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Blood Flow Rate during Orthostatic Pressure Changes in the Pulp Skin of the First Toe

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Objectives: *Determination of the local regulation of cutaneous blood flow through nutritive capillaries and through arteriovenous anastomoses of the pulp of the first toe in response to passively induced orthostatic blood pressure changes in normal subjects and in patients with occlusive atherosclerotic disease.*
Material: *Six normal subjects, seven patients with unilateral, crural intermittent claudication and six patients with unilateral, chronic critical ischaemia.*
Methods: *Blood flow rates were measured in supine subjects by the heat washout method (the sum of blood flow rate in arteriovenous anastomoses and blood flow rate in nutritive capillaries) and by the* ^{133}*Xenon washout method (blood flow rate in nutritive capillaries) after local, atraumatic labelling. Measurements were made with (a) the toe passively elevated to 50 cm above heart level, (b) at heart level and (c) passively lowered to 50 cm below heart level.*
Results: *Autoregulation of nutritive blood flow was present in normal subjects and in claudicants, but the local sympathetic veno-arteriolar axon reflex was absent in both groups. In patients with critical ischaemia blood flow rate was the same in the supine position and during lowering in arteriovenous anastomoses and in nutritive capillaries. The arteriovenous anastomoses had distinct and characteristic reaction patterns in response to lowering in each of the three examined groups and to elevation in normal subjects and in patients with intermittent claudication (not measured in patients with critical ischaemia)*
Conclusions: *The microvascular responses to changes of orthostatic blood pressure differed among the three groups (normal subjects, patients with intermittent claudication, patients with critical chronic leg ischaemia). The heat washout method may be used to detect the functional significance of occlusive atherosclerotic disease.*

Key Words: Atherosclerosis; Arteriovenous anastomoses; Blood flow; Capillaries; Critical ischaemia; Intermittent claudication.

Introduction

The local regulation of blood flow through nutritive capillaries in the periphery of extremities has been studied in detail in skin, subcutaneous adipose tissue and skeletal muscle,[1,2] but it is not known how the arteriovenous anastomoses (the shunt vessels) react to orthostatically induced pressure changes in normal subjects or in patients with occlusive atherosclerotic disease.

Since intermittent claudication (functional ischaemia) is a symptom of relative blood flow insufficiency and inadequate substrate delivery in relation to local metabolic needs during exercise, and since critical ischaemia is a symptom of inadequate resting blood flow, local disturbances in the regulation of shunt vessel perfusion might play a role for the development of the symptoms and signs of arterial insufficiency. During critical ischaemia it would seem to be harmful if even a small part of the total blood supply should bypass nutritive capillaries and go through shunt vessels.

For these reasons the present study was undertaken in normals, claudicants and patients with chronic critical ischaemia to examine the absolute and fractional blood flow rates through nutritive capillaries and through arteriovenous anastomoses in the skin of the toe pulps and to assess the microvascular responses in terms of blood flow rate regulations to orthostatically induced blood pressure changes.

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Patients

The examinations were performed in a prospective, open study on three groups of volunteers:

(A) Six normal subjects (two males; median age 43 years, range 33–62) without symptoms or clinical signs of arterial disease. All had four pedal pulses and normal systolic ankle blood pressures;
(B) Seven patients (three men; 69 years, range 53–84) with unilateral intermittent claudication of the calf muscles (Fontaine group II a) and an ankle blood pressure index (= segmental systolic ankle blood pressure/systolic arm blood pressure) above 0.30 on the symptomatic side; and
(C) Six patients (three men; 69 years, range 58–80) with unilateral chronic critical ischaemia as defined by persistently recurrent ischaemic rest pain that had required analgesia for more than 2 weeks and with a segmental ankle systolic blood pressure below 50 mmHg on the symptomatic side (none had ulceration or gangrene).[3]

The protocol was approved by the local ethical committee, and all subjects gave their informed, written consent.

Methods

The patients were questioned for symptoms and examined for signs of arterial and venous diseases. Patients with venous diseases or with diabetes mellitus were excluded from the study. Systemic (arm) blood pressures were recorded by conventional sphygmomanometry, and the segmental systolic ankle blood pressures were measured by the strain gauge method.

The subjects were resting on an examination couch in supine position in a room with a temperature of 21–24°C. The blood flow rate through the skin of the pulp of the first toe was measured by the two methods outlined below (a) with the leg elevated passively so the toe was fixed and immobilised 50 cm above heart level, (b) with the toe at heart level and (c) with the toe 50 cm below the heart level. Elevation was not performed on patients with critical ischaemia because of increased pain.

Heat washout method

The method has recently been introduced and described in detail.[4] A Clark type electrode E 5250 (Radiometer a/s, Denmark) was used.[5,6] The electrode was constructed with a thermostatically controlled cap to ensure heat delivery in one direction only (to the skin). The electrode was fixed on the pulp of the first toe by a double adhesive, ring shaped membrane and silk tape, and a baseline temperature was recorded. Next, the probe was heated (usually for about 5 min) until the underlying skin reached a steady state temperature of 41°C as evidenced by constant heat dissipation from the probe. The heat was turned off, and the temperature, T, was recorded every 10 s until a stable base line temperature, T_b, was obtained after 6–10 min, $\Delta T = T - T_b$ for each recorded temperature measured every 10s was plotted against time in a semilogarithmic diagram. The fractional rate constant, k_h, of ΔT vs time was used to calculate blood flow rate, f_h from: $f_h = k_h \times \lambda_h \times 100$ (ml/100 g/min) where λ_h is the cutaneous tissue to blood partition coefficient of heat.[7] A λ_h-value of 1.0 ml/g was used for simplicity (exact value 0.954).

The heat washout method detects the sum of the blood flow rate through arteriovenous anastomoses (shunt vessels) plus the blood flow rate through the nutritive, cutaneous capillaries.

^{133}Xenon washout method

The measurements were made at unheated skin temperature (around 35.5°C). 0.1–0.2 ml ^{133}Xe (555 MBq/ml, Nordion, Europe, S. A.) dissolved in isotonic saline was introduced between the skin of the pulp of the first toe and a 20 μm thick gas tight, circular Mylar membrane with an outer diameter of 3.8 cm that was attached to the skin by double adhesive tape at its circumference to create a small diffusion chamber of 1.9 cm diameter. After 3 min the solution was withdrawn, the membrane was removed, the area wiped off, and surplus ^{133}Xe blown away. In this way atraumatic labelling of the skin by ^{133}Xe was obtained.[8,9]

The γ-rays of ^{133}Xe were recorded for 40 min with a sampling integration time of 10 s by a NaI(Tl) scintillation detector (diameter 40 mm, thickness 6 mm) connected to a γ-spectrometer adjusted to the 81 keV photopeak, a ratemeter and a computer. The collimation was wide, and the distance between skin and detector was kept constant (5–10 cm). The count values were corrected for background activity and plotted against time in a semilogarithmic diagram. After curve resolution the cutaneous blood flow rate, f_x, was calculated from $f_x = k_x \times \lambda_x \times 100$ (ml/100 g/min) where k_x is the fractional washout rate constant of ^{133}Xe from cutaneous tissue, and λ_x is the cutaneous tissue to blood partition coefficient for ^{133}Xe (0.7 ml/g).[7]

Table 1. The results of the measurement of the blood flow rate in the pulp of the first toe in normal subjects with (1) the heat washout method using the Radiometer electrode and (2) the ^{133}Xenon washout method.

	Normal subjects								
Patient No.	50 cm above heart level			At heart level			50 cm below heart level		
	Heat ml/100 g/min		^{133}Xe	Heat ml/100 g/min		^{133}Xe	Heat ml/100 g/min		^{133}Xe
	Right	Left	Right	Right	Left	Right	Right	Left	Right
1	20.8	23.3	5.8	53.2	60.6	6.5	53.9	59.2	13.2
2	29.2	26.7	9.7	50.7	47.3	9.0	44.2	59.4	9.2
3	32.2	27.8	9.0	40.8	49.9	11.7	46.5	52.8	6.4
4	23.3	20.9	8.1	42.9	42.6	10.8	46.9	44.1	10.4
5	25.6	28.4	13.5	57.2	50.6	10.2	63.7	47.9	6.3
6	24.0	25.0	9.3	46.0	49.6	12.1	46.0	46.1	5.8
$\bar{x}$	25.3		9.2	49.8		10.5	47.4		7.8
S.E.	0.99		1.0	1.70		0.8	1.95		1.2

Blood flow rate was measured at three positions of the toe pulp in relation to the reference level of the heart in supine patients: heart level, 50 cm above the heart and 50 cm below heart level. $\bar{x}$ = median value, and S.E. = standard error of the mean.

Table 2. The results of the measurements in patients with intermittent claudication.

	Patients with intermittent claudication								
Patient No.	50 cm above heart level			At heart level			50 cm below heart level		
	Heat ml/100 g/min		^{133}Xe	Heat ml/100 g/min		^{133}Xe	Heat ml/100 g/min		^{133}Xe
	Sympt	Asympt	Sympt	Sympt	Asympt	Sympt	Sympt	Asympt	Sympt
1	26.6	47.3	–	31.7	33.6	10.4	57.0	40.4	21.1
2	42.6	29.6	7.6	32.0	31.6	13.9	83.2	59.4	7.1
3	22.8	23.8	9.7	28.5	79.4	10.8	47.3	74.4	12.1
4	–	27.4	–	27.2	91.6	–	41.1	123.8	–
5	–	39.7	–	30.9	58.1	–	49.8	34.0	11.5
6	18.5	18.0	–	45.4	58.3	8.4	57.4	54.3	7.1
7	3.0	40.9	14.7	47.6	61.1	12.2	49.9	55.5	11.3
$\bar{x}$	22.8	29.6	9.7	31.7	58.3	10.8	49.9	55.5	11.4
S.E.	6.39	3.97	2.1	3.11	8.28	0.9	5.14	11.3	2.1

Sympt signifies symptomatic leg, asympt the asymptomatic. – indicates that blood flow could not be determined for technical reasons. Blood flow rate was measured at three positions of the toe pulp in relation to the reference level of the heart in supine patients: heart level, 50 cm above the heart and 50 cm below heart level. $\bar{x}$ = median value, and S.E. = standard error of the mean.

The ^{133}Xenon washout method determines blood flow rate through the nutritive, cutaneous capillaries.

Results

The results are given in Tables 1–3. Figure 1 presents the absolute blood flow rates obtained by the heat washout method at the various levels in the three investigated groups. In Fig. 2 blood flow rates as measured with heat washout are plotted as the blood flow rates at the given test level above or below the heart divided by the value measured at the reference (heart) level in order to show the fractional change in perfusion rate in the two test positions in relation to the reference level.

Heat washout method (blood flow rate in nutritive capillaries plus blood flow rate in arteriovenous anastomoses)

In group A, normal subjects, blood flow was halved when the toe was elevated to 50 cm above heart level,

Table 3. The observations in patients with critical ischaemia.

Patient No.	50 cm above heart level			At heart level			50 cm below heart level		
	Heat ml/100 g/min		^{133}Xe	Heat ml/100 g/min		^{133}Xe	Heat ml/100 g/min		^{133}Xe
	Sympt	Asympt	Sympt	Sympt	Asympt	Sympt	Sympt	Asympt	Sympt
1	–	19.8	–	26.0	48.4	21.6	13.2	46.2	15.2
2	–	37.6	–	23.5	44.6	13.9	19.8	35.2	13.9
3	–	22.1	–	18.5	38.1	13.1	24.5	48.5	15.7
4	–	16.1	–	28.5	42.2	8.8	39.3	51.9	–
5	–	16.0	–	15.7	34.5	11.9	22.8	62.4	15.3
6	–	50.4	–	23.6	32.6	12.1	30.4	44.2	6.8
$\bar{x}$	–	21.0	–	23.6	40.2	12.6	23.7	47.4	15.2
S.E.	–	5.70	–	1.94	2.48	1.8	3.67	3.67	1.7

All patients had aggravation of their ischaemic rest pain during elevation to 50 cm above heart level, so it was considered unethical to proceed with measurements at that level. Blood flow rate was measured at three positions of the toe pulp in relation to the reference level of the heart in supine patients: heart level, 50 cm above the heart and 50 cm below heart level. $\bar{x}$ = median value, and S.E. = standard error of the mean.

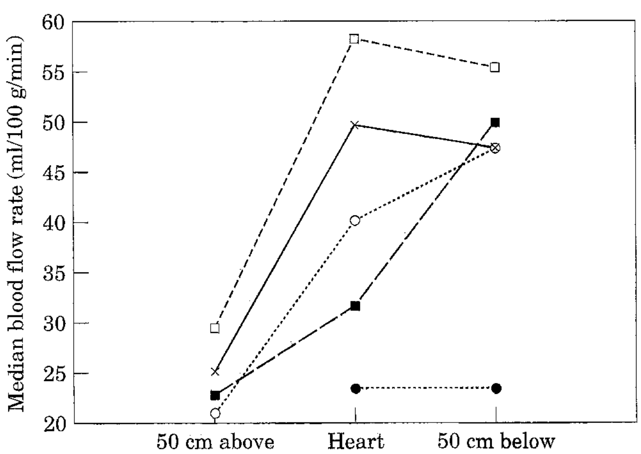

Fig. 1. The median blood flow rate in ml/100 g/min as function of the position of the pulp of the first toe in relation to the heart as measured with the heat washout method. (x) normal subjects; (□) claudicants: asymptomatic side; (■) claudicants: symptomatic side; (○) critical ischaemia: asymptomatic side; (●) critical ischaemia: symptomatic side.

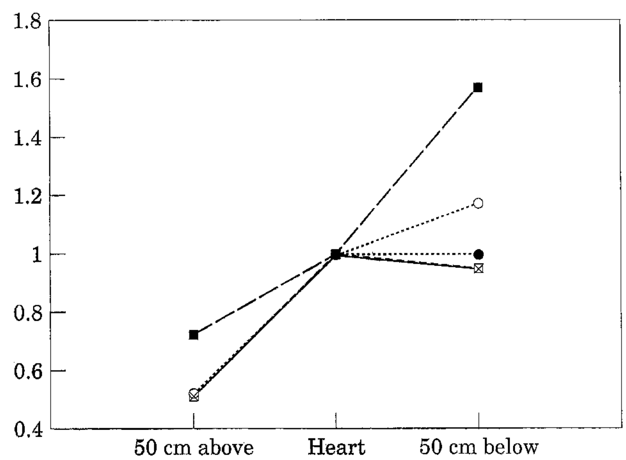

Fig. 2. Median blood flow rates as measured with the heat washout technique are plotted as the blood flow rate at the given test level above or below the heart divided by the value measured at the reference heart level in order to illustrate the fractional change in perfusion rate at the two test positions. (x) normal subjects; (□) claudicants: asymptomatic side; (■) claudicants: symptomatic side; (○) critical ischaemia: asymptomatic side; (●) critical ischaemia: symptomatic side.

but it remained the same as compared with heart level value when the toe was placed 50 cm below the heart. In group B, patients with intermittent claudication, blood flow rate was halved at elevation on the asymptomatic side, but on the symptomatic side it fell only to 72% of the reference level value of the heart. Below heart level blood flow rate on the asymptomatic side remained constant as compared to heart level, but on the symptomatic side blood flow rate was increased by a factor of 1.6. In group C, patients with critical ischaemia, blood flow rate was halved on the asymptomatic side by elevation, but it increased by 18% as compared to heart level when the toe was placed at 50 cm below the heart. During lowering, change in blood flow rate was not observed on the side with critical ischaemia.

^{133}Xe washout method (nutritive vessel blood flow rate)

Blood flow remained practically the same (around 10 ml/(100 g/min)) regardless of the position of the pulp.

Discussion

Measurements of absolute values of nutritive blood flow rate in the skin of the distal extremities in areas without arteriovenous anastomoses at heart level have shown that it is not possible to distinguish on this basis between normals, claudicants or patients with critical ischaemia.[10] This was also the case for the present set of experiments. However, expressing the measured blood flow rate in a peripheral tissue at a given test level above or below the reference level (the heart level) as a fraction of the blood flow rate at the reference level, it is possible to detect the influence of orthostatic pressure changes on blood flow rate in a normalised manner (heart level fractional blood flow rate = 1). By this technique two major mechanisms for local control of the peripheral circulation in skin, subcutaneous tissue and skeletal muscle have been discovered: (a) local autoregulation of blood flow rate and (b) the local sympathetic veno-arteriolar axon reflex.[10–12]

The reason why patients with ischaemic rest pain get relief from their pain when they hang the leg down is due to the fact that autoregulation, and also the veno-arteriolar reflex in the forefoot, are defective in the precapillary arterioles (nutritive blood flow) because of ischaemic paralysis of the smooth muscle cells in the arteriolar media layer.[13] Using nail fold capillary video microscopy it has been shown that patients with relief of rest pain while sitting did not always have a higher capillary perfusion but did have a higher density of perfused capillaries in the sitting position.[14] These experiments are not directly comparable to the present series, where the limb was moved passively down to the test level in the supine subject to minimize central vasoregulatory modulations of blood flow.

These previous studies were performed in areas without shunt vessels. For this reason the present techniques were used to study blood flow rate changes in response to orthostatically induced pressure changes in the toe pulp where arteriovenous anastomoses are numerous. The arteriovenous anastomoses are shunt vessels with a lumen of 20–70 μm and a thick wall[15] with well developed smooth muscle cells that are richly supplied by nerve fibres.[16] Their diameter is influenced neither by local metabolites nor by regional temperature changes.[17] The main function of arteriovenous anastomoses is to eliminate heat from the blood by shunting blood directly from the arterial to the venous side of the circulation and, in this way, they participate in temperature regulation. Since nutritive capillaries are bypassed by this mechanism, these vessels might play an important role in the cutaneous circulation of patients with critical ischaemia.

Normal subjects

In normal subjects the nutritive blood flow rates of cutaneous and subcutaneous adipose tissue as well as skeletal muscle tissue are autoregulated,[11,12] so perfusion coefficients remain constant in spite of arterial perfusion pressure changes within a characteristic pressure interval. In the distal leg of a supine individual nutritive blood flow rates are constant in these somatic tissues when the leg is elevated or lowered passively in relation to the heart in the interval from about 30 cm above the heart level to 30 cm below that level.

When the leg is elevated above 30 cm over heart level, blood flow rate in nutritive vessels decreases in proportion to the orthostatic reduction of arterial perfusion pressure head since venous blood pressure is constant and about 0 mmHg at heart level and further above the heart. When the leg is lowered both the arterial and the venous blood pressures increase in parallel with the increase of orthostatic pressure. At 30 cm below the heart the perfusion is suddenly reduced to about half of the value at heart level. This oedema reducing phenomenon is due to the local sympathetic veno-arteriolar axon reflex, where dilatation of veins triggers the reflex that induces local vasoconstriction of resistance vessels.[10] When the leg is lowered further down, blood flow rate remains constant at some 50% of the value at heart level.

The results of the present study with the ^{133}Xe washout method showed that the nutritive capillary blood flow rate of the skin in the pulp of normal toes remained constant within the entire examined pressure range. It follows that the veno-arteriolar axon reflex was not present in this anatomical region considering that the vascular resistance at 50 cm below the heart was identical to that at heart level.

Autoregulation of blood flow was present in the nutritive vessels in the pulp area even to 50 cm above the heart level. As described below the perfusion through arteriovenous anastomoses decreased during elevation corresponding to the reduction of orthostatic blood pressure. The perfusion through the capillary network is maintained due to local redistribution of blood flow since the capillaries will constitute a relatively larger fraction of the perfused volume. In this way the pulp can keep autoregulation of the perfusion in nutritive vessels at a level that is higher than that in other parts of the foot.

The shunt vessel blood flow rate at 50 cm above the heart decreased to half of that at heart level whereas shunt blood flow rate at 50 cm below the heart was identical to that at heart level as showed by the heat washout method. Since the shunt vessels have a thick muscular wall, they have low compliance, so they will not dilate during lowering. For this reason the resistance is not diminished, and blood flow rate is not expected to increase.

Patients with intermittent claudication

In patients with intermittent claudication the nutritive cutaneous blood flow in the distal symptomatic leg during rest has been shown to be similar to that of normal persons. This was also the case in the present study. In addition, the results of the examinations of both the symptomatic and the asymptomatic leg showed a behaviour with respect to regulation of local blood flow rate in the examined area that was identical to that of normal persons, i.e. presence of autoregulation but absence of veno-arteriolar reflex.

At elevation of the toe to 50 cm above heart level, blood flow rate through shunt vessels on the symptomatic side fell only to 72% of the value at heart level. The absolute value of shunt vessel blood flow rate was about half of that found in normal subjects and in the asymptomatic leg of these claudicants. This is probably because there was a larger resistance in the major arteries of these symptomatic, atherosclerotic legs which will tend to give smaller shunt vessel blood flow rates at a given blood pressure at all levels.

During lowering to 50 cm below heart level shunt vessel blood flow rate increased by a factor of 1.6 on the symptomatic side which was in contrast to the unchanged blood flow rate in normal subjects and on the asymptomatic side of these patients. Blood flow rate as measured by heat washout in the asymptomatic leg in patients with intermittent claudication showed higher values at all levels as compared to normals. This is probably due to the reduced wall thickness (atrophy) induced by relatively lower arterial blood pressure that results in increased compliance.

Patients with critical ischaemia

As previously stated, patients with critical ischaemia developed increased pain during elevation so it was considered unethical to pursue measurements in that position. It is obvious, though, that blood flow during elevation must be zero or very close to that value.

During lowering at 50 cm below the heart the blood flow rate in the pulp of the asymptomatic leg increased by 18% as compared to the value at heart level, an intermediary response between the normal findings and the behaviour of the claudicants. It is difficult to interpret this finding in clinical terms since possible symptoms of claudication may be masked by critical ischaemia on the contralateral side. It must be speculated that some degree of impaired local blood flow regulatory response is present even on the asymptomatic side of patients with critical ischaemia.

On the symptomatic side no increase was observed during lowering. Besides, the absolute values of the shunt vessel blood flow rate were only around half the value of that in normals. The absolute value of the shunt perfusion coefficient was low (about 10 ml/100 g/min) as obtained by subtraction of the nutritive blood flow rate measured by ^{133}Xe washout from the total blood flow rate measured by the heat washout method. Thus, only half of the total blood flow rate passed through shunt vessels in these patients. An increase was not observed during lowering which shows that the increase in effective perfusion pressure due to increased compliance in arterioles, as expected, was extremely low or absent due to atherosclerotic occlusions.

In conclusion, the present study shows that autoregulation of nutritive blood flow is present in the pulp of toes in normal subjects and in claudicants, but that the local sympathetic veno-arteriolar axon reflex is absent. The arteriovenous anastomoses exhibit distinct, characteristic reaction patterns in response to passive elevation and lowering of the extremity in the three examined groups. Determination of the local responses to changes in orthostatic blood pressures might permit objective assessment of patients with arterial insufficiency.

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References

1 Henriksen O, Paaske WP. Local regulation of blood flow in peripheral tissue. *Acta Chir Scand* 1995; **502**: 63–74.
2 Eickhoff JH, Henriksen O. Local regulation of subcutaneous forefoot blood flow during orthostatic changes in normal subjects,

in sympathetically denervated patients and in patients with occlusive arterial disease. *Cardiovasc Res* 1985; **19**: 219–227.
3 Second European consensus document on chronic critical leg ischaemia. *Eur J Vasc Surg* 1992; **6**, Suppl. A: 2.
4 MIDTTUN M, SEJRSEN P, COLDING-JØRGENSEN M. Heat washout: A new method for measuring blood flow rate in areas with and without arteriovenous anastomoses. *Clin Physiol* 1996; **16**: 259–274.
5 JASZCZAK P, SEJRSEN P. A combined tc-PO_2 and skin blood flow sensor. *Acta Anaesthesiol Scand* 1985; **29**: 623–628.
6 JASZCZAK P, SEJRSEN P. Determination of skin blood flow by ^{133}Xe washout and by heat flux from a heated tc-PO_2 electrode. *Acta Anaesthesiol Scand* 1984; **28**: 482–489.
7 KETY SS. Measurement of regional circulation by the local clearance of radioactive sodium. *Am Heart J* 1949; **38**: 321–328.
8 SEJRSEN P. Atraumatic labeling of skin by inert gas: Epicutaneous application of xenon 133. *J Appl Physiol* 1968; **24**: 570–572.
9 SEJRSEN P. Blood flow in cutaneous tissue in man studied by washout of xenon-133. *Circ Res* 1969; **25**: 215–229.
10 HENRIKSEN O. Local sympathetic reflex mechanism in regulation of blood flow in human subcutaneous adipose tissue. *Acta Physiol Scand* 1977; (Suppl. 450): 1–48.
11 HENRIKSEN O, NIELSEN SL, PAASKE WP. Autoregulation of blood flow in human adipose tissue. *Acta Physiol Scand* 1973; **89**: 531–537.
12 HENRIKSEN O, NIELSEN SL, PAASKE WP. Autoregulation of blood flow in human cutaneous tissue. *Acta Physiol Scand* 1973; **89**: 538–543.
13 HENRIKSEN O. Orthostatic changes of blood flow in subcutaneous tissue in patients with arterial insufficiency of the legs. *Scand J Clin Lab Invest* 1974; **34**: 103–109.
14 UBBINK DT, JACOBS MJHM, SLAAF DW, TANGELDER GJWJM, RENEMAN RS. Capillary recruitment and pain relief on leg dependency in patients with severe lower limb ischaemia. *Circulation* 1995; **85**: 223–229.
15 GRANT RT, BLANT EF. Observations on arteriovenous anastomoses in human skin and in the bird's foot with special reference to the reaction to cold. *Heart* 1931; **15**: 385–411.
16 MOLYNEUX GS. The role of arteriovenous anastomoses in the peripheral circulation. *Proc Roy Soc* 1977; **88**: v–xiv.
17 HALES JRS, FAWCETT AA, BENNETT JW, NEEDHAM AD. Thermal control of blood flow through capillaries and arteriovenous anastomoses in skin of sheep. *Pflügers Archiv* 1978; **378**: 55–63.

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