Comparison of dye-dilution and plethysmographic blood flow measurements: an evaluation of the influence of invasive techniques on blood flow and on arterial and femoral venous substrate variables in man

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SUMMARY

1. In eight healthy volunteers we compared leg blood flow, as determined in a calf segment by strain-gauge plethysmography, with the flow measured by a constant-rate infusion of Indocyanine Green dye into the femoral artery. The representativeness of the calf segment was evaluated by complementary measurements with additional strain gauges attached around the proximal and distal crural and the distal thigh segments (n = 6). Furthermore, we investigated the influence of the catheterization procedure and a simulated vascular puncture, as well as repeated venous occlusions, on blood flow and on arterial and femoral venous substrate concentrations and blood gases (n = 8).

2. The leg blood flow measured by dye dilution was 0.31 ± 0.03 litre/min (mean ± SEM). The blood flow in the calf segments was 14.8 ± 1.6 ml min⁻¹ litre⁻¹ and no difference between the legs was observed. Extended to the whole leg the plethysmographic blood flow was 0.17 ± 0.01 litre/min and thus lower (43 ± 7%, P < 0.001) than the flow determined by the indicator-dilution method. Blood flow in the legs was not influenced by catheterization or sham punctures of the vessels or by repeated venous occlusions.

3. The concentrations of glucose, lactate and glycerol, as well as blood gas variables, in arterial and femoral venous blood did not change during the study or decreased so slightly (pH and lactate) that the arteriovenous difference was not influenced.

4. We conclude that the blood flow of the total leg cannot be satisfactorily estimated from strain-gauge plethysmography of a single calf segment. Strain-gauge plethysmography can therefore not be recommended for quantitative studies of substrate turnover in the leg tissues applying the Fick principle. Catheterization of the femoral vessels, or manipulations close to them with a thin cannula or repeated venous occlusions, has no significant effect on leg blood flow and substrate exchange.

Key words: blood gases, catheterization, dye dilution, femoral artery, femoral vein, free fatty acids, glucose, glycerol, lactate, leg blood flow, strain-gauge plethysmography.

Abbreviations: PO₂, partial pressure of oxygen; SO₂, oxygen saturation.

INTRODUCTION

The adaptation to physical exercise of blood flow, oxygen uptake and exchange of energy substrates in leg tissues has been thoroughly investigated [1-8]. For the measurement of leg blood flow, the method described by Jorfeldt & Wahren [4] is commonly used, but other methods have also been applied [8-10].

To an increasing extent attention is being paid to the exchange of energy substrates in leg tissue even at rest, especially in connection with trauma, malnutrition, cancer and other cachectic conditions. This interest is related to the great muscle mass of the leg and the significance of the skeletal musculature as a carbohydrate and protein store.

All investigations of net exchange in leg tissues employ the Fick principle, i.e. the uptake or release of a metabolite is calculated from the blood flow and the arteriovenous concentration difference. This procedure is valid only if the blood flow, the arterial concentration and the arteriovenous difference are constant [11]. Furthermore, the blood flow must be representative for the same...
vascular area as the arteriovenous difference. For studies at rest, blood flow can easily be determined non-invasively by venous occlusion plethysmography, where the volume changes are most conveniently recorded with a strain gauge [12]. Blood flow determined that way is, however, relevant at most only for the limb segment enclosed in the plethysmograph. The arteriovenous difference, on the other hand, is commonly obtained by analysis of blood samples drawn from the femoral artery and vein and, if so, it refers to the tissues of the whole leg. Catheterization or puncture of the femoral vessels may, by changes in sympathetic tone, alter the blood flow, the arterial concentrations of substrates and the local metabolism.

None of these potential sources of error has been studied in detail experimentally and the present investigation was designed to elucidate in healthy volunteers: (i) if it is appropriate to estimate the total leg blood flow from measurements of segmental blood flow by strain-gauge plethysmography and leg volume; (ii) if catheterization, puncture of the femoral vessels or repeated venous occlusions alter blood flow, arterial and femoral venous substrate concentrations or blood gases.

MATERIALS AND METHODS

Study A

Eight healthy male subjects were studied after an overnight fast. All subjects were informed of the nature, purpose and possible risks of the study before they consented to participate. The experimental protocol was reviewed and approved by the Ethics Committee of the Medical Faculty, University of Linköping. Mean values (± SEM) for age, height, weight and leg volume of the subjects were 31.5 ± 2.2 years, 1.80 ± 0.01 m, 78.5 ± 3.6 kg and 11.0 ± 0.7 litres, respectively.

Leg volume was estimated from anthropometric data considering the leg as being built up by the foot, two frustrums of cones with their circular basis at the level of the maximal circumference of the calf, a cylinder from 10 cm below to the upper border of the patella and finally the frustrum of a cone with the circular basis at the level of the pubis.

The investigation was performed in the morning with the subjects in the supine position wearing a T-shirt. Areas surrounding the groins were covered by surgical dressings. The room temperature was 20–21°C. The legs were raised about 20° and supporting pillows were placed under the thighs and heels. A mercury-in-silastic rubber strain gauge was wrapped around the calf at the level of maximal circumference. The relative change of the maximal circumference of the vessels, influenced blood flow and leg exchange of gases and substrates. Blood was drawn for the same analyses as previously and thereafter repeated sham punctures of the vessels were performed over a period of about 4 min. During the last minutes of this period, the sixth set of plethysmographic determinations took place as well as blood sampling. Another set of blood samples was drawn directly after the sham manipulations.

The next step in the protocol was included to determine whether manipulations with a thin cannula very close to the femoral artery and vein, simulating puncture of the vessels, influenced blood flow and leg exchange of gases and substrates. Blood was drawn for the same analyses as previously and thereafter repeated sham punctures of the vessels were performed over a period of about 4 min. During the last minutes of this period, the sixth set of plethysmographic determinations took place as well as blood sampling. Another set of blood samples was drawn directly after the sham manipulations.

Finally, the seventh set of plethysmographic determinations was completed preceded and followed by blood sampling from the veins and artery, respectively. Also this final section of the protocol was included to study the possible influence of the venous occlusions on the variables measured.

The oxygen content of the blood samples was calculated after determination of the oxygen saturation (SO2) of the haemoglobin (OSM 2; Radiometer, Copenhagen, Denmark), the haemoglobin concentration using the cyanmethaemoglobin technique [14] and the partial pressure of oxygen (PO2). Blood gases and acid–base
variables were measured with an electrode technique (ABL 2; Radiometer, Copenhagen, Denmark). Glucose, lactate and glycerol concentrations in blood were determined fluorimetrically by enzymatic procedures previously described [13]. The investigation took 50–60 min to complete.

Study B

This part of the investigation was undertaken to evaluate the impact of the level at which the strain gauge was positioned on the relative volume change of the leg segment encircled by the gauge. For this, six healthy young female members of the laboratory staff were studied by two modifications of the same plethysmographic technique as in study A using two apparatuses, i.e. in all four channels.

(a) The four strain gauges were placed on the same leg at different levels: maximal circumference of the calf (1), distal thigh 10 cm proximal to the upper border of the patella (2), distal calf at the midpoint between strain 1 and the medial malleolus (3) and at the proximal calf halfway between strain 2 and the apex of the patella (4). Strain gauges (1) and (2) were connected to one apparatus and strain gauges (3) and (4) to the other. The occlusion cuff was wrapped around the proximal thigh.

(b) All the four strain gauges were attached parallel and close to each other around the calf of one leg at the level of the maximal circumference. The occlusion cuff was placed around the distal part of the thigh. This moment was included to rule out possible differences between strain gauges or recording channels.

Statistics

The data are expressed as means ± SEM unless otherwise stated. The paired Student's t-test, F-test and two-way analysis of variance were used. P values of less than 0.05 were considered statistically significant.

RESULTS

Study A

Blood flow determined by plethysmography (Fig. 1). There was no significant difference between the control leg and the study leg in any one of the seven situations or when analysed over the whole study period. Before catheter insertion, the mean blood flow for both legs was 17.8 ± 2.8 ml min⁻¹ litre⁻¹. After insertion of the first venous catheter the flow was reduced by 18% in both legs (P < 0.05). During the rest of the study the flow did not change significantly and the mean was 14.8 ± 1.6 ml min⁻¹ litre⁻¹. The heart rate did not change significantly during the study (54 ± 2 beats/min) but there was a decrease of 3 beats/min after insertion of the first catheter.

Leg blood flow determined by dye dilution (Fig. 1). After insertion of all three catheters and 3 min after the commencement of dye infusion, the leg blood flow was determined and found to be 0.36 ± 0.05 litre/min. Five minutes later, immediately after the fifth set of plethysmographic determinations, the value found was 17% lower (P < 0.01). From this time no further significant change was observed and the mean leg blood flow throughout the study was 0.31 ± 0.03 litre/min.

Leg blood flow estimated by plethysmography. Leg blood flow was here estimated as the product of leg volume and blood flow determined plethysmographically. During the period of dye infusion no significant changes were seen and the mean plethysmographically estimated leg blood flow was 0.17 ± 0.01 litre/min. When the last three sets of plethysmographic estimations of the leg blood flow were compared with the succeeding dye-dilution measurements, the following ratios were obtained: 0.56 ± 0.07, 0.57 ± 0.06 and 0.57 ± 0.08, respectively. Thus, compared with the dye-dilution technique, assessment of leg blood flow from segmental determinations by strain-gauge plethysmography underestimated the flow by 43 ± 7 (sd 19)%.

Influence of catheter insertion and sham puncture of femoral vessels on blood flow. The blood flow determined by plethysmography was not influenced by insertion of the arterial catheter (14.9 ± 1.8 ml min⁻¹ litre⁻¹ before and 15.3 ± 1.7 ml min⁻¹ litre⁻¹ after; P > 0.05) and it was not affected by manipulations with a thin cannula in the area around the vessels mimicking puncture of them. The leg blood flow (dye dilution) was 0.31 ± 0.03, 0.30 ± 0.02 and 0.27 ± 0.02 litre/min before, during and after the manipulation period, respectively (P > 0.05). The corresponding values for blood flow (plethysmography) were 15.7 ± 1.2, 15.1 ± 1.2 and 15.3 ± 1.3 ml min⁻¹ litre⁻¹, respectively (P > 0.05). Statistical testing of the differences between the two legs showed no influence of the catheter insertion or of the manipulations.

Influence of venous occlusion on leg blood flow. The leg blood flow determined by dye dilution immediately

![Blood flow](image-url)
before and after the last set of six venous occlusions was $0.28 \pm 0.03$ and $0.30 \pm 0.04$ litre$^{-1}$ min$^{-1}$, respectively ($P > 0.05$).

$\text{SO}_2$, blood gas variables and concentrations of energy substrates in the femoral veins and artery (Figs. 2 and 3). Neither catheterization of the femoral artery nor manipulations with a cannula close to the femoral vessels induced any changes in the femoral vein of $\text{SO}_2$, $\text{PO}_2$, glucose, lactate and glycerol, i.e. for no one of the blood variables analysed. Repeated venous occlusions (the fifth and last set of plethysmographic determinations) did not induce any significant changes in the gas and substrate variables studied in femoral venous blood. $\text{SO}_2$ and $\text{PO}_2$ of the femoral venous and arterial blood did not change during the study and no differences were observed between study and control legs. The meanSEM for femoral venous $\text{SO}_2$ was 2.8%.

The glucose and glycerol concentrations in arterial blood did not change during the observation periods. The mean femoral venous glucose concentration was 0.04 mmol/l lower in the study leg compared with the control leg ($P < 0.01$). Corresponding to this, the arteriovenous concentration difference was approximately 13% higher in the study leg than in the control leg. The difference did not change throughout the study. The glycerol concentration in femoral venous blood showed no differences between the legs. The lactate concentrations decreased slightly (0.04–0.05 mmol/l) in arterial ($P < 0.01$) as well as in femoral venous ($P < 0.05$) blood during the study. The arteriovenous difference was unchanged and there was no difference between the legs. The total variability of glucose, lactate and glycerol in femoral venous blood showed no differences between the legs and was 0.15, 0.05 and 0.008 mmol/l (meanSEM), respectively.

Study B

The results are presented in Table 1. Close to identical values for blood flow were obtained with the four strain gauges when they were applied parallel and close to each other around the calf of one leg. In contrast, measurements performed simultaneously at different levels of the same leg demonstrated significant discrepancies. At the distal crural level, the blood flow was only $54 \pm 5\%$ of the flow measured at the maximal circumference of the calf. The corresponding value for the proximal part of the calf was $79 \pm 8\%$ and for the distal thigh $118 \pm 8\%$.

**DISCUSSION**

The two methods for leg blood flow determinations studied are very different in character. The indicator-dilution method described by Jorfeldt & Wahren [4] measures the blood flow of leg tissues distal to the catheter positions at the level of the inguinal ligament. Applying the plethysmographic techniques, the measure of blood flow refers to a segment of the limb. If this segment is taken as representative of the whole leg, the leg blood flow can be estimated by extending the flow per unit volume to

![Graph](image1)

**Fig. 2.** Femoral venous $\text{SO}_2$ before and after catheter insertions into the femoral vein (v) and artery (a) of the study leg and the femoral vein of the contralateral leg (r), and before and after manipulations close to the femoral vessels of the study leg with a thin cannula (sham puncture) ($n = 8$). ●, Study leg; ○, control leg.

![Graph](image2)

**Fig. 3.** Femoral venous concentrations of glucose, lactate and glycerol before and after catheter insertions into the femoral vein (v) and artery (a) of the study leg and the femoral vein of the contralateral leg (r), and before and after manipulations close to the femoral vessels of the study leg with a thin cannula (sham puncture) ($n = 8$). ●, Study leg; ○, control leg.
the total volume of the leg. The indicator-dilution method has been validated when used as a reference for evaluation of other methods [9, 15], but no comparisons with non-invasive methods have been performed.

In the present study leg blood flow estimated from leg volume and venous occlusion plethysmography was lower by 43 ± 7% than the blood flow measured by dye dilution. Only a few studies have been designed to compare venous occlusion plethysmography with other techniques for blood flow determination in man. Longhurst et al. [16] were able to measure forearm blood flow at rest and during steady-state grip exercise with the aid of an electromagnetic flowmeter and by strain-gauge plethysmography simultaneously. In studies of hand blood flow, Levy et al. [17] demonstrated a close correlation between Doppler blood flowmetry and venous occlusion plethysmography using a water-filled plethysmograph. In both studies slightly higher flow values were obtained by plethysmography than with the method under comparison.

Flow measurements with strain-gauge devices and air- or water-filled plethysmographs have been compared in several investigations. In general, the strain-gauge technique yields slightly lower flow values than the other methods [18-20], but a good agreement has also been reported [21, 22].

The most probable explanation for the discrepancy in the present study between leg blood flow values obtained by the dye-dilution method and by strain-gauge plethysmography is that the segmental blood flow determined from the volume increase of the very narrow segment under the strain gauge on venous occlusion is not representative of the tissues of the whole leg.

After venous occlusion, the volume of the leg distal to the collecting cuff will increase at a rate corresponding to the blood flow entering this part of the leg. The major part of the blood accumulates in the veins and the volume expansion of a particular segment will therefore be dependent on the relative venous volume within the segment and the compliance of these veins in relation to other veins in the area shut off from venous return. The relative volume expansion was smallest at the distal crus where only a minor part of the segment consists of soft tissues, which comprise most of the distensible veins. Similar results were obtained by others [22]. In the distal thigh, on the other hand, which has a large component of muscle as well as cutaneous and subcutaneous tissues, the relative volume expansion was more than twice as high. In the present work, measurements on the calf in study B underestimated the volume expansion of the distal thigh by about 20% and probably the expansion of the proximal thigh by a greater extent. As the thigh constitutes about two-thirds of the leg volume, the small calf segment circumscribed by the strain gauge is far from representative of the total leg.

It would therefore appear justified to conclude that the blood flow of the total leg cannot be satisfactorily estimated from strain-gauge plethysmography of a single calf segment since it underestimates total leg blood flow. Strain-gauge plethysmography can therefore not be recommended for quantitative studies of substrate turnover in leg tissues. In this context it can be claimed that strain-gauge plethysmography still may be useful for studies of relative changes which, for instance, have been induced by some type of intervention. This statement would be true if there is a constant relationship between the volume expansion of the narrow segment under the strain gauge and the whole leg. This is probably not true for the following reasons. First, the relationship between the venous volume and the transmural venous pressure is not linear. Secondly, this relationship is not identical in the different parts of the venous system. Thirdly, it may be shifted by variations in the sympathetic tone. The non-linearity of the curve implies that the level of the transmural venous pressure at the onset of venous occlusion influences the following volume expansion. Evidence has been presented that cutaneous veins are more reactive to sympathetic stimuli than are the veins of the deep limb tissues [23, 24]. As the ratio between cutaneous and deep tissues varies along the leg, it is very likely that changes in sympathetic activity influence the relationship between the volume expansion in a small calf segment and that of the leg as a whole. For these reasons it is not certain that strain-gauge plethysmography can be used for studies of relative changes in total leg blood flow, although this assumption has not been tested directly.

After insertion of the first venous catheter, the blood flow as determined by plethysmography declined. The change was almost identical on both sides and additional catheterization manoeuvres were not accompanied by further changes in blood flow, arguing against the catheterization per se as the causative factor. The evidence is more in favour of a psychological adaptation to the experimental situation. In the present study the leg

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Table 1. Segmental blood flow measured by strain-gauge plethysmography at four levels of the leg

<table>
<thead>
<tr>
<th>Strain gauge…</th>
<th>Blood flow (ml min⁻¹ litre⁻¹)</th>
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<tbody>
<tr>
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<td>1</td>
</tr>
<tr>
<td>All in calf position</td>
<td>13.8 ± 2.1</td>
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<tr>
<td>Different positions</td>
<td>13.2 ± 1.9</td>
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<td>(calf)</td>
<td>(distal thigh)</td>
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<td></td>
<td><em>P</em>&lt;0.05</td>
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Results are means ± SEM. Statistical significance for values at different positions compared with calf values are presented.
blood flow obtained after 8 min or more of dye infusion was 0.31 ± 0.03 litre/min. This value is similar, but slightly lower, than that reported elsewhere for healthy volunteers when the same technique was used [4, 6, 7, 13, 25, 26].

Blood flow as determined by dye infusion was slightly but significantly higher 3 min after the commencement of dye infusion than 5 min later. A corresponding decline in the flow determined plethysmographically was not observed. The most probable explanation for this discrepancy in our results is that a steady state for the indicator was not fully achieved at the 3 min measurements. This is in contrast to the original paper by Jorfeldt & Wahren [4] in which the authors claimed, on an empirical basis, that a steady state was reached 3 min after commencement of the infusion.

It is crucial for the indicator-dilution techniques with constant infusion of the indicator that a steady-state situation is achieved, in which the flow of indicator into the vascular system under study equals the flux out from it [11, 27]. An absolute steady state is seldom reached in biological systems. The rate at which an acceptable relative steady state is reached is determined by the time constant $V/F$ [27]. In the present study of leg blood flow at rest, $F$ (leg plasma flow) was of the order of 0.2 litre/min and $V$ (the plasma volume located in the leg) was estimated as approximately 0.3 litres corresponding to a time constant of 1.5 min. With this time constant, the blood flow was overestimated at 3 min of infusion compared with 8 min by a factor 1.13, which is in close agreement with the current results. Aiming at a systematic error induced by the lack of steady state in this system of less than 5%, the first set of sampling should not take place until after three times the time constant, i.e. not before 4.5 min after commencement of the infusion. On the basis of the present results and theoretical considerations, we recommend that, at rest, 5 min should be allowed to elapse from the start of infusion until blood sampling for dye analysis is performed.

In studies of regional exchange of substrates by measurements of blood flow and arteriovenous concentration differences for the substrates, it is fundamental that there is a steady state for blood flow, arterial concentrations and arteriovenous concentration differences [11]. The arterial concentrations of the variables studied were constant with the exception of lactate, which showed a decrease. This was so small, however, that a temporal correction for it influenced the arteriovenous difference for lactate by less than the estimated analytical error for the arteriovenous difference [11]. A relative steady state [27] was consequently at hand also for lactate.

Since blood flow and arterial concentrations were constant, the lack of change in arteriovenous difference in the present study indicates that a metabolic steady-state condition also prevailed. We therefore conclude that after adaption to the experimental situation, a steady-state prevails for leg blood flow and arterial concentrations and metabolism of various substrates in leg tissues, and that this condition is not influenced by manipulations, such as catheter insertions or puncture of the femoral vessels which might modify the sympathetic tone, or repeated venous occlusions. Furthermore, the present study indicates that strain-gauge plethysmography underestimates leg blood flow compared with the dye-dilution technique and can therefore not be recommended for quantitative studies of substrate turnover in leg tissues.

ACKNOWLEDGMENT

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REFERENCES


