.100\ ml^{-1}$).

Table 19: Arterial blood pressure (ABP), heart rate (HR), body temperature (B.T.), foot skin temperature (Sk. T.), body weight (B. Wt.) and the postural changes in skin blood flow (SBF) measured in 3 women during the menstrual, follicular and luteal phases of 2 consecutive ovulatory cycles. Values are given as mean ± SEM. H, with the foot horizontal, D, during the 4th min with the foot dependent. The postural fall in flow was calculated as (H - D) x 100/H. C.V., mean coefficient of variation.

	Menstrual Phase			Follicular Phase			Luteal Phase		
	Cycle 1	Cycle 2	C.V.(%)	Cycle 1	Cycle 2	C.V.(%)	Cycle 1	Cycle 2	C.V.(%)
ABP (mm Hg)									
Systolic	108.3 ± 1.7	109.3 ± 0.7	1.5	105.3 ± 2.4	105.7 ± 3.5	1.1	102.7 ± 1.8	101.7 ± 2.0	3.0
Diastolic	67.7 ± 1.5	69.3 ± 0.7	3.1	73.3 ± 2.9	71.7 ± 2.0	1.6	65.3 ± 2.7	66.7 ± 1.7	3.6
Mean	81.2 ± 1.5	82.6 ± 0.7	2.4	84.0 ± 2.7	83.0 ± 2.5	0.8	77.8 ± 2.0	78.3 ± 1.4	3.3
HR (beats/min)	71.3 ± 2.9	70.0 ± 1.2	4.0	66.7 ± 2.7	68.0 ± 1.2	2.8	73.3 ± 1.3	72.7 ± 2.4	3.2
B.T. (°C)	36.63 ± 0.09	36.67 ± 0.09	0.3	36.63 ± 0.03	36.63 ± 0.07	0.3	36.93 ± 0.03	37.0 ± 0.06	0.1
B. Wt. (Kg)	50.2 ± 4.9	50.0 ± 4.8	0.7	50.3 ± 4.8	50.2 ± 5.0	0.8	50.5 ± 4.8	50.7 ± 5.0	0.7
Dorsum of Foot									
Sk. T. (°C)	27.2 ± 0.7	27.5 ± 0.9	2.0	25.9 ± 0.2	26.5 ± 0.5	1.8	27.8 ± 0.9	28.5 ± 0.7	3.7
SBF (H)	10.9 ± 3.0	11.7 ± 3.3	5.9	12.5 ± 3.5	14.5 ± 3.7	11.3	12.7 ± 4.4	15.1 ± 5.0	13.8
SBF (D)	3.2 ± 0.4	3.1 ± 0.2	10.5	2.2 ± 0.5	2.5 ± 0.4	10.8	6.0 ± 1.8	6.8 ± 2.0	9.1
(H-D) x 100/H	64.4 ± 12.2%	67.8 ± 9.7%	8.2	74.4 ± 14.4%	76.3 ± 12.1%	7.8	42.9 ± 17.6%	46.9 ± 15.5%	4.7
Pulp of Big Toe									
Sk. T. (°C)	24.1 ± 1.9	24.5 ± 1.6	2.7	21.8 ± 0.9	23.1 ± 0.9	4.2	25.3 ± 1.8	25.8 ± 1.0	3.5
SBF (H)	25.3 ± 3.0	28.1 ± 4.3	14.3	34.9 ± 2.0	41.4 ± 1.1	12.1	59.7 ± 33.1	55.4 ± 22.3	15.6
SBF (D)	6.2 ± 0.6	6.6 ± 1.2	12.1	2.9 ± 0.3	3.5 ± 0.5	13.2	30.5 ± 17.4	27.7 ± 13.6	10.3
(H-D) x 100/H	74.0 ± 5.8%	75.5 ± 5.9%	4.7	91.8 ± 0.4%	91.6 ± 1.1%	8.5	47.2 ± 10.9%	50.5 ± 9.2%	7.6

Table 20: The postural changes in skin blood flow (mV) measured during the luteal phase of the menstrual cycle in 4 women who reported premenstrual oedema as compared with the corresponding changes in 11 women without oedema. The increase in body weight from the late follicular to the midluteal phase is also shown. H, with the foot horizontal, D, during the 4th min with the foot dependent. The postural fall in flow was calculated as (H-D) x 100/H.

	Women Without Oedema (n = 11)	Women with Oedema (n = 4)	Statistical Significance
Dorsum of the Foot			
Skin Blood Flow (H)	12.3 ± 2.2	15.1 ± 1.4	NS
Skin Blood Flow (D)	3.9 ± 0.4	6.5 ± 0.7	P<0.01
Postural Fall in Flow	64.0 ± 5.8%	56.9 ± 3.0%	NS
Pulp of the Big Toe			
Skin Blood Flow (H)	34.5 ± 13.5	189.4 ± 81.7	P<0.05
Skin Blood Flow (D)	13.4 ± 7.5	81.8 ± 37.0	P<0.05
Postural Fall in Flow	66.0 ± 5.3%	52.0 ± 8.0%	NS
Increase in Body Weight (Kg)	0.41 ± 0.06	1.1 ± 0.1	P<0.001

Section 2 POSTURAL VASOCONSTRICTION IN WOMEN RECEIVING COMBINED ORAL CONTRACEPTIVE THERAPY

Introduction

Combined oral contraceptive steroids produce profound effects upon the cardiovascular system (Segal & Atkinson, 1973). Cardiac output and blood volume are markedly elevated (Lehtovirta, 1974), and variations in peripheral blood flow (Keates & Fitzgerald, 1975), increased venous distensibility (Goodrich & Wood, 1964), increased capillary hydrostatic pressure (Tooke et al, 1981) and modifications in vascular smooth muscle reactivity (Altura & Altura, 1977) have also been reported. However, the effect of these synthetic steroids on the postural control mechanisms is still unclear.

Premenstrual oedema (Thorn, 1957; Reid & Yen, 1981) appears to be particularly prevalent in women receiving combined oral contraceptive preparations (Tooke et al, 1981). Clinical studies indicate that the use of such preparations may ameliorate some premenstrual symptoms, but other symptoms including a persistent increase in body weight tend to be aggravated (Nilsson & Solvell, 1967). Since the postural increase in precapillary resistance is thought to act as an oedema preventing mechanism (Sejrsen et al, 1981a), the attenuation of such a postural response might be implicated in the pathogenesis of premenstrual oedema. This assumption gains much support from the observation that impaired postural

vasoconstriction was common in conditions characterized by high prevalence of peripheral oedema (Rayman et al, 1986; Williams et al, 1987).

Since it has been demonstrated (Chapter IV, Section 1) that the postural vasoconstrictor response is markedly attenuated during the luteal phase of the normal menstrual cycle particularly in women with premenstrual oedema, the aim of the present study was to investigate the effect of combined oral contraceptive therapy on the postural response and its relationship to the swelling rate in the dependent foot.

Methods

Fifteen healthy women aged 23-35 years taking combined oral contraceptive pills for a total duration of 1.5 - 14 years (Table 21) were studied on day 7 (the last of the 7 pill-free days) and day 28 (the last day of pill ingestion). Another woman aged 31 years taking a continuous progesterone-only contraceptive preparation (Norethisterone 350μg) for 3 months was also studied just before and on the last day of treatment. None of the women was taking any drugs other than the contraceptive preparations during the course of study. Fifteen healthy men aged 21 - 37 years were also studied on 2 separate occasions at least one week apart.

From a menstrual history, eight of the women reported ankle swelling and feeling tight shoes or tight rings recurring 5 - 8

Table 21: The combined oral contraceptive preparations used. n, Number of women. The women used the last preparation for one month to 5.5 years.

n	Last Preparation Used	Oestrogen Content	Progestogen Content	Total duration of pill use (years)
11	Microgynon 30 or Ovranette	Ethinyl Oestradiol 30µg	Levonorgestrel 150µg	2-14
1	Eugynon 30	Ethinyl Oestradiol 30µg	Levonorgestrel 250µg	9
1	Marvelon	Ethinyl Oestradiol 30µg	Desogestrel 150µg	8
1	Conova 30	Ethinyl Oestradiol 30µg	Ethynodiol Diacetate 2mg	1.5
1	Trinordiol	Ethinyl Oestradiol 30-40µg	Levonorgestrel 50-125µg	4

days before the onset of menstruation. On physical examination, bilateral pitting oedema of the ankles was documented, but there was no clinical evidence of any cardiac, hepatic or renal disease. Each subject wore similar clothing on repeat visits.

Measurement of the postural changes in foot skin blood flow

In 12 women and 12 aged-matched men, the postural vasoconstrictor response in the foot was assessed by measuring the postural changes in foot skin blood flow using laser Doppler flowmetry as described in Chapter II. On each visit, skin blood flow was measured with the subject supine, first with the foot maintained at heart level, then with the foot placed passively 50 cm below the heart. Recordings were made from the dorsum of the foot and the pulp of the big toe. One of the women stopped the combined oral contraceptive therapy after recording the postural response on day 28 of the cycle, and the postural changes in flow were examined during the menstrual, follicular and luteal phases of the cycle immediately following cessation of therapy.

Measurement of Foot Swelling Rate

In 3 women (on days 7 and 28) and 3 men (on the 2 visits), changes in foot circumference on dependency were measured using strain gauge plethysmography (as described in Chapter II) at the time of blood flow measurements.

On each visit, brachial arterial blood pressure (by auscultation), heart rate (radial pulse), sublingual temperature (by a mercury clinical thermometer), foot skin temperature (by a sensitive thermocouple) and body weight (by an ordinary scale) were also measured. In 6 of the women, experiments were repeated on days 7 and 28 of 2 consecutive cycles.

RESULTS

No significant differences were found in arterial blood pressure and heart rate measured on day 28 as compared with day 7 in women receiving the combined oral contraceptive preparations, or in all the variables measured in the men on the 2 visits (Table 22). In contrast, the women showed significant increases in body temperature, foot skin temperature and body weight on day 28 compared with day 7. Whereas there was a moderate nonsignificant increase in skin blood flow measured at heart level, the mean flow values measured in the dependent foot on day 28 were significantly higher than those obtained on day 7 (Figure 37). The response was qualitatively similar in the 2 skin areas tested, but the flow values were much greater in the toe pulp than in the dorsum of the foot. Moreover, the postural fall in flow (%) was significantly attenuated on day 28 as compared with day 7 (Table 22).

It was also noted that the percentage increase in flow measured

Table 22: The postural changes in foot skin blood flow (as mV) in 12 women taking combined oral contraceptives studied on days 7 and 28 of the cycle, as compared with the changes obtained in 12 age-matched men studied on 2 separate visits. The changes in body temperature and body weight are also included. H, with the foot horizontal, D, during the 4th min with the foot dependent. *P<0.01, **P<0.001 (as compared with H). The postural fall in flow was calculated as (H-D)/H%. NS, nonsignificant. Values are expressed as mean ± SEM.

	Women on oral contraceptives			Male Subjects		
	Day 7	Day 28	P	1st Visit	2nd Visit	P
Dorsum of the foot						
Skin Temperature (°C)	30.0 ± 0.3	31.2 ± 0.3	<0.001	30.1 ± 0.2	29.8 ± 0.3	NS
Skin blood flow (H)	17.3 ± 3.2	25.8 ± 4.5	NS	13.5 ± 1.1	15.9 ± 1.1	NS
Skin blood flow (D)	4.0 ± 0.8**	8.5 ± 1.3*	<0.01	2.8 ± 0.3**	3.3 ± 0.4**	NS
Postural fall in flow	75.5 ± 2.6%	61.1 ± 5.1%	<0.05	78.4 ± 1.8%	79.3 ± 1.9%	NS
Pulp of the big toe						
Skin Temperature (°C)	27.2 ± 0.6	30.0 ± 0.6	<0.001	27.1 ± 0.7	26.9 ± 0.7	NS
Skin blood flow (H)	148.0 ± 37.4	282.5 ± 74.5	NS	105.2 ± 26.5	101.4 ± 25.0	NS
Skin blood flow (D)	20.1 ± 5.1*	85.3 ± 32.3	=0.01	18.0 ± 4.9*	15.6 ± 3.9*	NS
Postural fall in flow	85.4 ± 2.8%	67.9 ± 7.0%	<0.05	84.1 ± 2.1%	85.4 ± 1.9%	NS
Body Temperature (°C)	36.63 ± 0.06	36.84 ± 0.04	<0.001	36.8 ± 0.05	36.74 ± 0.05	NS
Body Weight (Kg)	67.1 ± 2.3	68.2 ± 2.4	<0.001	70.8 ± 1.3	70.7 ± 1.3	NS

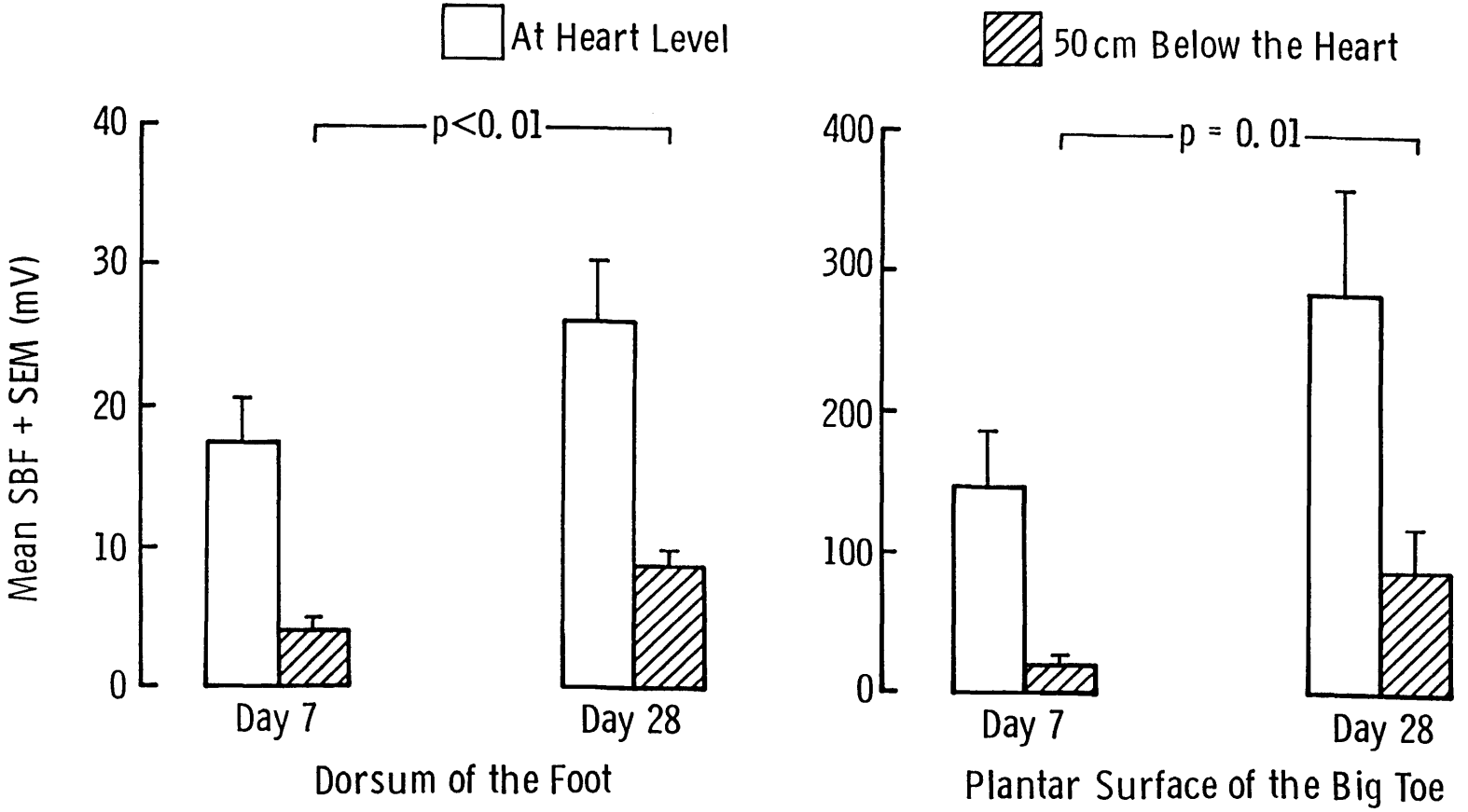


Figure 37: The postural changes in skin blood flow (SBF) measured in the dorsum of the foot and the plantar surface of the big toe on days 7 and 28 of the cycle in 12 women on combined oral contraceptive therapy.

in the dependent position in women on day 28 was significantly greater than the percentage increase in horizontal flow (Dorsum: 207.9 ± 45.2% vs 85.0 ± 35.5%, P=0.01, Toe pulp: 840.8 ± 588.4% vs 281.0 ± 201.8%, P=0.05). In addition, a positive correlation was found between the total duration of oral contraceptive therapy and the blood flow values measured in the dependent position on the dorsum of the foot on day 28 of the cycle (Figure 38).

The flow responses obtained before and on the last day of treatment in the woman on progesterone-only contraceptive, were comparable to those obtained on days 7 & 28 in women on the combined preparations. Thus, in the dorsum of the foot, skin blood flow measured in the horizontal and dependent positions and the postural fall in flow (%) were 10.4 mV, 2.4mV, 76.9% before, and 44 mV, 18.8 mV, 57.3% on the last day of therapy, respectively. The corresponding values in the toe pulp were 36.8 mV, 6.4 mV, 82.6%, and 122.7 mV, 59.7 mV, 51.3% respectively.

In the woman who stopped the combined oral contraceptive therapy, the postural fall in flow (%) measured in both skin areas on days 7 & 28 of the last treatment cycle was similar to that obtained in the whole group (Dorsum: 95.1% & 58.8%, Toe pulp: 86.9 % & 37.6%, respectively), whereas the pattern of response obtained during the menstrual, follicular and luteal phases of the cycle following cessation of therapy was similar to that obtained in women with normal ovulatory cycles (Dorsum:

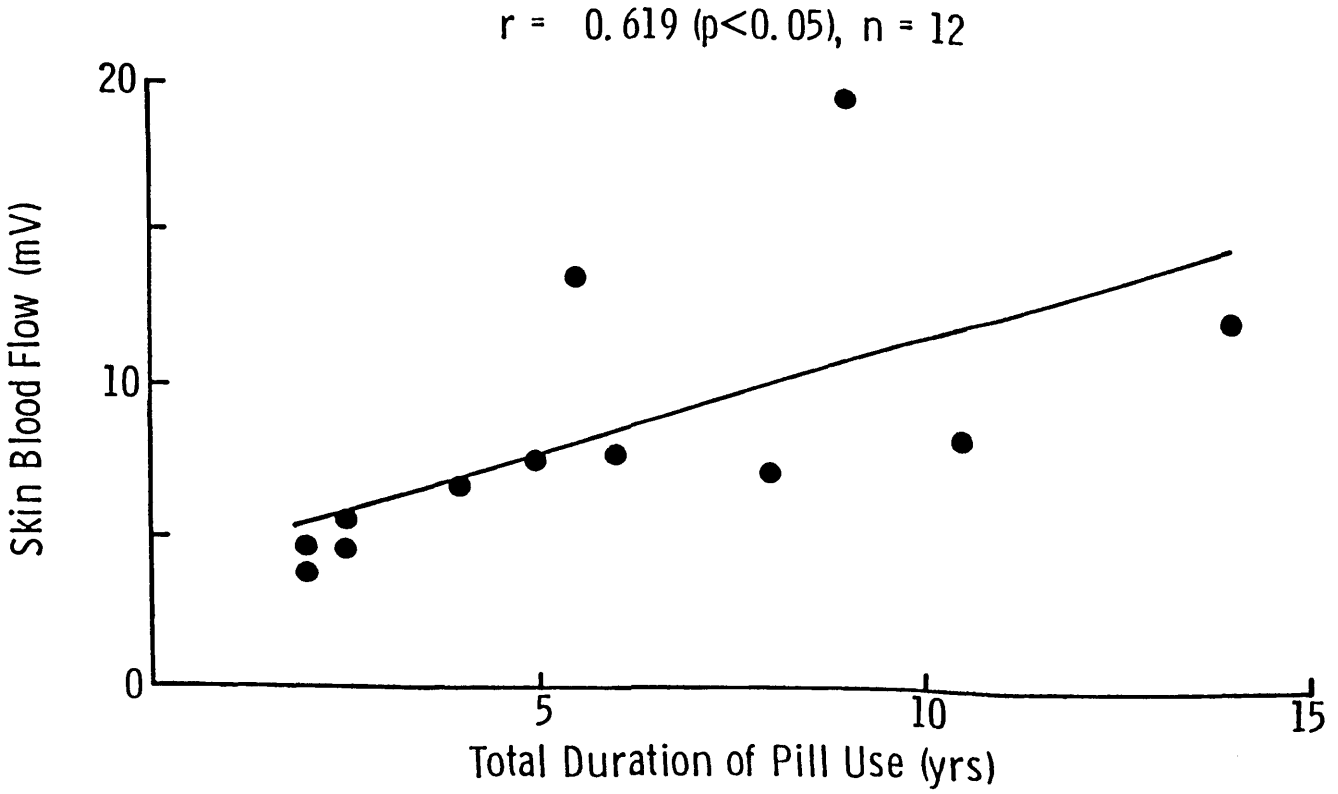


Figure 38: The relationship between the duration of therapy in 12 women receiving combined oral contraceptive preparations and skin blood flow measured in the dependent position on the dorsum of the foot on day 28 of the cycle.

83.2%, 86.6% & 51.5%, Toe pulp: 82.9%, 92.6% & 48.0% respectively).

The different parameters assessed in women taking combined oral contraceptives on day 7 were not significantly different from those obtained in the men, whereas on day 28 of the cycle, skin temperature and skin blood flow measured in the horizontal and dependent positions were significantly higher and the postural fall in flow (%) was significantly lower ($P<0.05$ - $P<0.01$) than the corresponding values measured in the men on either visit (Table 22).

In women who reported premenstrual ankle oedema, the flow values tended to be higher than those obtained in the rest of women. Indeed, in the toe pulp, women with oedema showed significantly higher dependent flow on day 28 than women without oedema (132.2 ± 48.7 vs 19.5 ± 6.7 mV, $P<0.05$). Also, the mean increase in body weight and the mean duration of pill exposure were significantly greater in women with oedema than the rest of women (1.6 ± 0.2 vs 0.5 ± 0.2 Kg, $P<0.01$, 7.7 ± 1.4 vs 3.4 ± 0.8 years, $P<0.02$, respectively).

The 6 women studied on days 7 and 28 of 2 consecutive cycles showed a reproducible characteristic cyclical pattern (Table 23).

In the foot swelling rate experiments, the men showed consistent responses on the 2 visits (Figure 39). In contrast,

Table 23: The postural changes in foot skin blood flow (as mV) in 6 women taking combined oral contraceptives measured on days 7 and 28 of 2 consecutive cycles. The changes in body temperature and body weight are also shown. H, with the foot horizontal, D, during the 4th min with the foot dependent. The postural fall in flow was calculated as (H-D)/H%. n, Number of subjects. Values are expressed as mean ± SEM.

	Cycle 1		Cycle 2		Mean Coefficient of variation	
	Day 7	Day 28	Day 7	Day 28	Days 7	Days 28
Dorsum of the foot (n=3)						
Skin Temperature (°C)	29.9 ± 1.0	30.8 ± 0.5	29.7 ± 0.7	31.0 ± 0.5	1.3%	0.7%
Skin blood flow (H)	19.4 ± 7.2	23.4 ± 5.3	17.7 ± 5.2	24.8 ± 5.4	9.2%	7.9%
Skin blood flow (D)	4.1 ± 1.3	8.6 ± 2.5	4.0 ± 1.2	8.4 ± 1.7	7.3%	8.1%
Postural fall in flow	78.7 ± 2.4%	62.1 ± 7.2%	77.9 ± 1.3%	64.0 ± 6.7%	6.3%	3.3%
Pulp of the big toe (n=3)						
Skin Temperature (°C)	28.2 ± 0.8	30.3 ± 1.3	28.0 ± 0.7	30.1 ± 0.9	1.2%	1.4%
Skin blood flow (H)	143.3 ± 20.2	307.1 ± 199.7	139.7 ± 32.2	267.0 ± 167.1	9.1%	9.7%
Skin blood flow (D)	22.8 ± 8.1	88.7 ± 56.3	20.9 ± 7.0	83.3 ± 51.3	6.5%	6.3%
Postural fall in flow	83.8 ± 6.1%	68.8 ± 3.4%	84.3 ± 5.9%	66.9 ± 2.9%	9.4%	4.5%
Body Temperature (°C)	36.58 ± 0.06	36.8 ± 0.04	36.53 ± 0.03	36.75 ± 0.04	0.3%	0.2%
Body Weight (Kg)	66.9 ± 3.3	67.8 ± 3.4	66.7 ± 3.1	67.9 ± 3.1	0.4%	0.7%

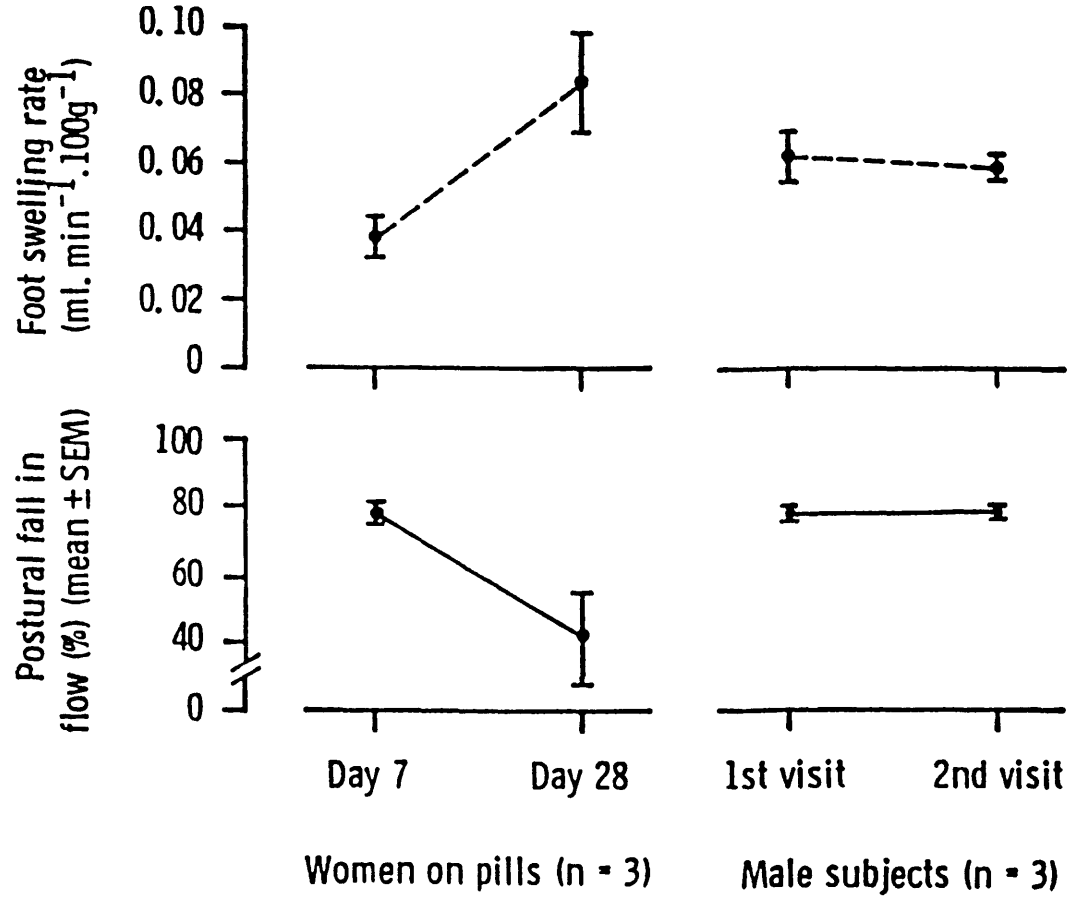


Figure 39: The relationship between the postural fall in skin blood flow and the simultaneously recorded foot swelling rate in the dependent foot in 3 women taking combined oral contraceptives studied on days 7 and 28 of the cycle as compared with the changes obtained in 3 age-matched men studied on 2 separate occasions.

women using the combined oral contraceptive steroids showed a markedly attenuated postural fall in flow (%) associated with a significantly higher foot swelling rate on day 28 as compared with day 7 (42.0 ± 14.4% vs 79.2 ± 3.0%, 0.085 ± 0.014 vs 0.039 ± 0.005 $ml.min^{-1}.100ml^{-1}$., $P<0.05$) (Figure 39).

There was no significant difference in the responses obtained with the different combined oral contraceptive preparations used in the present study.

DISCUSSION

1. Postural Vasoconstriction in Women During the Normal Menstrual Cycle

Significant physiological variations in the postural vasoconstrictor response and the swelling rate of the dependent foot have been demonstrated at different stages of the normal ovulatory menstrual cycle. These variations appear to be related, in some way, to the physiological fluctuations in the blood levels of the female sex hormones. Most important is the observation that postural vasoconstriction is partially augmented during the follicular phase, but markedly attenuated during the luteal phase of the cycle particularly in women with premenstrual oedema.

The partially augmented postural vasoconstrictor response during the follicular (oestrogenic) phase of the cycle might be ascribed to an oestrogen-induced potentiation of vascular smooth muscle contraction (Lloyd, 1959; Altura, 1975). On the other hand, the marked impairment of the response during the luteal phase of the cycle in skin areas with and without arteriovenous anastomoses is unlikely to be wholly attributable to an overriding reflex thermoregulatory vasodilatation due to increased body temperature, since it has been shown (Chapter III, Section 1) that during indirect heating the postural response is impaired in the toe pulp (with anastomoses) but preserved in the dorsum of the foot (without anastomoses).

Although the exact mechanism of the partially impaired postural vasoconstriction during the luteal phase of the cycle remains unclear, it has been shown that female sex hormones not only strongly influence vascular smooth muscle reactivity to several vasoactive agonists (Hettiaratchi & Pickford, 1968; Eccles & Leathard, 1985), but can also modify the mechanisms involved in the synthesis, release or disposition of norepinephrine as well as the process of excitation-contraction coupling of the vascular smooth muscle (Altura & Altura, 1977; Vanhoutte et al, 1981). This explanation is also supported by the finding that the use of combined oral contraceptive steroids is associated with markedly impaired postural vasoconstriction (Chapter IV, Section 2).

The significantly reduced diastolic and mean arterial pressures observed in women during the luteal phase of the normal menstrual cycle (Table 18) might reflect a reduced resting peripheral vascular tone, and thus a reduced resting vascular resistance. This could provide further indirect evidence as to the mechanism by which the postural increase in precapillary resistance was markedly attenuated during the luteal phase.

The biphasic basal body temperature responses observed in ovulatory cycles (Figure 31, Table 18) are consistent with the findings reported in earlier studies (Marshall, 1963). It is thought that the higher basal body temperature during the luteal phase of the cycle is due to the influence of progesterone (Davis & Fugo, 1948) acting probably on

hypothalamic heat regulating centres (Landau, 1973). This interpretation is supported by the absence of any significant change in body temperature during the late phase of anovulatory cycles (Table 17).

The fluctuations in serum oestradiol and progesterone concentrations seen on the experimental days at the different phases of the normal menstrual cycle in the present study are in agreement with previous reports (Landgren et al, 1980), whereas the positive correlation obtained between serum oestradiol concentrations and foot skin blood flow (Figure 33) emphasizes the modulating influence played by oestrogen on peripheral blood flow and vascular tone (Reynolds & Foster, 1939; Reynolds, 1941; Lim & Walters, 1970). However, it is not known which of the 2 hormones (oestrogen or progesterone) might be involved in the attenuation of the postural response during the luteal phase of ovulatory cycles. Nevertheless, the absence of any impairment of the response during the latter phase of anovulatory cycles, when serum progesterone concentration remained unchanged, suggests that progesterone might be critically involved in the mechanisms leading to the inhibition of the postural vasoconstrictor response. This conclusion is also supported by the finding that progesterone inhibits the pressor action of angiotensin in the rat (Hettiaratchi & Pickford, 1968).

2. Postural Vasoconstriction in Women Receiving Combined Oral Contraceptive Therapy

In this study it was demonstrated that the use of combined oral contraceptive steroids is associated with a marked attenuation of the postural vasoconstrictor response together with a significant increase in the swelling rate of the dependent foot.

Since the postural response was impaired in areas with and without arteriovenous anastomoses, such impairment is unlikely to be wholly ascribed to an overriding reflex thermoregulatory vasodilatation due to increased body temperature, as the postural vasoconstrictor response has been shown to be preserved in areas without anastomoses during indirect heating (Chapter III, Section 1). Although the mechanism of impaired postural vasoconstriction during combined oral contraceptive therapy remains unclear, several potential explanations may be advanced: it is known that female sex hormones can modulate adrenergic neuromuscular transmission (Vanhoutte et al, 1981) and can modify the mechanisms involved in vascular smooth muscle contraction (Altura & Altura, 1977). Furthermore, oestrogen and progesterone can substantially influence the reactivity of the vascular muscle to several vasoactive agonists (Hettiaratchi & Pickford, 1968; Altura, 1975; Eccles & Leathard, 1985).

Although it is not known which of the 2 hormones (oestrogen or

progesterone) may be involved in the impairment of the postural vasoconstrictor response in women taking combined oral contraceptive steroids, the finding of attenuated postural vasoconstriction in the woman using progesterone-only contraceptive, and the absence of any impairment of the postural response in women with anovulatory cycles (with no increase in serum progesterone) suggest that progesterone is the hormone more likely to be involved. However, the exact role played by each of these hormones in modifying the mechanisms of postural vasoconstriction remains to be elucidated.

The observation that foot blood flow measured in the dependent position was positively correlated with the total duration of combined oral contraceptive therapy is interesting. Although the data do not provide a reasonable explanation for such a relationship, one possibility is that the long term use of these synthetic steroids might modify the cellular metabolism in the vascular wall (Altura & Altura, 1977) which might result in alterations in the concentration of some of the local humoral mediators. This suggestion is supported by the finding of enhanced prostacyclin formation in the saphenous veins in women under chronic treatment with combined oral contraceptives (Sinzinger et al, 1980). Prostacyclin, being one of the vasodilator prostaglandins, can increase the local blood flow either directly (Moncada & Vane, 1978) or by modifying the constrictor response to norepinephrine in the vascular wall (Vanhoutte et al, 1981).

The Relation Between Postural Vasoconstriction and Foot Swelling Rate

In contrast to the consistent responses obtained in men on the 2 visits, women with normal ovulatory cycles or those taking combined oral contraceptive pills showed an inverse relationship between the percentage fall in flow on dependency and foot swelling rate (Figures 36 & 39, Table 24) i.e. more effective postural vasoconstriction was associated with lower foot swelling rate and vice versa.

Since both skin temperature and foot swelling rate were reduced during the follicular but increased during the luteal phase of the cycle, it may be argued that the observed fluctuations in foot swelling rate could be related to the fluctuations in resting skin temperature, presumably, by changing the mercury resistance of the strain gauge. Indeed, a positive correlation was found between resting foot skin temperature and foot swelling rate in both men (Figure 14) and women (Figure 40). However, despite the similarity of mean skin temperature in the men, and in both groups of women during the luteal phase, the women showed higher foot swelling rate than the men (Table 24). Also, the change in foot swelling rate per degree centigrade change in skin temperature (i.e. the regression coefficients in Figures 14 & 40) was much greater in women than in men (0.0096 vs 0.0055 $ml.min^{-1}.100ml^{-1}.^{\circ}C^{-1}$). Furthermore, the strain gauge was left to equilibrate with the skin for at least 20 minutes before setting the gauge resistance to a new reference

Table 24: The relationship between the postural changes in skin blood flow (recorded on the dorsum of the foot, as mV) and foot swelling rate (FSR) measured during the 10th-20th min after the foot was lowered 50cm below the heart in 4 women studied during the menstrual, follicular and luteal phases of their normal cycles, 3 women taking combined oral contraceptives studied on days 7 & 28 of their cycles, and 3 men studied on 2 separate visits. H, with the foot horizontal, D, during the 10th-20th min with the foot dependent. The postural fall in flow was calculated as (H-D)/H%.

Women with normal cycles	Menstruation	Follicular	Luteal
Skin Temperature (°C)	27.7 ± 1.0	26.8 ± 1.1	28.5 ± 0.9
Skin Blood Flow (H)	14.8 ± 3.5	17.2 ± 2.0	21.8 ± 4.6
Skin Blood Flow (D)	4.1 ± 0.7	2.6 ± 0.4	10.3 ± 1.3
Postural Fall in Flow	71.4 ± 2.4%	85.2 ± 1.9%	49.0 ± 5.9%
FSR ($ml.min^{-1}.100ml^{-1}$)	0.057 ± 0.015	0.030 ± 0.004	0.085 ± 0.012

Women on oral contraceptives	Day 7	Day 28
Skin Temperature (°C)	27.1 ± 1.3	28.7 ± 0.8
Skin Blood Flow (H)	18.4 ± 5.8	26.1 ± 7.1
Skin Blood Flow (D)	3.7 ± 1.1	13.1 ± 1.4
Postural Fall in Flow	79.2 ± 3.0%	42.0 ± 14.4%
FSR ($ml.min^{-1}100ml^{-1}$)	0.039 ± 0.005	0.085 ± 0.014

Male subjects	1st Visit	2nd Visit
Skin Temperature (°C)	28.8 ± 0.8	28.6 ± 0.9
Skin Blood Flow (H)	22.0 ± 4.4	21.4 ± 2.7
Skin Blood Flow (D)	4.8 ± 1.1	4.2 ± 0.7
Postural Fall in Flow	78.7 ± 1.0%	80.6 ± 1.0%
FSR ($ml.min^{-1}.100ml^{-1}$)	0.062 ± 0.007	0.059 ± 0.004

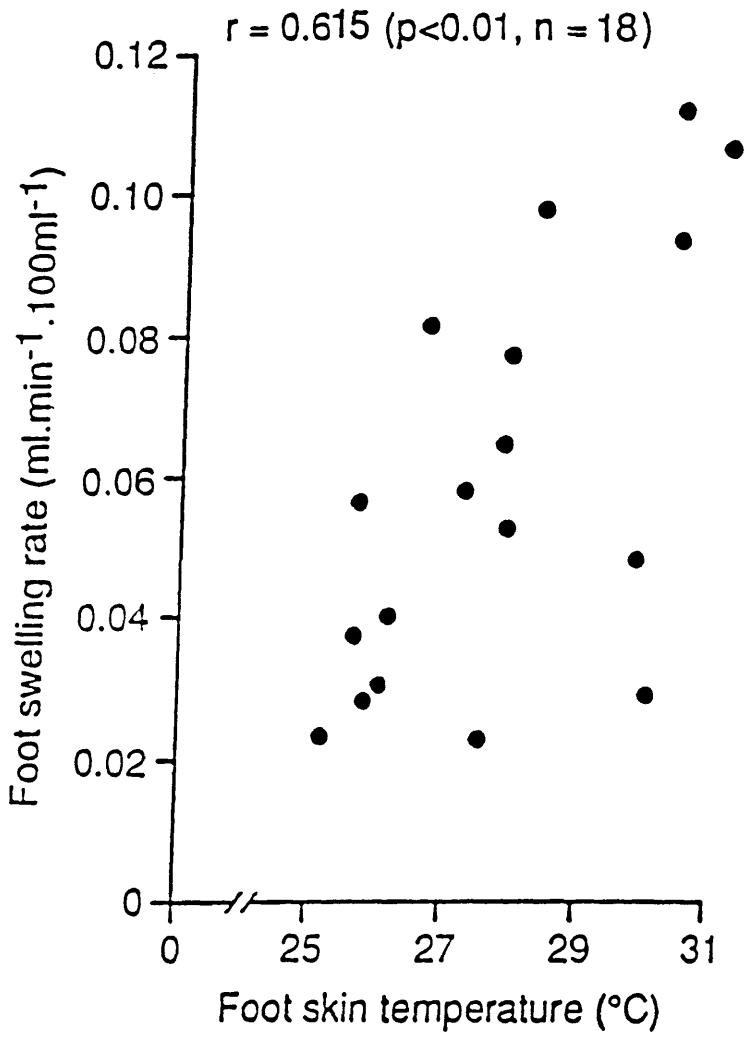


Figure 40: The relationship between resting foot skin temperature (recorded on the dorsum of the foot) and foot swelling rate measured during the 10th - 20th min after the foot was passively lowered 50cm below the heart (18 measurements in 7 women studied at different stages of their cycles).

level using the autozeroing system. Indeed, it has been shown that errors resulting from the change in gauge resistance due to the change in gauge temperature become negligible if the gauge is allowed to reach thermal equilibrium with the skin (Youdin & Reich, 1976); a requirement which has been fulfilled in the present experiments.

Therefore, the observed changes in foot swelling rate in women during the menstrual cycle are less likely to be wholly attributable to changes in resting skin temperature. A more likely explanation, however, is that these changes in foot swelling rate might result from the associated alterations in the postural increase in precapillary resistance found in these women (Table 24).

The attenuated postural vasoconstrictor response during the luteal phase in women with normal cycles and women receiving combined oral contraceptive therapy might reflect a lesser increase in precapillary resistance which would eventually result in a greater increase in capillary hydrostatic pressure on dependency than would have been expected if the postural response was more effective. This could explain the higher filtration rate observed in these women during the luteal phase of the cycle, and hence could provide an interpretation to the incidence of premenstrual ankle swelling in some of these women.

Although an attenuated postural response was consistently

observed during the luteal phase in nearly all women, manifest ankle oedema occurred only in some of the women. However, the consistent increase in body weight in all women suggests that at least some of the women who did not develop manifest oedema might have had an occult oedema. This conclusion is supported by the observation that foot swelling rate measured during the luteal phase in these women was higher than that obtained in the men (Figures 36 & 39, Table 24) who did not show any significant change in body weight.

Although premenstrual swelling has been previously suggested to be related to changes in capillary permeability (Jones et al, 1966) or alterations in the vasopressin and/or the renin-angiotensin-aldosterone system (Reid & Yen, 1981), the actual mechanism of this condition is not yet completely understood. However, the impaired postural vasoconstrictor response and the relatively higher dependent flow observed in women with premenstrual oedema during the luteal phase of the cycle suggests that sex hormone-induced changes in peripheral vascular reflexes could be an important factor contributing to the complex pathogenesis of this condition. This hypothesis is supported by the observations of increased capillary filtration coefficient during the premenstrual period in women with premenstrual syndrome (Wong et al, 1972), excessive increase in leg volume on standing in patients with idiopathic oedema (Kuchel et al, 1970), and the marked improvement of periodic orthostatic oedema when the patients were treated with vasoconstrictor drugs (Streeten & Conn, 1959).

It might be speculated that the attenuated postural vasoconstriction observed in women during the luteal phase of the cycle could result in excessive pooling of blood in the dependent extremities. As a consequence of a greater reduction in central blood volume, activation of the vasopressin and/or the renin-angiotensin-aldosterone systems, with a subsequent decrease in urinary volume and urinary sodium excretion might ensue (Edwards & Bayliss, 1973), thus leading to salt and water retention and an aggravation of the oedema state (Kuchel et al, 1970).

In conclusion, this study has confirmed that female sex hormones have a modulating influence on peripheral blood flow and vascular tone. Moreover, it has provided evidence supporting the view that the postural increase in precapillary resistance might be involved in the protection against the development of dependent oedema (Sejrsen et al, 1981a).

Chapter V

THE MECHANISM OF POSTURAL VASOCONSTRICTION IN THE FOOT

Chapter V

THE MECHANISM OF POSTURAL VASOCONSTRICTION IN THE FOOT

Introduction

It has been demonstrated in Chapters III & IV that foot blood flow is reduced on standing or when the extremity is passively lowered below heart level in supine subjects. However, the mechanism underlying the postural vasoconstrictor response in the foot is still unclear.

Previous work suggests that postural vasoconstriction is unaffected by acute spinal sympathetic blockade, but abolished 4 days after sympathectomy. It is also blocked by local infiltration with lidocaine and phentolamine, indicating the involvement of a local sympathetic axon reflex mechanism (Henriksen & Sejrsen, 1976; Henriksen, 1977; Henriksen & Paaske, 1980).

It has also been suggested that the increase in hydrostatic pressure in the dependent extremities, by distending the arterial walls, could initiate a local myogenic contraction of the vascular smooth muscle (Bayliss, 1902; Mellander et al, 1964), whereas consideration of the central haemodynamic responses to postural change implicates the involvement of central reflexes (Brigden et al, 1950; Blomqvist & Stone, 1983), although such involvement is controversial (Delius et

al, 1972; Rowell et al, 1973).

Therefore, the aim of the present study was to investigate the relative contribution of both the central and local mechanisms to the postural vasoconstrictor response in the skin of the human foot, and to further elucidate the nature of the local mechanism involved in this response.

Methods

Four protocols were carried out on a total of 102 adult subjects aged 18-78 years.

Measurement of the postural changes in foot skin blood flow:

In all protocols, the postural vasoconstrictor response in the skin of the foot was examined by measuring the postural changes in foot skin blood flow using laser Doppler flowmetry as described in Chapter II.

Foot skin blood flow (as mV) was measured first with the subject lying in the supine position and both feet maintained at heart level for at least 2 minutes (H), then with one foot lowered passively 50cm below the heart for at least 4 minutes (D), with the rest of the body remaining in the horizontal position. The postural fall in flow (%) was calculated as (H-D) x 100/H. Recordings were made from one or 2 skin areas: (i) the dorsum of the foot and (ii) the plantar surface of the

big toe.

Of the 4 protocols to be described, protocols 1 and 2 were designed to investigate the relative contribution of local and central control mechanisms to the postural vasoconstrictor response, whereas protocols 3 and 4 were designed to elucidate the nature of the local mechanisms involved.

Protocol 1: Foot blood flow in the contralateral limb on leg lowering:

a) In 45 subjects, the changes in flow in anatomically-identical skin areas on the dorsum of the foot and the pulp of the big toe were simultaneously recorded from the left foot subjected to the postural challenge and the right foot maintained in the horizontal position using 2 laser Doppler flowmeters.

b) In 12 other subjects, the changes in arterial blood pressure (upper arm) and heart rate were recorded for 2 minutes before, for 4 minutes during, and for 4 minutes after the orthostatic manoeuvre described above. Recording started after at least 10 minutes of supine rest using a Vital Signs Monitor (Model 845 XT, Critikon, Dinamap, Florida, USA). Mean arterial pressure was calculated as diastolic + 1/3 pulse pressure. The mean coefficients of variation from 12 duplicate measurements using this instrument were 3.0, 3.1 & 3.4% for systolic pressure, diastolic pressure and heart rate respectively.

Protocol 2: The effect of acute lumbar sympathetic blockade on postural vasoconstriction:

The postural changes in skin blood flow were measured on the dorsum of the foot and the pulp of the big toe (as described above) before and 30 minutes after acute lumbar sympathetic blockade induced by lumbar (L4/5 interspace) epidural anaesthesia (using 10ml of 4.9×10^{-2} mol/l lignocaine) in 10 otherwise healthy patients undergoing investigation for chronic low back pain. In 2 of these patients, the simultaneous changes in flow in the horizontal right foot and the dependent left foot were also recorded before and after sympathetic blockade. Using a sensitive thermocouple, skin temperature was measured from the 2 recording sites, and arm blood pressure and heart rate were also recorded before and 30 minutes after blockade with the patient supine and both feet at heart level.

Protocol 3: The effect of local anaesthesia on postural vasoconstriction:

The postural changes in skin blood flow were recorded on the dorsum of the foot in 19 subjects before and 15 minutes after S. C. infiltration of the recording site in one foot with 0.1 ml of different concentrations of lignocaine (3.7×10^{-4} - 7.4×10^{-2} mol/l) to induce an acute local nervous blockade, and 0.1 ml of physiological saline into an anatomically-identical skin site on the dorsum of the contralateral foot acting as a control. The right and left foot were randomly chosen for

either saline or lignocaine infiltration. When the postural response was tested in one foot before and after infiltration, the other foot was kept horizontal.

Protocol 4: Investigation of the involvement of a myogenic component in postural vasoconstriction:

Since the increase in venous pressure in the dependent extremity has been proposed to be the main stimulus initiating the arteriolar constriction (the venoarteriolar axon reflex) observed on dependency (Gaskell & Burton, 1953; Henriksen, 1977), in 13 subjects the reduction in skin blood flow measured on the dorsum of the foot and the pulp of the big toe when a venous occlusion of 40 mmHg is applied (with the cuff around the ankle or the base of the toe) with the foot maintained at heart level, was compared with the reduction in flow (measured from the same skin area) associated with lowering the foot 50cm below the heart (equivalent to a rise in venous pressure of approximately 37 mmHg (Henriksen & Sejrsen, 1977). The flow was measured with the venous occlusion applied for at least 4 minutes. About 5 minutes after the release of occlusion, when the flow almost resumed its preocclusion level the foot was lowered in the dependent position for 4 minutes, and the postural reduction in flow was recorded.

In 3 other subjects, the changes in skin blood flow in the dorsum of the foot during venous occlusion with the foot kept at heart level were measured before and 15 minutes after acute

local nervous blockade induced by S.C. infiltration of the recording site in one foot with 0.1ml of lignocaine (7.4 x 10^{-2}mol/l). An anatomically-identical skin site on the dorsum of the contralateral foot was infiltrated with 0.1ml of physiological saline acting as a control.

RESULTS

Relative Contribution of Local and Central Control Mechanisms to the Postural Vasoconstrictor Response in the Foot:

When the changes in skin blood flow in the dorsum of the foot and the pulp of the big toe were simultaneously recorded from both feet when one foot was lowered below heart level, there was a progressive fall in flow in the dependent left foot, with a concomitant, almost constant but less marked, reduction in flow in the horizontal right foot, suggesting that a central mechanism is involved (Figure 41). When the left foot was returned to heart level, there was an initial nonsignificant increase in flow during the first min above resting values in that foot followed by a gradual return to almost original levels within 3-4 minutes, whereas resting flow in the horizontal right foot was almost restored (Figures 41 & 42). The response was qualitatively similar in both skin areas tested, but the mean flow values measured in the toe pulp throughout the experiment were about 6-8 times greater than the corresponding values measured on the dorsum of the foot, reflecting probably the greater number of arteriovenous

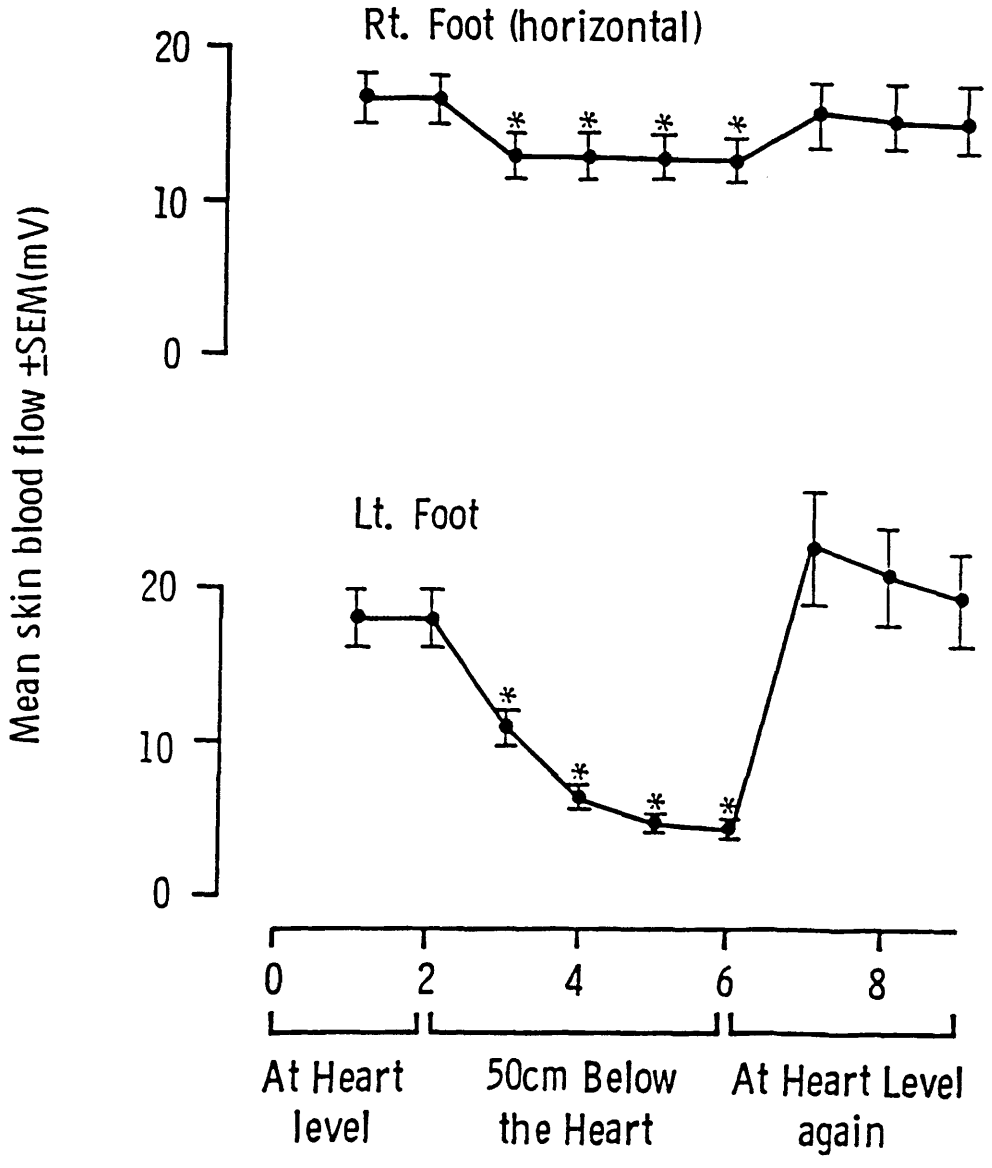


Figure 41: Effect of lowering the left foot (lower curve) 50 cm below the heart on skin blood flow measured on the dorsum of both feet in 45 normal subjects. *P<0.001 (as compared with the preceding mean rest flow).

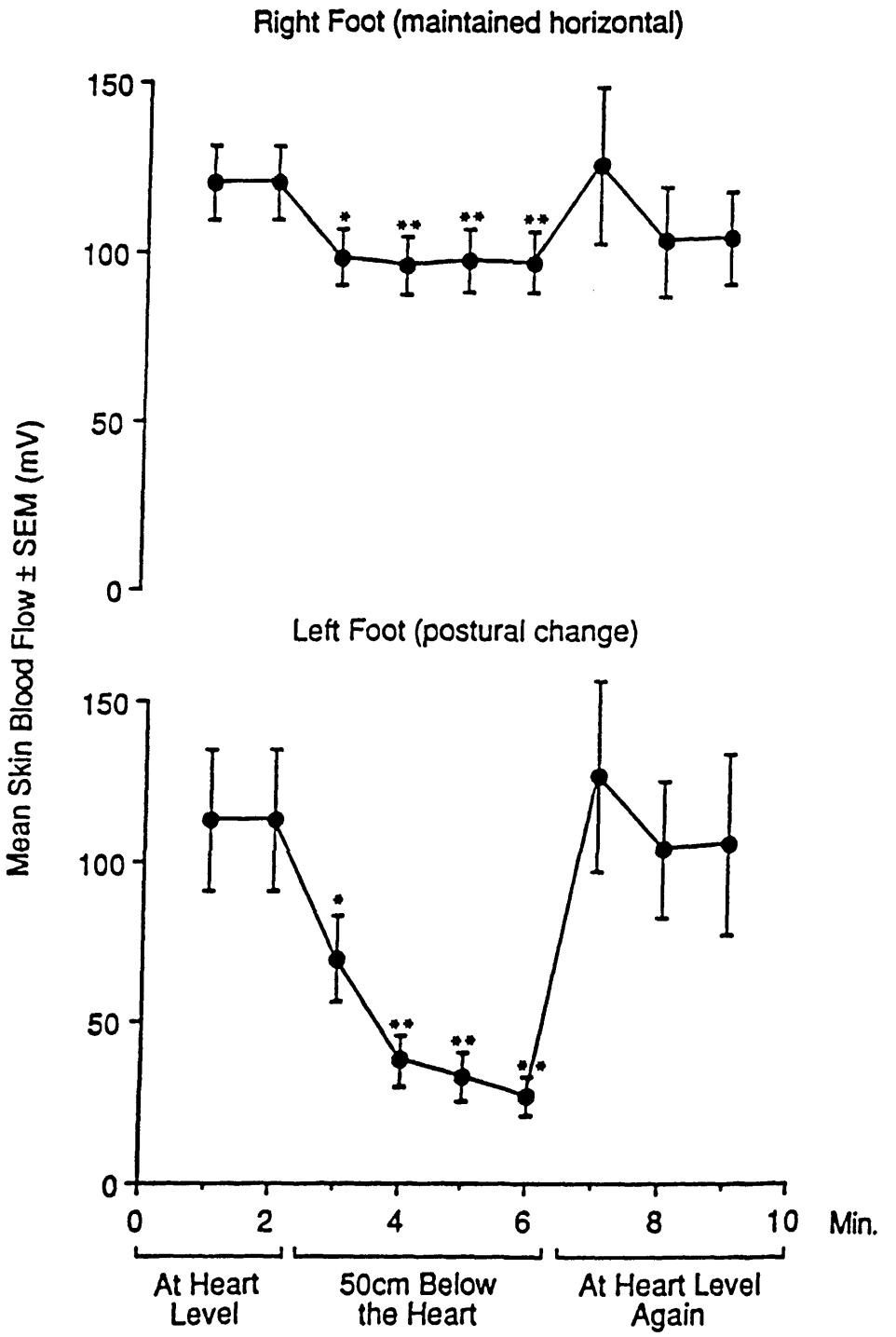


Figure 42: Effect of lowering the left foot (lower curve) 50 cm below the heart on skin blood flow measured on the plantar surface of the big toe of both feet in 45 normal subjects. *P<0.01, **P<0.001 (as compared with the preceding mean rest flow).

Table 25: Effect of lowering the left foot 50cm below the heart (with the right foot kept horizontal) on the percentage fall in skin blood flow in both feet (calculated as the % difference in flow values measured in each foot when both feet were horizontal and when the left foot was placed in the dependent position). n, number of experiments, *P<0.01 (as compared with right foot).

		Dorsum of the Foot (n=45)		Pulp of the Big Toe (n=45)	
		Right Foot	Left Foot	Right Foot	Left Foot
Time of	1	23.8 ± 2.5%	20.8 ± 11.5%	13.6 ± 4.5%	19.5 ± 8.2%
dependency	2	23.6 ± 2.8%	56.9 ± 4.2%*	17.9 ± 3.3%	61.0 ± 3.7%*
of the left	3	24.7 ± 2.5%	68.6 ± 2.4%*	19.9 ± 3.7%	72.6 ± 3.1%*
foot (min.)	4	24.4 ± 2.7%	71.2 ± 2.0%*	18.2 ± 2.8%	78.6 ± 2.4%*

anastomoses in the toe pulp (Grant & Bland, 1931). A similar pattern was obtained when the right foot was dependent and left foot kept horizontal.

During the first minute of dependency of the left foot, both feet showed similar percentage fall in flow. However, from the 2nd to 4th minute, the mean percentage fall in flow in the dependent left foot was significantly greater than that obtained in the horizontal right foot (Table 25), suggesting that both central and local mechanisms are involved in flow reduction in the dependent foot.

Changes in Arterial Blood Pressure and Heart Rate:

During the first minute after lowering one foot 50cm below the heart with the rest of the body remaining in the horizontal position, there was a small but significant reduction in systolic and mean arterial pressures, with no significant change in diastolic blood pressure or heart rate (Figure 43). During the 4th minute of dependency, there was a small significant reduction in systolic pressure, a small but significant increase in diastolic pressure, with the mean arterial pressure almost resuming its original resting level. When the foot was returned to heart level, resting pressures were gradually restored. Heart rate showed a small significant increase during the 4th minute of dependency and first minute when the foot was returned to heart level, then gradually restored its resting values (Figure 43).

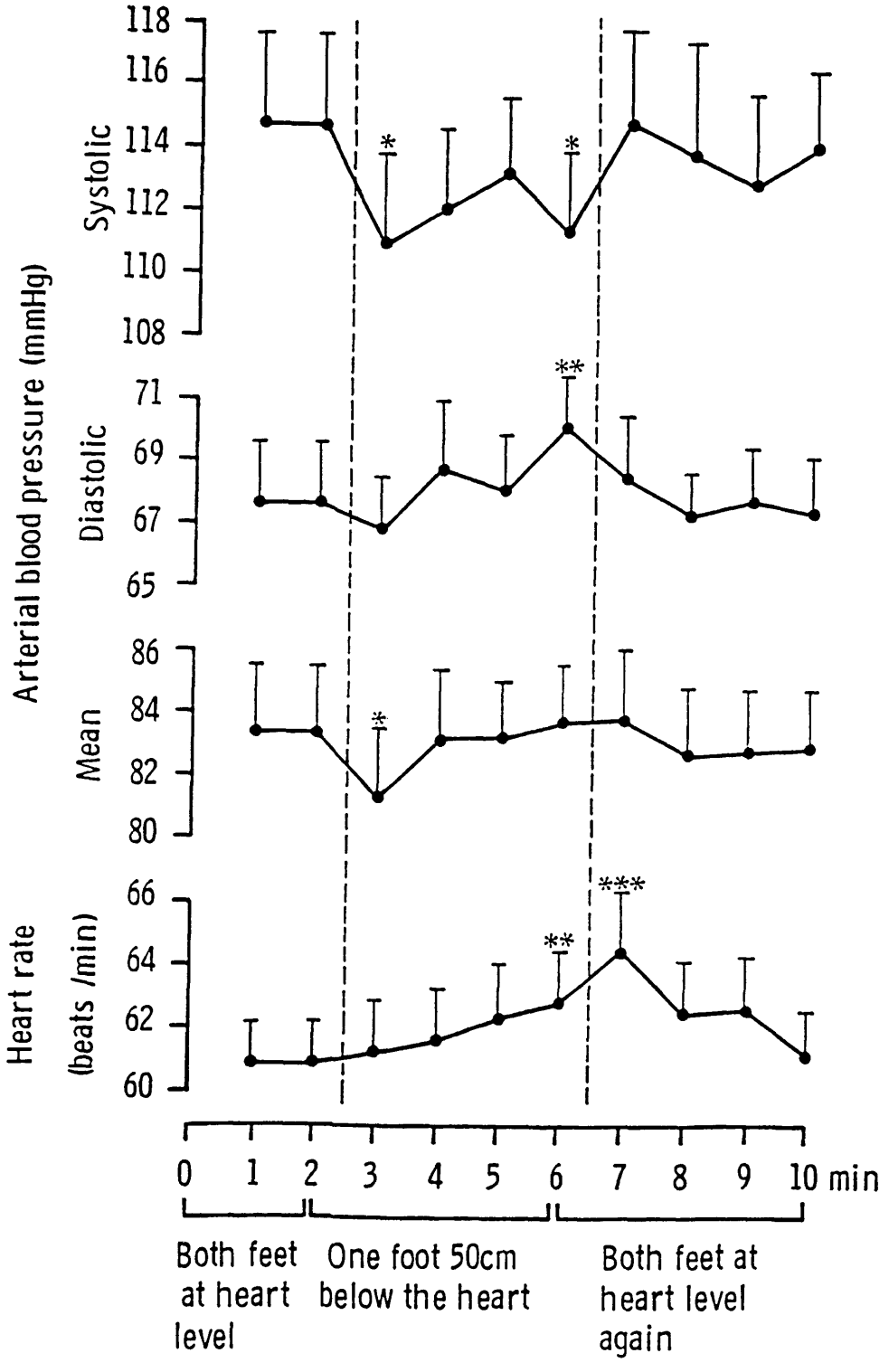


Figure 43: The changes in arterial blood pressure and heart rate associated with lowering one foot 50 cm below the heart (with the rest of the body remaining in the horizontal position) in 12 normal subjects. Values are given as means + SEM. *P<0.05, **P<0.01, ***P<0.001 (as compared with mean values obtained with both feet at heart level before the postural change).

Effect of Lumbar Sympathetic Blockade:

The significant increase in resting skin temperature (Figure 44) and skin blood flow (Table 26) after epidural anaesthesia indicated an effective sympathetic blockade (Roe & Cohn, 1973), with the toe pulp showing much greater increments in temperature and flow after blockade than the dorsum of the foot. Figure 45 presents an example of the tracings showing the postural changes in skin blood flow obtained before and 30 minutes after sympathetic blockade. On lowering the foot, the flow fell significantly in the 2 skin areas both before and after sympathetic blockade (Figure 46). Although the percentage of the postural fall in flow was partially attenuated after blockade as compared with preblockade, the absolute fall in flow after blockade was similar to or even slightly greater than that before blockade (Table 26).

In 2 of the patients, the changes in flow in the horizontal right foot associated with lowering the left foot below heart level were also simultaneously recorded before and after sympathetic blockade. Blood flow fell in the foot placed below heart level after blockade (dorsum: from 14 to 5 mV, big toe: from 371 to 296.3 mV) though to a lesser extent compared with pre-blockade (dorsum: from 5.6 to 1.3 mV, big toe: from 95.8 to 12.8 mV). Although the horizontal right foot showed a concomitant reduction in flow when the left foot was lowered below heart level before sympathetic blockade (dorsum: from 11.4 to 8.2 mV, big toe: from 117.6 to 71.7 mV), blood flow

Table 26: Effect of acute lumbar sympathetic blockade on the postural changes in foot skin blood flow (as mV) in 10 patients undergoing epidural anaesthesia. H, with the foot horizontal, D, during the 4th min with the foot dependent. The postural fall in flow was calculated as (H-D) x 100/H. Values are given as means ± SEM. *P<0.02, **P<0.01 (as compared with H). n, Number of experiments. NS, nonsignificant.

	Before Blockade	After Blockade (30 min)	Statistical Significance
Dorsum of the Foot (n=10):			
Skin Temperature (°C):	29.1 ± 0.5	31.3 ± 0.8	P<0.01
Skin Blood Flow (H):	19.4 ± 4.6	26.6 ± 4.9	P<0.05
Skin Blood Flow (D):	3.6 ± 0.7**	11.1 ± 1.7**	P<0.001
Postural fall in flow:	77.7 ± 3.2%	54.5 ± 4.6%	P<0.01
Pulp of the Big Toe (n=10):			
Skin Temperature (°C):	25.4 ± 1.0	31.6 ± 1.1	P<0.001
Skin Blood Flow (H):	106.8 ± 33.3	325.2 ± 77.4	P<0.01
Skin Blood Flow (D):	22.2 ± 11.7**	216.7 ± 66.5*	P<0.01
Postural fall in flow:	85.7 ± 2.5%	41.9 ± 10.0%	P<0.01
Arterial Blood Pressure (mmHg)			
Systolic:	116.8 ± 1.8	118.6 ± 2.1	NS
Diastolic:	74.5 ± 1.8	70.4 ± 2.5	NS
Mean:	88.6 ± 1.8	86.5 ± 2.0	NS
Heart Rate (beats/min):	67.2 ± 1.7	69.6 ± 1.2	NS

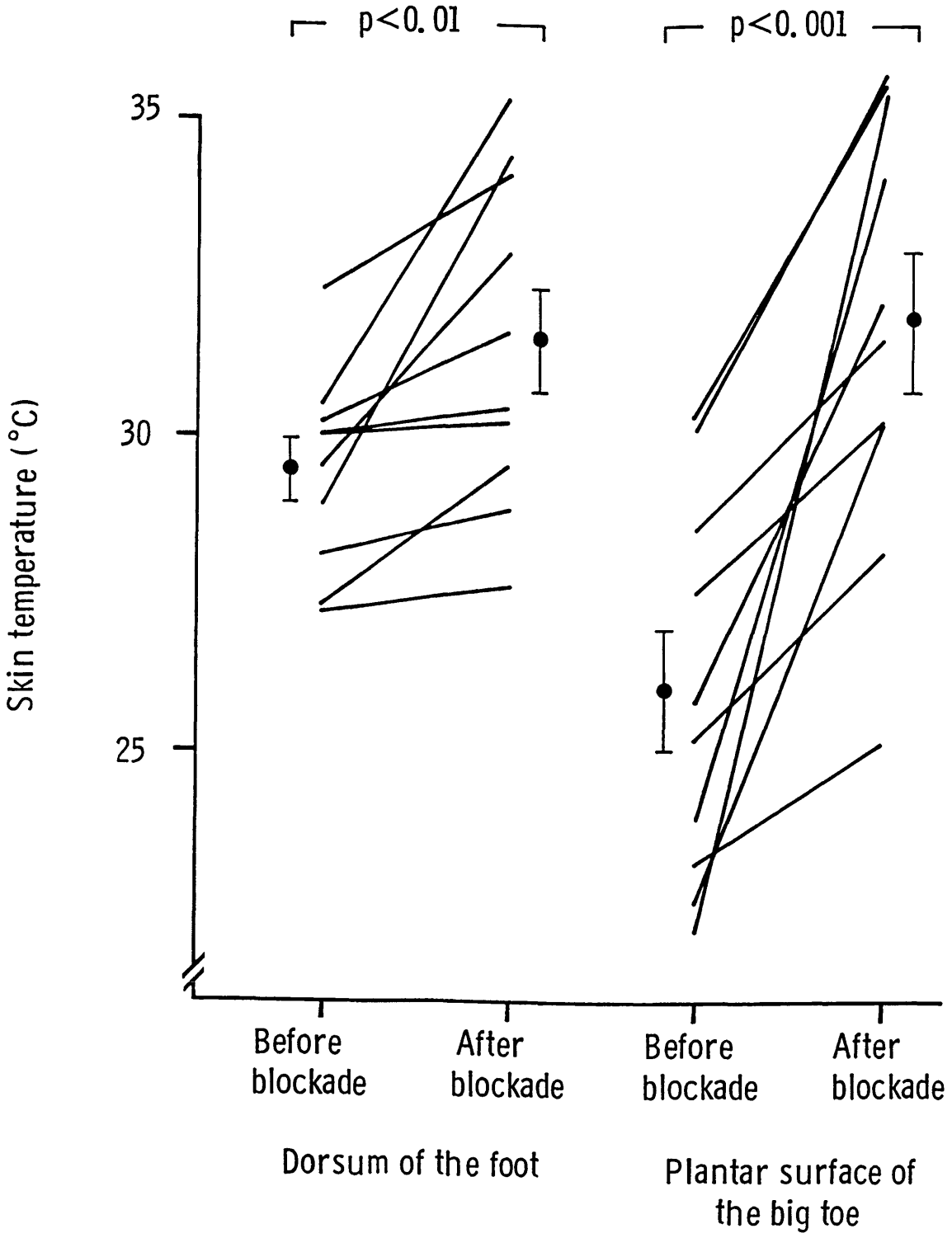


Figure 44: Foot skin temperature measured in 2 skin areas before and 30 min after acute lumbar sympathetic blockade in 10 patients with epidural anaesthesia.

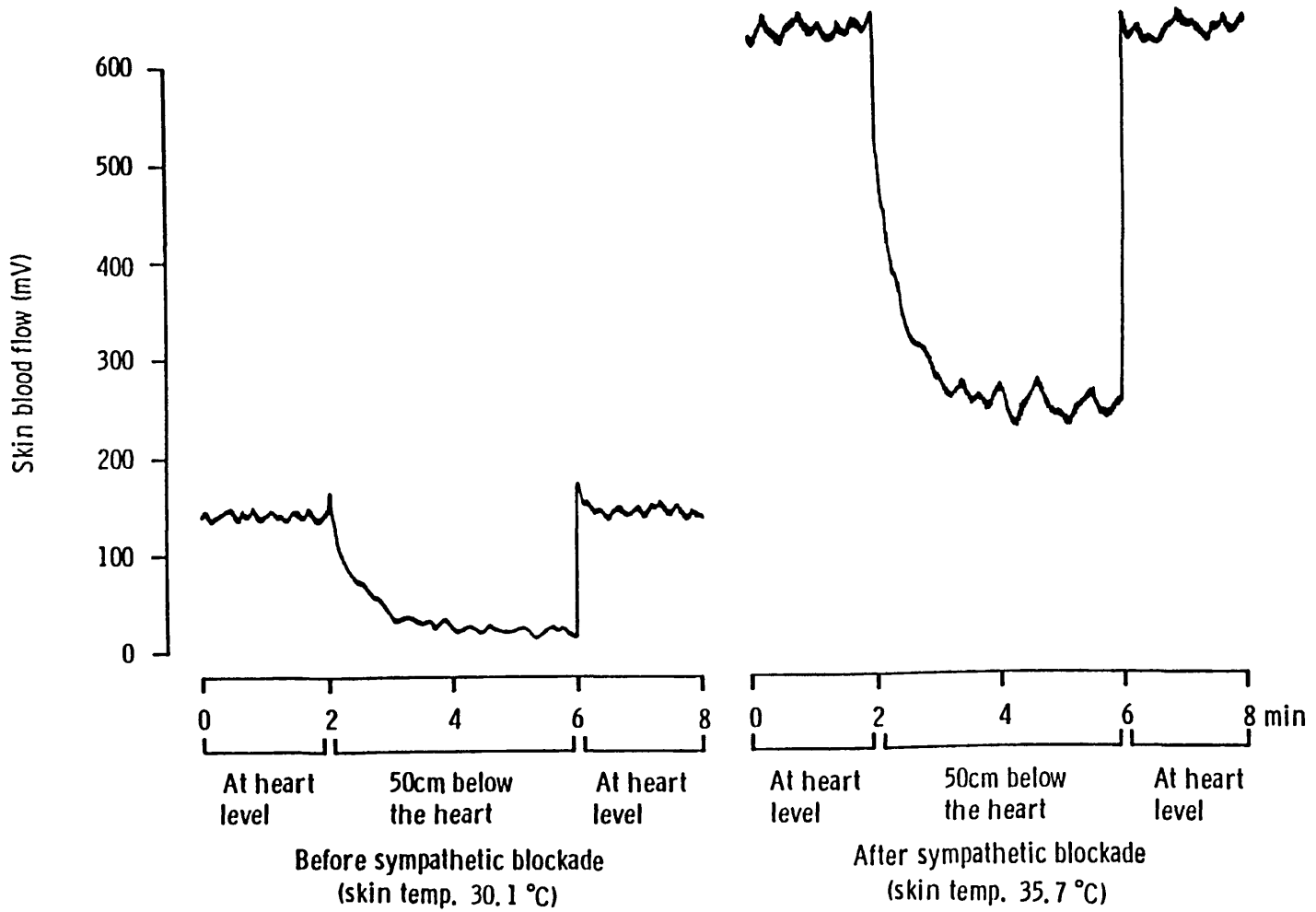


Figure 45: An example of the tracings obtained using laser Doppler flowmetry showing the postural fall in skin blood flow measured in the pulp of the big toe before and 30 minutes after acute lumbar sympathetic blockade (epidural anaesthesia) in a male patient aged 60 years.

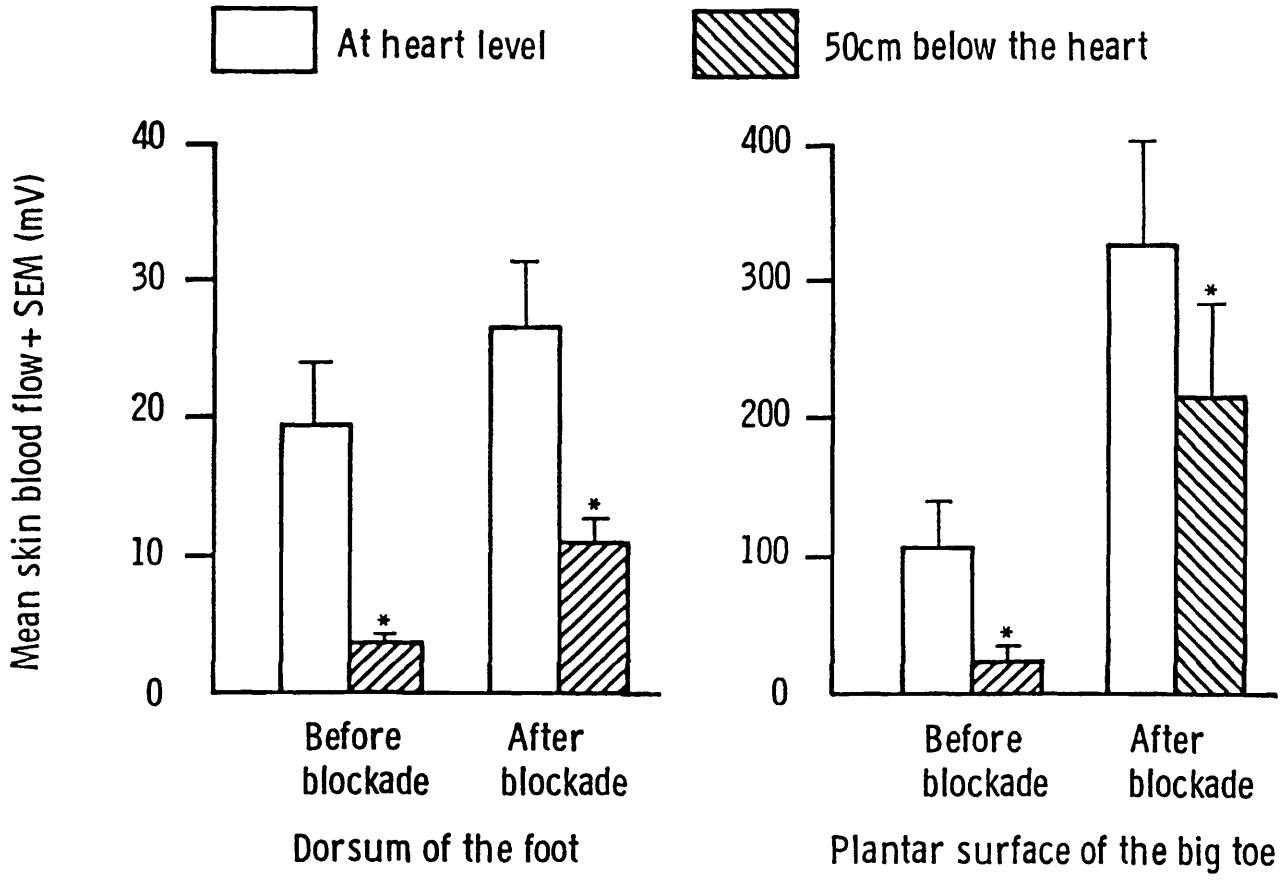


Figure 46: The postural changes in foot skin blood flow measured in 2 skin areas before and 30 min after acute lumbar sympathetic blockade in 10 patients with epidural anaesthesia. *P<0.01 as compared with mean flow values measured at heart level.

remained virtually constant after blockade (dorsum: from 21.7 to 20.0 mV, big toe: from 331.2 to 333.2 mV).

Effect of Local Nervous Blockade:

An effective local nervous blockade was indicated by the complete anaesthesia of the recording site 10-15 minutes after lignocaine infiltration.

Foot skin blood flow showed a significant increase in both the horizontal and dependent positions 15 minutes after either saline or lignocaine (7.4×10^{-2} mol/l) infiltration (Table 27) with greater hyperaemia after lignocaine than that after saline ($P<0.001$). However, in the saline-infiltrated (control) foot, the flow fell significantly both before and after saline infiltration (Figure 47, Table 27). In contrast, in the lignocaine infiltrated foot (with local nervous blockade), the flow fell significantly when the foot was lowered below heart level before lignocaine infiltration, but increased by 14% during the 4th minute in the dependent position after infiltration, above that obtained at heart level (Figure 48, Table 27) i.e. the postural vasoconstrictor response was preserved in the control foot but totally abolished in the foot with local nervous blockade. It was also noted that on lowering the lignocaine-infiltrated foot below heart level, there was initially, within the first minute, a rise in flow of 27% (Figure 48) followed by a gradual fall to a steady mean value during the 4th minute (Table 27) which was still

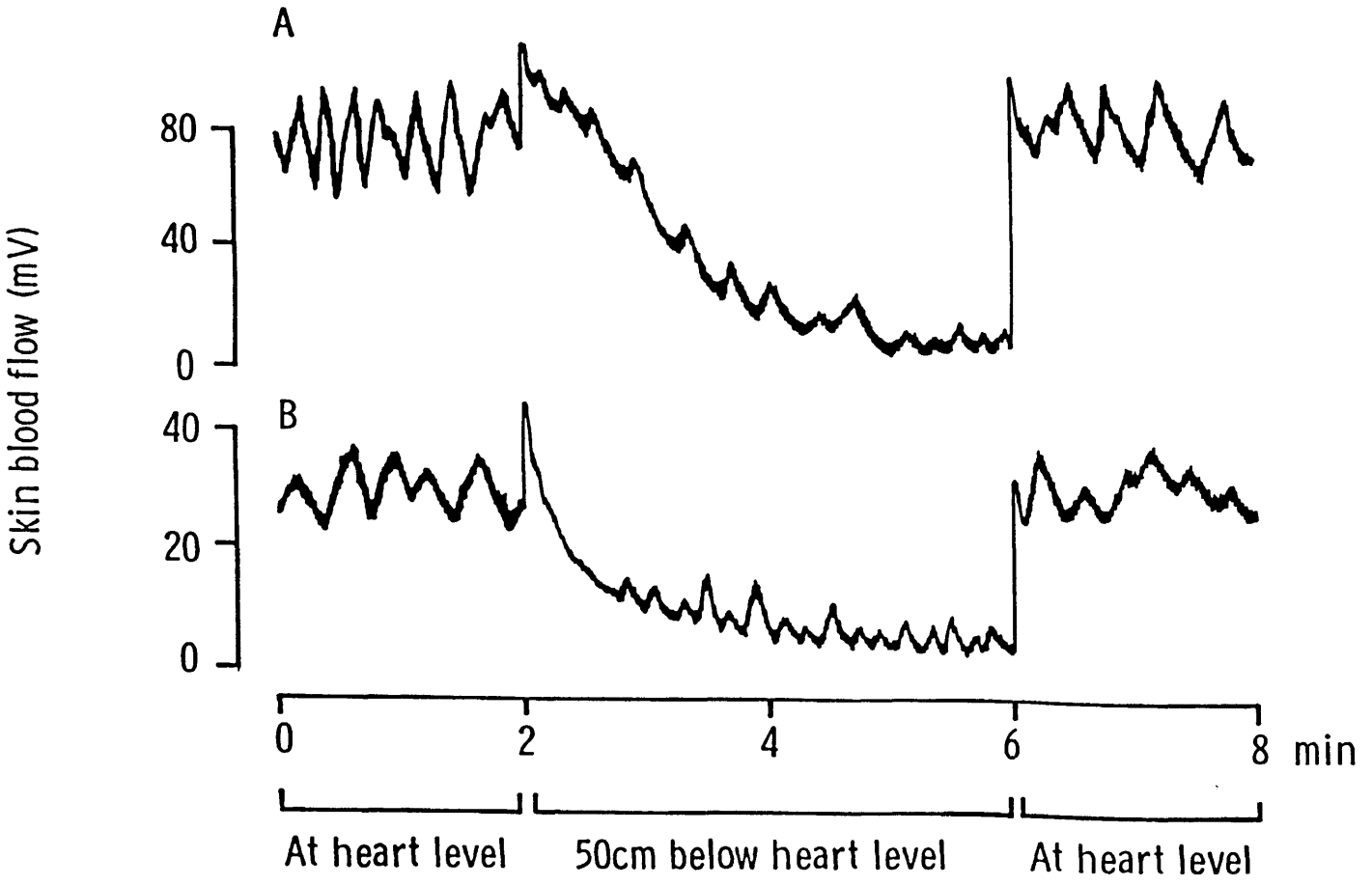


Figure 47: An example of the tracings obtained using laser Doppler flowmetry showing the postural changes in skin blood flow measured on the dorsum of the foot before (B) and 15 min after (A) S.C. infiltration of the recording site with 0.1 ml of physiological saline.

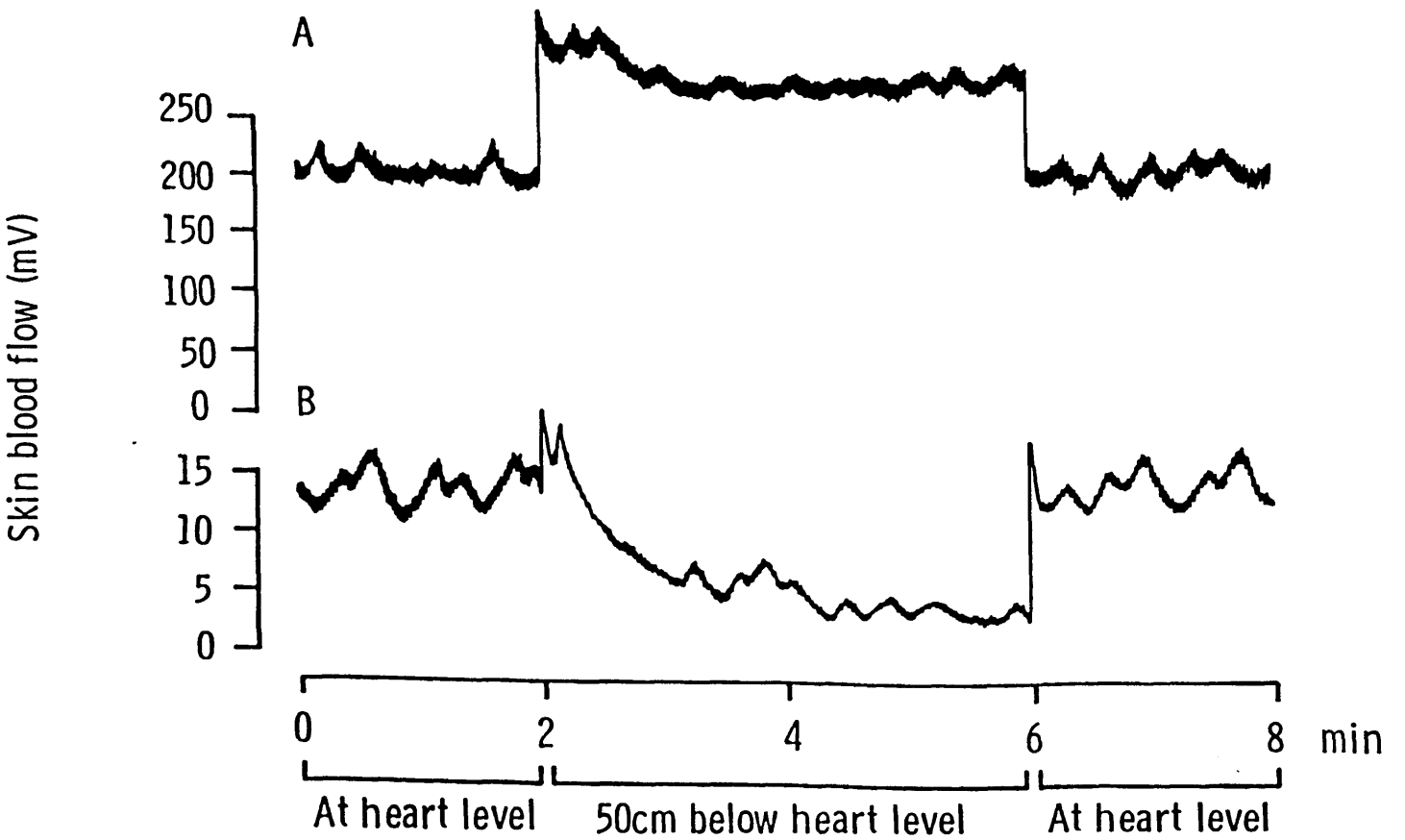


Figure 48: An example of the tracings obtained using laser Doppler flowmetry showing the postural changes in skin blood flow measured on the dorsum of the foot before (B) and 15 min after (A) S.C. infiltration of the recording site with 0.1 ml lignocaine 7.4 x 10^{-2} mol/l.

Table 27: The postural changes in skin blood flow (SBF, as mV) measured on the dorsum of the foot before and 15 minutes after S.C. infiltration of the recording site in one foot with 0.1 ml of lignocaine, and 0.1 ml of physiological saline into an anatomically identical skin site in the contralateral foot. H, with the foot horizontal, D, during the 4th min. with the foot dependent. +P<0.05, ++P<0.001 (as compared with H), *P<0.05, **P<0.01, ***P<0.001 (as compared with mean values obtained before infiltration). The postural fall in flow was calculated as (H-D) x 100/H. The minus sign of the postural fall in flow indicates increased flow in the dependent above that in the horizontal position. n, Number of experiments.

	Before Infiltration	After Infiltration
Lignocaine hydrochloride:		
7.4×10^{-2} mol/l (n=10):		
SBF (H):	20.3 ± 3.0	295.6 ± 37.3***
SBF (D):	5.6 ± 1.1++	333.1 ± 39.4***++
(H-D) x 100/H:	72.6 ± 2.3%	-14.2 ± 3.4%**
3.7×10^{-2} mol/l (n=2):		
SBF (H):	29.7 ± 1.4	290.3 ± 31.3
SBF (D):	6.1 ± 0.1+	303.5 ± 21.5*
(H-D) x 100/H:	79.3 ± 1.3%	-5.0 ± 3.9%*
3.7×10^{-3} mol/l (n=4):		
SBF (H):	26.3 ± 11.3	70.6 ± 27.6
SBF (D):	4.9 ± 0.7	66.7 ± 22.3
(H-D) x 100/H:	75.6 ± 4.4%	-0.3 ± 5.0%*
3.7×10^{-4} mol/l (n=3):		
SBF (H):	27.9 ± 15.3	58.6 ± 17.3**
SBF (D):	3.8 ± 1.3	48.8 ± 17.5*
(H-D) x 100/H:	82.7 ± 3.3%	19.4 ± 7.0%
Physiological Saline (n=10):		
SBF (H):	21.2 ± 2.5	102.5 ± 21.1**
SBF (D):	5.1 ± 1.2++	32.3 ± 10.9*++
(H-D) x 100/H:	77.1 ± 4.0%	71.9 ± 4.4%

significantly higher than the mean flow value obtained at heart level.

Since local anaesthetics not only block propagation of nerve impulse but might also directly relax the vascular smooth muscle (Altura & Altura, 1974), lower concentrations of lignocaine were used (Table 27). At 3.7×10^{-2} mol/l lignocaine, the results were essentially the same as those obtained at 7.4×10^{-2} mol/l. At much lower concentrations (3.7×10^{-3} & 3.7×10^{-4} mol/l), the hyperaemia associated with lignocaine infiltration was greatly reduced. When lignocaine was used at 3.7×10^{-3} mol/l, blood flow remained virtually constant on lowering the foot below heart level, whereas when it was used at 3.7×10^{-4} mol/l, the flow fell by 19% after infiltration as compared with 83% before infiltration (Table 27).

<u>Comparison between the effect of venous occlusion with the foot at heart level, and lowering the foot 50cm below the heart:</u>

During venous occlusion of 40mm Hg, with the foot at heart level, skin blood flow was significantly reduced from 22 $\pm$ 4 to 11 $\pm$ 3 mV in the dorsum of the foot and from 341 $\pm$ 71 to 176 $\pm$ 45 mV in the pulp of the big toe (Figure 49). After release of venous occlusion, the flow almost restored its preocclusion resting level within 3-5 minutes. On lowering the foot 50cm below the heart, the flow decreased to 8 $\pm$ 3 mV in the dorsum of the foot and to 91 $\pm$ 29 mV in the toe pulp; flow values

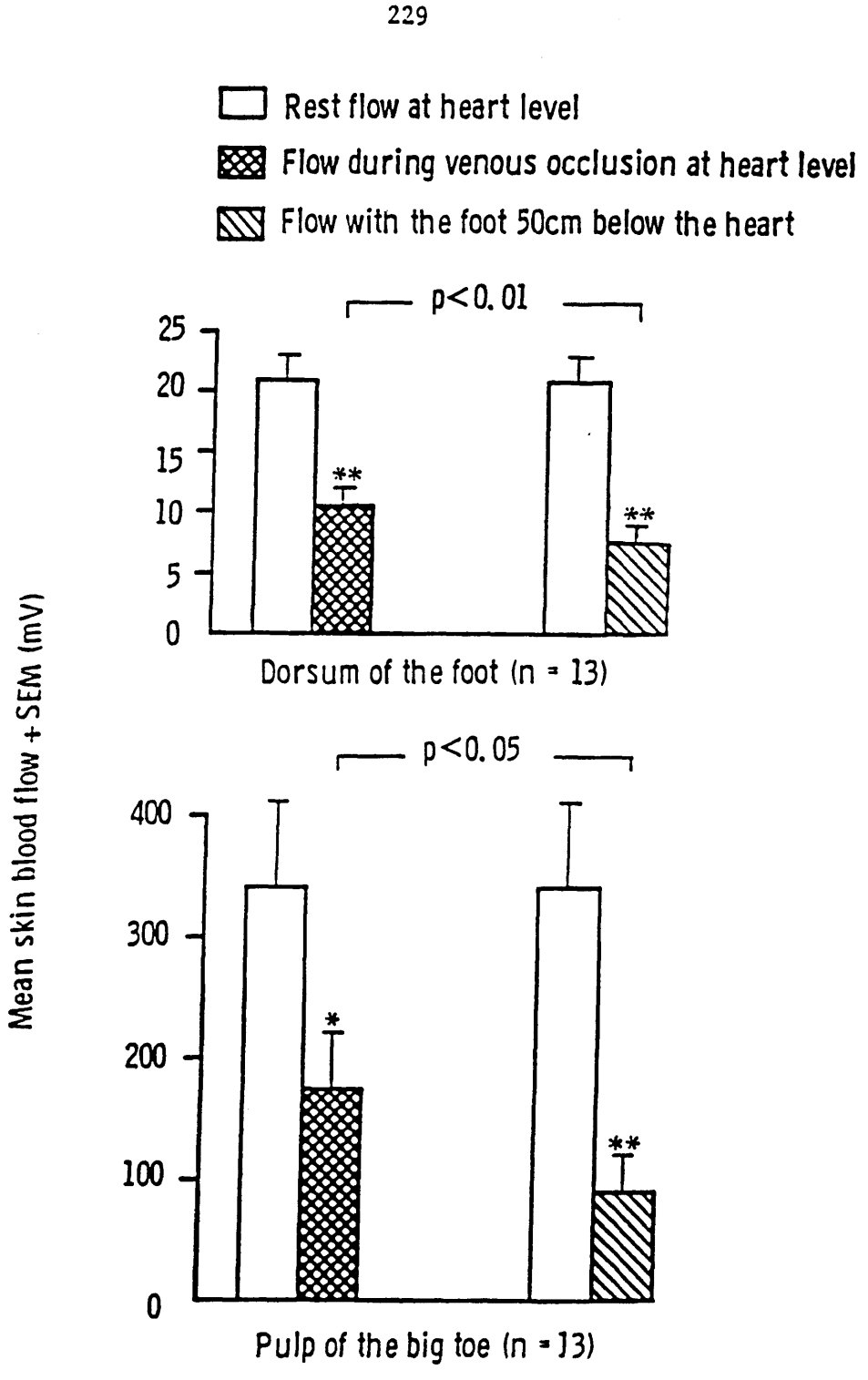


Figure 49: A comparison between the reduction in skin blood flow when the foot is lowered 50 cm below the heart and the reduction in flow induced by venous occlusion of 40 mm Hg with the foot maintained at heart level, measured on the dorsum of the foot and the pulp of the big toe in 13 normal subjects. *P<0.01, **P<0.001 (as compared with the preceding mean rest flow).

which were significantly lower than the values obtained during venous occlusion (Figure 49). Moreover, the percentage fall in flow was significantly greater during dependency than during venous occlusion (Dorsum: 67.2 ± 4.2% vs 48.0 ± 5.4%, $P<0.01$; Big toe: 75.0 ± 3.9% vs 51.6 ± 5.9%, $P<0.01$).

Effect of Venous Occlusion During Local Nervous Blockade:

In the saline-infiltrated foot, the flow fell during venous occlusion by 50.1 ± 10.6% and 63.4 ± 6.1% before and after saline infiltration respectively. In the lignocaine-infiltrated foot, the flow fell during venous occlusion by 63.4 ± 6.9% before, and by only 12.2 ± 4.6% after infiltration ($P<0.05$) (Figure 50).

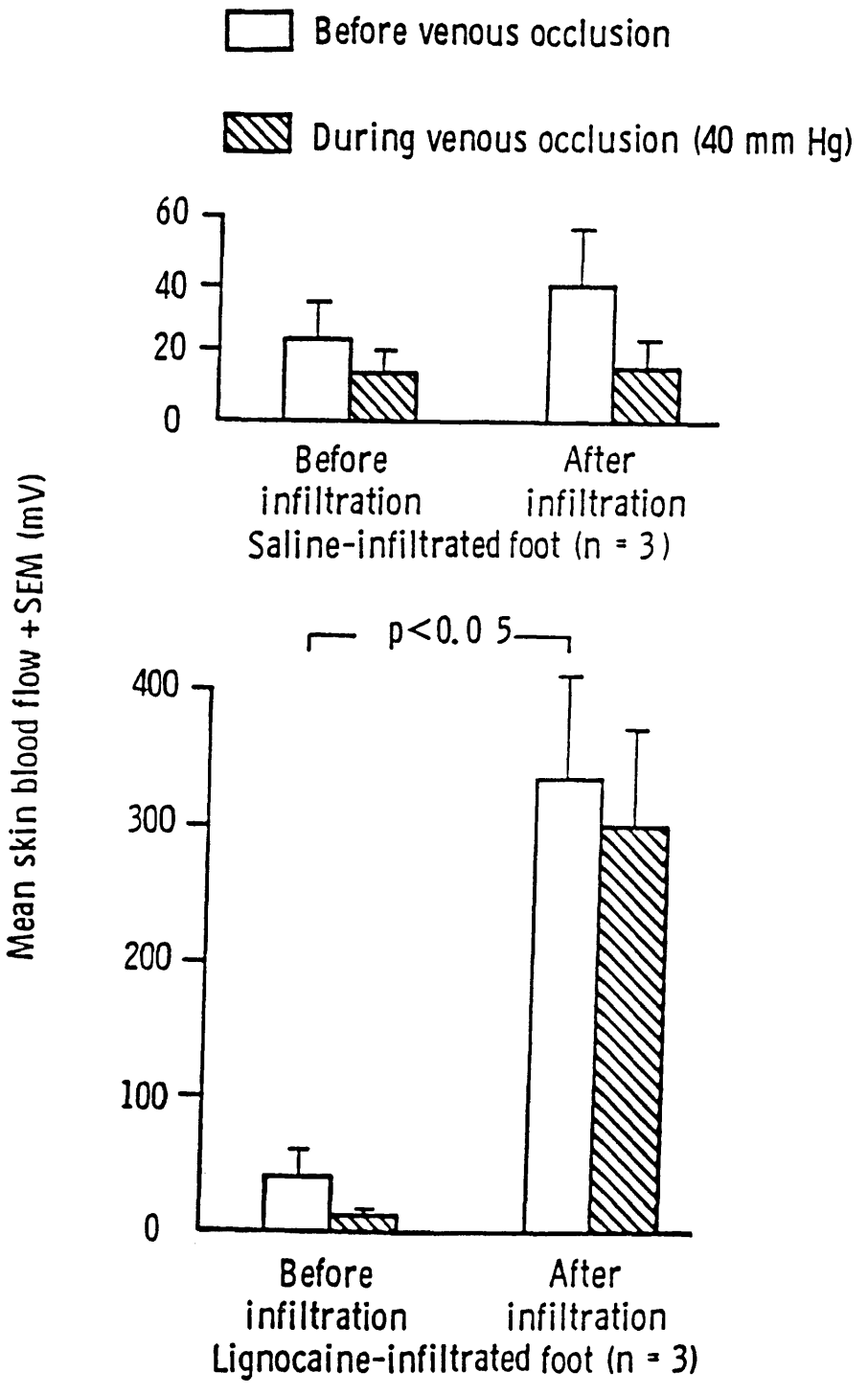


Figure 50: The changes in skin blood flow during venous occlusion measured on the dorsum of the foot before and 15 min after S.C. infiltration of the recording site in one foot with 0.1 ml lignocaine 7.4×10^{-2} mol/l, and an anatomically identical skin site in the contralateral foot with 0.1 ml of physiological saline. n, number of experiments.

DISCUSSION

This study has demonstrated that the postural vasoconstrictor response in the human foot is primarily mediated by local neurogenic and myogenic mechanisms supplemented, in part, by a central component mediated mainly via sympathetic efferent nerves.

The relative contribution of central and local mechanisms to postural vasoconstriction:

The concomitant reduction in flow in both feet when one foot was lowered below heart level suggests that a central mechanism is involved. This might be a haemodynamic (e.g. a reduction in cardiac output) or a neurogenic mechanism (e.g. increased sympathetic nervous activity) (Blomqvist & Stone, 1983). A contribution from a haemodynamic mechanism appears to be of little significance since there was almost no reduction in flow in the horizontal foot when the other foot was placed in the dependent position after lumbar sympathetic blockade, thus indicating that the central contribution to the postural vasoconstrictor response is mainly mediated via sympathetic efferent nerves. This conclusion is also supported by the partially attenuated postural response in the dependent foot after lumbar sympathetic blockade.

The exact mechanism by which the sympathetic vasoconstrictor activity is increased when the foot is lowered below heart

level is not yet completely understood. However, the small reductions in systolic and mean arterial pressures observed during the first minute of dependency (Figure 43) might slightly reduce the activity of the high-pressure baroreceptors (Rowell et al, 1973). Alternatively, the venous pooling in the dependent extremity and the subsequent reduction in venous return and central venous pressure might elicit a low-pressure baroreceptor-mediated increase in sympathetic vasoconstrictor tone (Zoller et al, 1972).

The greater reductions in flow during the 2nd-4th minute in the dependent foot than the horizontal foot indicates that, in addition to the role played by the central mechanism, local control mechanisms produced a much greater increase in vascular resistance in response to the postural increase in local transmural pressure. The involvement of a local mechanism is also supported by the finding that the postural vasoconstrictor response, though slightly attenuated, was still preserved after sympathetic blockade.

Although humoral mechanisms, particularly catecholamines (Hickler et al, 1959) and renin-angiotensin system (Oparil et al, 1970), have been shown to augment the increase in systemic vascular resistance associated with greater degrees of postural change, it is not known whether these mechanisms might also assist the postural increase in precapillary resistance associated with lowering one extremity below heart level. However, a minor contribution from these mechanisms to the

vasoconstriction observed during the 3rd to 4th minute of dependency cannot be entirely excluded.

Nature of the local mechanism underlying postural vasoconstriction:

The abolition of the postural fall in flow during local nervous blockade indicates that the local mechanism involved is mainly neurogenic in nature, as originally proposed (Gaskell & Burton, 1953; Henriksen & Sejrsen, 1976). The failure of postural vasoconstriction after lignocaine infiltration is unlikely to result from an overriding injection hyperaemia since the saline-infiltrated foot, despite the hyperaemia, showed persistent postural vasoconstriction. This hyperaemia (produced mainly by locally-released vasodilator substances) could also partially account for the greater increase in horizontal flow measured on the dorsum of the foot after lignocaine infiltration than after lumbar sympathetic blockade (Tables 26 & 27).

It may be argued that the failure of postural vasoconstriction during lignocaine infiltration might have resulted from an inhibitory effect of lignocaine on the vascular smooth muscle, since most local anaesthetics have been shown to produce some relaxation of the vascular walls by a direct action (Altura & Altura, 1974). However, several observations from the present study make this possibility unlikely. First, when the lignocaine-infiltrated foot was lowered below heart level, the

flow was initially increased by 27% (Figure 48), then it was gradually reduced to 14% above that obtained at heart level; the reduction in flow being most probably the result of increased vascular smooth muscle activity in response to the increase in local transmural pressure. Second, the spontaneous fluctuations in flow (produced mainly by arteriolar vasomotion) were preserved after lignocaine infiltration (Figure 48).

Since the postural increase in precapillary resistance has been previously suggested to result mainly from a myogenic response (Mellander et al, 1964), different concentrations of lignocaine were used in the present study in order to unmask a possible contribution of a myogenic mechanism to the postural vasoconstrictor response. The use of Lidocaine at a concentration of 3×10^{-4} mol/l has been previously shown to block the constrictor response to venous stasis in the human hand (Henriksen & Sejrsen, 1976) and in the subcutaneous tissue of the human forearm (Henriksen, 1977) and also to block impulse transmission in autonomic nerves in the cat, but not to influence the spontaneous rhythmic contractions of the rat portal vein in vitro (Johansson & Ljung, 1967). When lignocaine was used at a similar concentration (3.7×10^{-4} mol/l) in the present study, most of the postural fall in flow was abolished but there was still a small reduction in flow (by 19%) in the dependent foot indicating that, in addition to the primary role played by the local neurogenic mechanism, other local mechanisms are also involved, most probably a local myogenic response (Bayliss, 1902; Mellander et al, 1964), which

has been suggested to take part in the autoregulation of skin blood flow (Henriksen et al, 1973). However, it is also possible that lignocaine might be diluted by the extracellular fluid so that when the low concentration (3.7×10^{-4} mol/l) was used it could be slightly less effective in vivo than in vitro (Johansson & Ljung, 1967).

The nature of the stimulus which triggers the local neurogenic and myogenic mechanisms to elicit the postural vasoconstrictor response has not yet been fully elucidated. However, it has been suggested that the increase in venous pressure associated with pooling of blood in the dependent extremity could initiate a veno-arteriolar axon reflex, thereby producing arteriolar vasoconstriction (Gaskell & Burton, 1953; Henriksen, 1977; Henriksen & Sejrsen, 1977). The observation that, during local nervous blockade, the flow fell by 12% during venous occlusion of 40mm Hg with the foot at heart level as compared with 63% before blockade, adds much support to the view that increased venous pressure provides the main stimulus for the local neurogenic vasoconstriction observed during the postural change. The persistent small reduction in flow during venous occlusion with local nervous blockade would be anticipated to result mainly from a reduced arterio-venous pressure gradient. However, the finding of a greater reduction in flow when the foot was lowered 50cm below heart level than during venous occlusion of 40 mmHg with the foot at heart level indicates that, despite the presence of almost similar degrees of venous pressure elevation in the 2 situations, not only the increase

in venous pressure but also the increase in arterial transmural pressure is implicated in the vasoconstriction obtained on dependency, which further supports a contribution of a local myogenic response to the main local neurogenic mechanism.

In summary, the postural vasoconstrictor response in the human foot has been shown to be produced mainly by a local neurogenic mechanism with a small contribution from a local myogenic response, in addition to a centrally-elicited sympathetic component.

Chapter VI

GENERAL DISCUSSION AND CONCLUSIONS

Chapter VI

GENERAL DISCUSSION AND CONCLUSIONS

This thesis describes some aspects of the physiological regulation of postural vasoconstriction in the human foot using laser Doppler flowmetry. The studies were designed to explore the nature of the interaction between the postural changes in foot skin blood flow and the cutaneous vasomotor alterations associated with thermoregulation, with particular emphasis on the difference in response between areas with and areas without arteriovenous anastomoses. Experiments were also performed to examine the variations in the postural control of foot skin blood flow in women at different stages of the menstrual cycle, as well as to elucidate the different regulatory mechanisms involved in postural vasoconstriction.

Laser Doppler Flowmetry

The non-invasive technique of laser Doppler flowmetry was used in the present studies to assess the relative changes in foot skin blood flow in a particular skin area in response to changes in body or limb posture under different physiological conditions. Laser Doppler flowmetry has been suggested to be a sensitive method for measuring acute changes in flow (Sundberg, 1984), and has been shown to correlate well with many of the conventional methods used for the investigation of peripheral blood flow (Holloway & Watkins, 1977; Tooke et al, 1983; Saumet et al, 1986).

The strong positive correlation demonstrated between laser Doppler flowmetry and the direct technique of television microscopy (Chapter III, Section 2), and the successful use of the laser Doppler flowmeter throughout the present investigation for the detection of relative changes in flow in response to specific stimuli, adds much support to laser Doppler flowmetry as a reliable method capable of recording rapid reflex changes in superficial skin microcirculation.

Furthermore, it has been shown that laser Doppler flowmetry is particularly appropriate for the study of local skin vasomotor reflexes and hence can be used in the indirect assessment of peripheral sympathetic nervous activity (Low et al, 1983). Also, the facts that the laser Doppler flowmeter gives a continuous flow record, does not disturb the local microcirculation to the region, and in contrast to other blood flow measuring techniques (Greenfield et al, 1963, Challoner, 1976), is not affected by the distension of the vascular bed under study (e.g. during dependency) make it suitable for the study of changes in local blood flow following changes in limb posture.

Postural Vasoconstriction in the human foot

It has been demonstrated in this thesis that, under resting conditions, cutaneous vasoconstriction occurs in the human foot during standing or when the extremity is lowered passively 50 cm below heart level in supine subjects. This postural response

has been shown to be strongly influenced by changes in body temperature, skin temperature (Chapter III), by the phase of the menstrual cycle and by the use of combined oral contraceptive steroids (Chapter IV). Moreover, multiple control systems appear to be involved in this response, including local neurogenic and myogenic, as well as central sympathetic mechanisms (Chapter V).

The finding that female sex steroids strongly modify the constrictor response to postural change (Chapter IV) suggests that humoral mechanisms might also be involved in postural vasoconstriction, thus adding another factor to the complex control mechanisms described above. Indeed, previous studies have shown that upright tilting in humans is associated with increased plasma levels of catecholamines, renin-angiotensin and arginine vasopressin (Hickler et al, 1959; Oparil et al, 1970; Davies et al, 1976). However, the changes in these humoral agents when one limb is lowered below heart level in supine subjects require further studies.

The experiments performed in Chapter V have demonstrated that a local neurogenic mechanism is involved in the postural fall in foot skin blood flow, but the exact nature of this mechanism is still unclear. Previous work suggests that local sympathetic fibres are involved in the constrictor response to venous stasis in the hand (Henriksen & Sejrsen, 1976) and forearm (Henriksen, 1977), as the response was abolished after alpha adrenergic blockade (by phentolamine infiltration). However,

no such experiments have been performed on the human foot during a postural change. Therefore, the possible involvement of a local sympathetic reflex mechanism in the postural vasoconstrictor response observed in the cutaneous tissue of the foot in this study remains to be elucidated.

The reflex pathway through which the local nervous mechanism elicits the postural increase in precapillary resistance is still unknown. Since Woollard (1926) found no evidence of any ganglia in peripheral blood vessels but found that a single nerve fibre may supply both the smooth muscle of the arterial wall and a nearby Paccinian corpuscle, it is possible that nervous activity aroused by pressure stimulation of the receptor may produce constriction in the artery by an axon reflex. This possibility appears unlikely as it has been shown that the constrictor response to venous stasis or postural change was unaffected after somaesthetic denervation (Henriksen, 1977). Although it has been suggested that the receptor site of the local reflex induced by venous stasis is probably located in small veins, whereas the effector site is in the precapillary arterioles (Henriksen, 1977), the true pathway of such a proposed local reflex (including its afferent and efferent limbs, and its nerve cell) remains obscure.

The percentage fall in foot skin blood flow on dependency observed in the present study using laser Doppler flowmetry appears to be greater than that previously reported for the human hand or forearm using ^{133}Xenon clearance (Henriksen &

Sejrsen, 1976, Henriksen, 1977). This might be due to differences in the vascular volumes or the flow components assessed by each method. Alternatively, it could be due either to a more sensitive vascular wall because of a more rich sympathetic nerve supply (Woollard & Phillips, 1933), or to a thicker and more powerful vascular smooth muscle in the foot than in the hand (Ryan, 1983). Paaske and Henriksen (1975) have shown that, with similar increments in transmural pressure in the hand and leg, the arteriolar constriction induced by the venoarteriolar reflex was more pronounced in vascular beds subjected to excessive hydrostatic loads such as the legs than in the hands. This difference in the degree of postural response between upper and lower extremities suggests that an adaptive structural change might develop in the blood vessels chronically exposed to a greater increase in transmural pressure (Folkow et al, 1958).

PHYSIOLOGICAL IMPLICATIONS OF POSTURAL VASOCONSTRICTION

a) Control of Arterial Blood Pressure During Standing

The increase in precapillary resistance induced by the local postural control mechanisms is thought to support the role played by the baroreceptors for the maintenance of arterial blood pressure during standing (Amberson 1943; Henriksen et al, 1983b). Failure of such a constrictor response might explain some cases of postural hypotension (Stead & Ebert, 1941).

b) Oedema Prevention Mechanism

As discussed earlier (Chapter I, Section 2D), the postural increase in precapillary resistance has been suggested to act as a safety factor against oedema formation in dependent extremities (Sejrsen et al, 1981a; Henriksen et al, 1983a). This hypothesis is supported by the finding of a positive correlation between foot skin blood flow measured in the dependent position and foot swelling rate (Figure 51), as recorded in 4 women with ovulatory menstrual cycles, 3 women taking combined oral contraceptive steroids and 3 men (Chapter IV). It is interesting to note that the positive correlation exists not only in the group as a whole ($r = 0.81$, $P<0.001$) but also in each of the 3 subgroups, with correlation coefficients of $r = 0.828$ ($P<0.001$), $r = 0.951$ ($P<0.01$) and $r = 0.86$ ($P<0.05$) respectively. Moreover, a negative correlation was found between the percentage fall in flow on dependency and foot swelling rate (Figure 52). These observations lead to the conclusion that the more the increment in precapillary resistance with changes in posture the less would be the swelling of the foot and vice versa. Thus, the lesser increase in precapillary resistance and the higher foot swelling rate observed in women during the latter phase of the cycle could explain the incidence of ankle oedema in some of these women during the premenstrual period (Chapter IV). Taking this into account, it would be interesting to examine the postural response at different stages of human pregnancy, as it has been reported that progesterone can cause relaxation of vascular

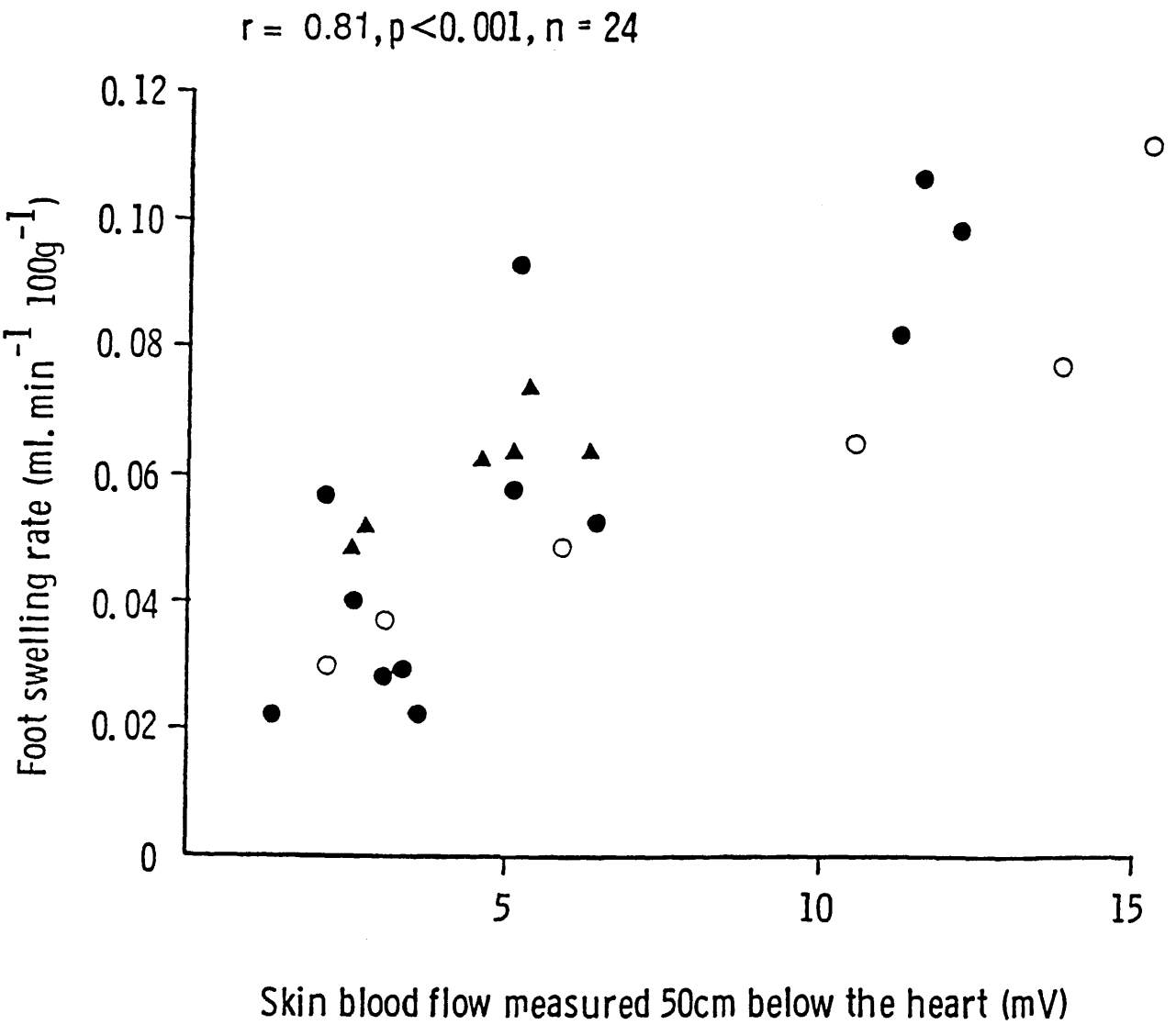


Figure 51: The relationship between skin blood flow recorded on the dorsum of the foot and foot swelling rate measured during the 10th-20th minute after the foot was lowered 50cm below the heart in 3 men (▲) studied at 2 separate occasions, 3 women taking combined oral contraceptive pills (o) studied on days 7 & 28 of their cycles, and in 4 women with normal ovulatory menstrual cycles (●) studied during the menstrual, follicular and luteal phases of their cycles.

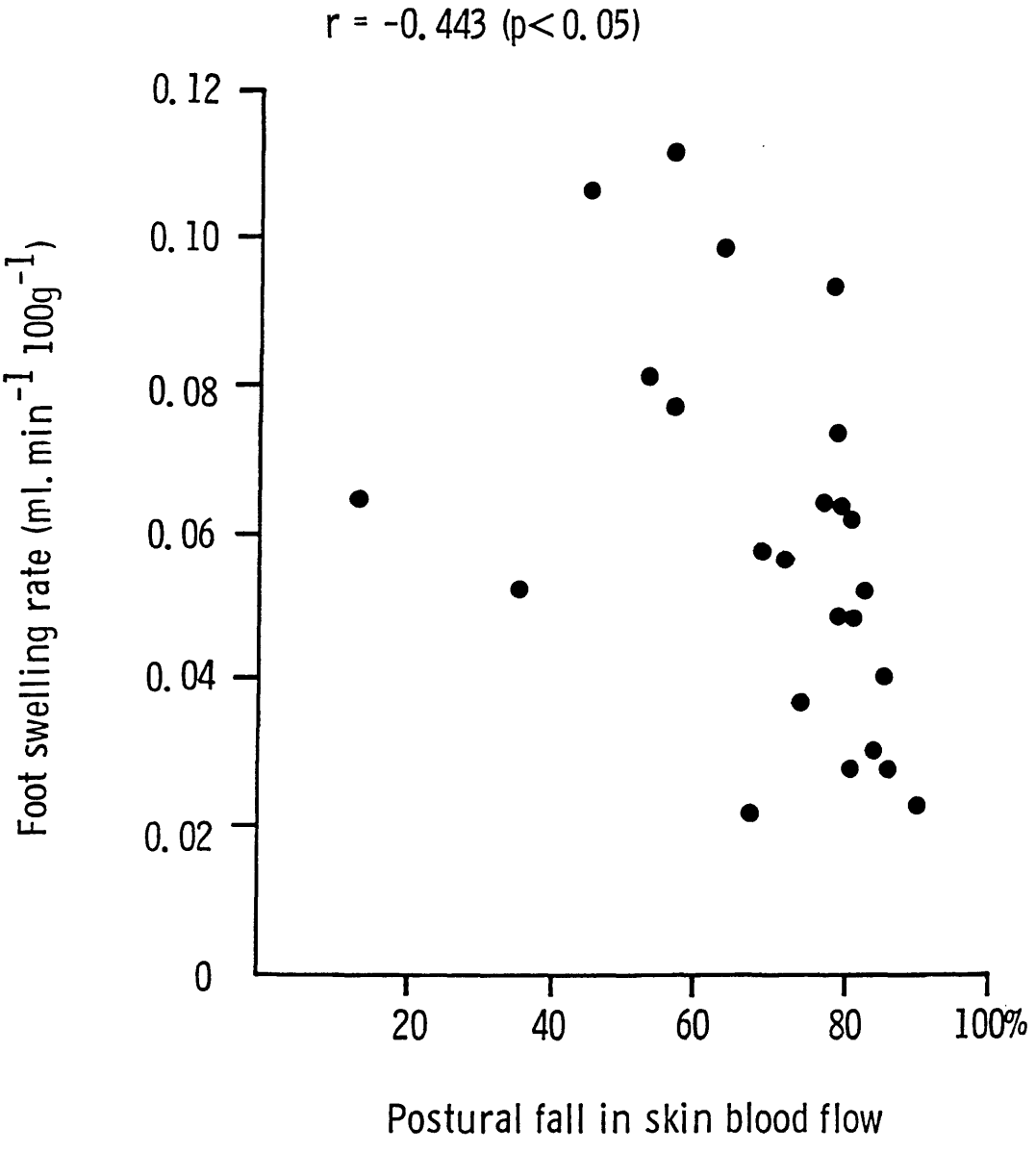


Figure 52: The relationship between the postural fall in skin blood flow (%) recorded on the dorsum of the foot, and foot swelling rate measured during the 10th-20th minute after the foot was lowered 50cm below the heart. (24 measurements in 10 subjects.)

smooth muscle during pregnancy (McCausland et al, 1961). Furthermore, normal pregnancy has been reported to be associated with not only increased peripheral blood flow (Herbert et al, 1958) but also increased capillary filtration rate (McLennan, 1943) and increased incidence of peripheral oedema (Thomson et al, 1967). Also, it is important to carry out well-controlled studies in order to elucidate which of the circulating female sex hormones might contribute to the control of arteriolar and venular tone, and whether these actions are mediated directly or via alterations in vascular smooth muscle reactivity (Altura & Altura, 1977).

c) Postural Intolerance in Hot Environments

On exposure to a thermal stress, the combined effects of attenuated postural vasoconstrictor response in areas rich in arteriovenous anastomoses (by increased core temperature and the release of sympathetic vasoconstrictor tone, Chapter III, Section 1), and the failure of the response in areas with and without anastomoses (by the local effect of heat, Chapter III, Section 3) when the skin temperature exceeds the physiological range, could result in displacement of a considerable amount of blood in the dependent extremities (venous pooling) with a subsequent fall in central blood volume, cardiac filling pressure, stroke volume and cardiac output (Rowell, 1983). This could partially explain postural syncope (orthostatic intolerance) on standing in the heat (Greenleaf et al, 1974, Rowell, 1983).

d) Swelling of the Extremities in Hot Environments

At higher skin temperatures, the failure of the postural increase in precapillary resistance and the subsequent increase in flow in the dependent limbs (Chapter III, Section 3), together with the rise in capillary hydrostatic pressure (Levick & Michel, 1978) and capillary filtration rate (Landis & Gibbon, 1933), might contribute to the swelling of the extremities commonly observed in a hot environment. This conclusion is supported by the observation of a more rapid rise in venous pressure in the legs after passive tilting in a hot environment than in the cold (Henry & Gauer, 1950), and by the finding of increased capillary filtration coefficient in human extremities by 40% when ambient temperature was increased from 20 to 35°C (Hyman & Wong, 1968).

e) Regional Sympathetic Innervation

Lumbar sympathetic blockade (Chapter V) resulted in a significant increase in blood flow measured at heart level both in skin areas with (toe pulp) and without (dorsum of the foot) arteriovenous anastomoses. This confirms that both areas are supplied with sympathetic vasoconstrictor fibres, and indicates that the sympathetic nervous system contributes to the maintenance of resting vasomotor tone in these areas. However, the significantly greater (3-fold) increase in flow in the toe pulp than the dorsum of the foot after sympathetic blockade might be due to opening of arteriovenous anastomoses in the toe

pulp. This finding provides an indirect evidence in support of a central sympathetic control over arteriovenous shunts in human extremities, as has been previously demonstrated in cats, dogs and sheep (Folkow, 1955; Spence et al, 1972; Hales et al, 1982). This could partly explain the markedly impaired postural vasoconstriction in the toe pulp when the sympathetic tone was released by indirect heating (Chapter III, Section 1).

PATHOPHYSIOLOGICAL IMPLICATIONS

a) Diabetes Mellitus

The abolition of the postural fall in skin blood flow observed in skin areas where shunt flow is prevalent (toe pulp), during the release of sympathetic vasoconstrictor tone induced by indirect heating (Chapter III, Section 1), and the partially attenuated postural vasoconstriction after sympathetic blockade (Chapter V) might explain, at least in part, why the postural control of foot skin blood flow is often impaired in diabetic patients with autonomic neuropathy, as in these patients the sympathetic tone may be partially lost (Rayman et al, 1986; Watkins & Edmonds, 1983).

b) Peripheral Arterial Occlusive Disease

Previous reports (Henriksen, 1974, Eickhoff, 1980) have demonstrated that the postural vasoconstrictor response in the foot and lower leg is lost in patients with peripheral arterial

occlusive disease (with rest pain). However, the mechanism of such impairment is still unclear. Since it has been shown in the present studies (Chapter V) that multiple control mechanisms are involved in the postural response, further studies are required to clarify whether these mechanisms are attenuated, lost or overridden by other local factors due to, for example, ischaemia in these patients.

c) Orthostatic Oedema

Idiopathic oedema (Kuchel et al, 1970) has been shown to be precipitated by upright posture. Patients with this form of oedema usually show excessive increase in leg volume on standing. It would be interesting to examine the postural response and foot swelling rate in these patients to see whether failure of postural vasoconstriction might be involved. Such a study would be required in view of the recent reports indicating that oedema caused by vasodilator therapy was associated with significantly impaired postural vasoconstriction (Williams et al, 1987).

In summary, the present studies have confirmed that, in normal subjects, not only total foot skin blood flow but also nutritional capillary blood flow is markedly reduced on standing or when the extremity is lowered below heart level, and have provided more evidence concerning the mechanisms involved in the constrictor response, including local neurogenic, myogenic and central sympathetic mechanisms. The

experiments have also shown that the vasoconstrictor response observed on dependency is strongly influenced by the thermoregulatory function and by the female sex hormones. Although these studies have clarified some aspects of the physiological control of foot skin blood flow with changes in posture, several questions remain to be answered, particularly the mechanism by which the postural increase in precapillary resistance limits the rate of oedema formation during standing, and how the thermoregulatory and humoral mechanisms modify the postural vasoconstrictor response in the human foot.

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