Limb and skeletal muscle blood flow measurements at rest and during exercise in human subjects

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The aim of the present review is to present techniques used for measuring blood flow in human subjects and advice as to when they may be applicable. Since blood flow is required to estimate substrate fluxes, energy turnover and metabolic rate of skeletal muscle, accurate measurements of blood flow are of extreme importance. Several techniques have therefore been developed to enable estimates to be made of the arterial inflow to, venous outflow from, or local blood flow within the muscle. Regional measurements have been performed using electromagnetic flow meters, plethysmography, indicator methods (e.g. thermodilution and indo-cyanine green dye infusion), ultrasound Doppler, and magnetic resonance velocity imaging. Local estimates have been made using ^{133}Xe clearance, microdialysis, near i.r. spectroscopy, positron emission tomography and laser Doppler. In principle, the aim of the study, the type of interventions and the limitations of each technique determine which method may be most appropriate. Ultrasound Doppler and continuous indo-cyanine green dye infusion gives the most accurate limb blood flow measurements at rest. Moreover, the ultrasound Doppler is unique, as it does not demand a steady-state, and because its high temporal resolution allows detection of normal physiological variations as well as continuous measurements during transitional states such as at onset of and in recovery from exercise. During steady-state exercise thermodilution can be used in addition to indo-cyanine green dye infusion and ultrasound Doppler, where the latter is restricted to exercise modes with a fixed vessel position. Magnetic resonance velocity imaging may in addition be used to determine blood flow within deep single vessels. Positron emission tomography seems to be the most promising tool for local skeletal muscle blood-flow measurements in relation to metabolic activity, although the mode and intensity of exercise will be restricted by the apparatus design.

Blood flow: Exercise: Muscle metabolism: Hyperaemia

Physical exercise exerts significant stress on human body functions, both with regards to its effect on the cardio-vascular system as well as on metabolic processes (Saltin & Rowell, 1980; Saltin & Gollnick, 1983; Saltin, 1985). In order to meet the elevated metabolic demands of active muscle, blood flow increases rapidly at the onset of exercise (Rådegran & Saltin, 1998; Fig. 1), and linearly in relation to work intensity (Fig. 2), to reach a peak level approximately 50-fold higher than that at rest (Andersen & Saltin, 1985). Moreover, blood flow continuously oscillates at rest, as an effect of the heart beat and blood pressure. During exercise these oscillations are even more pronounced, being also influenced by the intramuscular pressure variations (Fig. 3). However, the mean value for blood flow may still be considered as steady when these oscillations are short and repeated in a regular pattern. Furthermore, the flow needs to be averaged over a sequence of contraction–relaxation cycles, i.e. approximately 10 s, in order to minimize the normal physiological variation depending on the cyclic relationship between the cardiac cycle, pulse pressure and intramuscular pressure (Rådegran, 1997; Rådegran & Saltin, 1998).

Several techniques have been developed to enable estimates to be made of the arterial inflow to, venous outflow from, or local blood flow within the muscle (Table 1). Regional limb blood-flow measurements have been performed using electromagnetic flow meters (Cronestrand, 1970), plethysmography (Bygdeman & Pernow, 1978), indicator methods (Jorfeldt & Wahren, 1971; Andersen & Saltin, 1985), ultrasound Doppler (Rådegran, 1997) and magnetic resonance velocity imaging (Hundley *et al*. 1996). Local estimates have been made using ^{133}Xe clearance
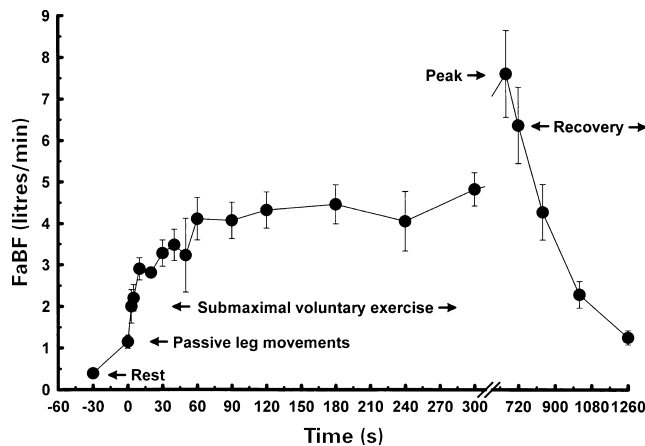

Fig. 1. Femoral artery blood flow (FaBF; ultrasound Doppler) in transition from rest, to passive leg movement, at onset of and during submaximal voluntary knee-extensor exercise, as well as at termination at peak effort, followed by post exercise. Values are means with their standard errors represented by vertical bars for *n* 7 determinations. (From Rådegran & Saltin, 1999.)

(Lassen *et al.* 1964), microdialysis (Hickner *et al.* 1994*a*; Rådegran *et al.* 1998), near i.r. spectroscopy (NIRS; Edwards *et al.* 1993; Boushel *et al.* 1997), positron emission tomography (PET; Ruotsalainen *et al.* 1997), and laser Doppler (Jensen *et al.* 1995). In addition, drop or volume counting, hot-wire anemometer and radioactively-labelled microsphere techniques have been used in animals. An overview will be given of techniques utilized in human subjects, with emphasis on recent methodological refinements which allow the most accurate blood-flow estimates.

Regional blood-flow measurements

Electromagnetic flow meters

Principle. The electromagnetic flow meters are based on induction of voltage changes proportional to the flow of blood (Kolin, 1936, 1941; Djordjevich & Sadove, 1981). As blood is a conductive liquid, an electromotive force is induced, which can be derived as: electromotive force $= B \times L \times u$, where B is the magnetic flux density (T), L is the distance between the electrodes (m), and u is the instantaneous blood velocity (m/s; Webster, 1978).

Practicalities. The electromagnetic flow probe is intra-operatively mounted directly on the vessel where flow is to be measured (Hall, 1969). Blood flow is calculated from the product of the cross-sectional area of the vessel and the mean flow velocity.

Strength and weaknesses. When the technique was developed it was unique, as instantaneous volumetric pulsatile flow could be calculated. Furthermore, it enabled measurements to be made of blood flow in single vessels at rest and during exercise after reconstruction for occlusive disease (Cronestrand, 1970; Vänttinen, 1975). The equipment is relatively inexpensive and running costs are relatively low. It is, however, too invasive as a standardized method for measuring blood flow in healthy human subjects. Thus, its primary application is restricted to species other than man.
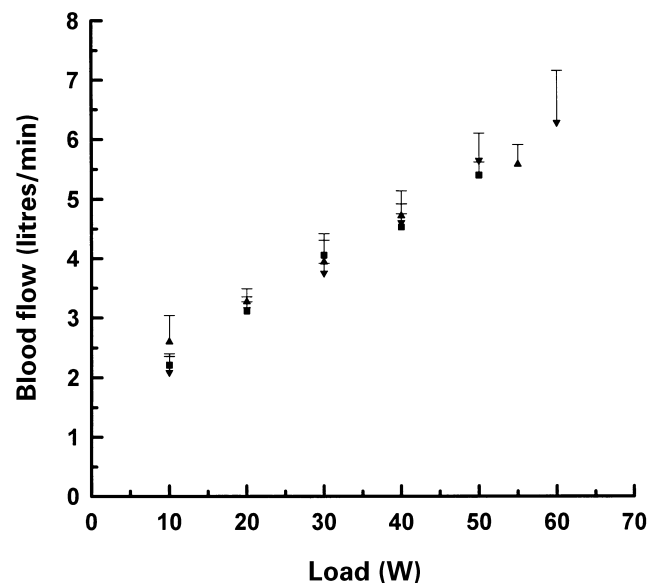

Fig. 2. Linear increase of arterial inflow to, and venous outflow from, the quadriceps muscle during steady-state knee-extensor exercise at incremental intensities. Measurements performed with ultrasound Doppler (averaged in relation to the contraction–relaxation cycles (■; *n* 10 determinations) or the electrocardiogram (▼; *n* 5 determinations); Rådegran, 1997) and thermodilution (▲; *n* 5 determinations; Andersen & Saltin, 1985) give similar results. Values are means with their standard errors represented by vertical bars.

Plethysmography

Principle. Venous occlusion plethysmography is based on determining the volume increase in a limb segment when venous outflow is temporarily arrested but arterial inflow is intact (Bygdeman & Pernow, 1978). The rate of limb swelling during occlusion is used to assess the rate of arterial inflow. Alternatively, the limb circumference can be used. With no change in the shape of the cross-sectional area with a certain volume increase, the percentage change in area at small changes will be twice the change of the limb circumference (Bygdeman & Pernow, 1978).

Practicalities. An air-filled plethysmograph was used previously (Bonde-Petersen & Siggaard-Andersen, 1967, 1969; Siggaard-Andersen & Bonde-Petersen, 1967; Siggaard-Andersen, 1970). The plethysmograph-manchett and pressure sensor were placed over the widest segment of the limb. A venous and arterial occlusion manchett were placed just proximal and distal to the plethysmograph-manchett, and occlusion pressures of approximately 50 and 250 mmHg were applied respectively. However, as increased precision in the circumference measurements was needed, the strain-gauge plethysmograph was developed, which utilizes a strain-gauge sensor placed around the widest part of the limb where flow is to be measured. Knowing the electrical calibration and registering the induced voltage change, the blood flow is calculated based on $\Delta V/V = 2\,\Delta O/O = \Delta R/R$, where V is the volume of the limb segment, O is the circumference, and R is the electrical resistance of the sensor. At a constant current through the sensor, the voltage will follow the variations in resistance, and the voltage changes will be proportional to the volume
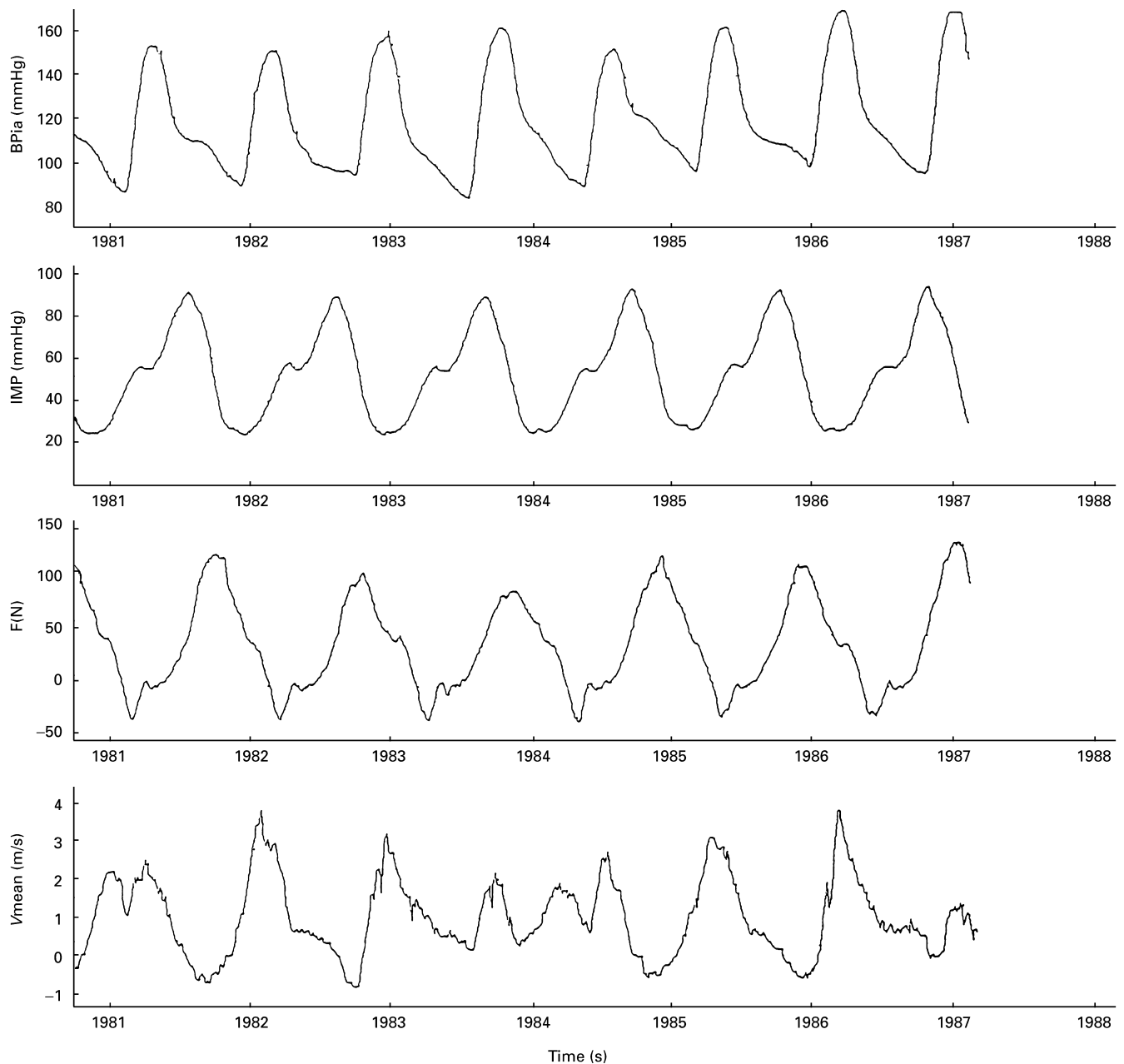
Fig. 3. A continuous tracing of femoral artery blood velocity (*V*mean, representing arterial inflow, ultrasound Doppler) during steady-state knee-extensor exercise in relation to the knee-extensor force (F), intramuscular pressure (IMP), and arterial blood pressure (BPia). (From Rådegran & Saltin, 1998.)

changes of the limb. The blood flow is then calculated from: blood flow (ml/min per litre tissue) = deflection size (after 10 s; mm) × 6 × 10/calibration deflection (mm; Bygdeman & Pernow, 1978).

Strength and weaknesses. The strength of plethysmography is that it is relatively easy to use and non-invasive. Also, the equipment is inexpensive and running costs are low. However, the limitations of the method are clear, as shown by following its technical development (Schäfer & Moore, 1896; Brodie & Russell, 1905; Hewlett & van Zwaluwenburg, 1909; Barcroft & Dornhorst, 1949; Whitney, 1953; Conrad & Green, 1961). The original model was cumbersome and required immobilization of the

subjects. It was sensitive to movement and induction of local vasomotor changes. Sealing of the limb without inducing tissue constriction was also problematic. Although subsequently the technique was markedly refined, the original fundamental assumptions still have to be fulfilled for plethysmographic flow to equal arterial inflow (Conrad & Green, 1961). The veins have to be completely occluded, allowing no leakage. The arterial inflow must not be altered initially and the occlusion artifact has to be small. These factors also have to stay constant over the time period of measurement. However, the occlusion pressure has been shown to substantially decrease arterial inflow (Byström *et al.* 1998). Two intrinsic occlusion errors have also been

Table 1. Techniques used for regional and local blood flow measurements in human subjects, indicating the type of measurements that may be performed and the relative cost aspects of the techniques

Blood flow techniques	Comments	Type of measurement	Cost of equipment	Running costs
Regional				
Electromagnetic flow meter	Invasive, probe mounted on vessel	Intra-operative usage	Inexpensive	Inexpensive
Plethysmography	Non-invasive, easy to use	Rest	Inexpensive	Inexpensive
ICG dye dilution	Invasive ICG-injection, blood withdrawal	Rest, submaximal and maximal exercise	Inexpensive	Expensive
Thermodilution	Invasive saline infusion, easy to use	Rest*, submaximal and maximal exercise	Inexpensive	Inexpensive
Ultrasound Doppler	Non-invasive, advanced to use, continuous sampling, high temporal resolution	Rest, transitional changes, submaximal exercise, fixed vessel position	Expensive	Relatively expensive
Magnetic resonance velocity imaging	Non-invasive, advanced to use, continuous sampling, high temporal resolution	Rest, light exercise, restricted by apparatus design	Very expensive	Very expensive
Local				
^{133}Xe clearance	Invasive ^{133}Xe injection	Non-linear at high intensities	Relatively expensive	Relatively expensive
Microdialysis ethanol outflow: inflow	Invasive insertion of 'delicate' microdialysis fibres	Under evaluation, disturbed by contractions	Relatively inexpensive	Relatively expensive
Near i.r. spectroscopy–ICG	Invasive ICG injection	Under evaluation	Relatively expensive	Relatively expensive
Laser Doppler	Invasive insertion of optical fibre	Under evaluation	Relatively expensive	Inexpensive
Positron emission tomography	Invasive, injection of positron emitting isotopes, advanced to use	Rest, light exercise, restricted by apparatus design	Very expensive	Very expensive

ICG, indo-cyanine green.
* Bolus injections at rest only reflect a short time period.

found to work in opposing directions, leading to a tendency towards inaccuracy (Conrad & Green, 1961). Furthermore, it is important to note that measurements performed after or in-between contractions only reflect an immediate post-exercise value and not exercise *per se*. Thus, due to its inherent limitations and the motion artifacts induced by exercise, it is limited to intermittent measurements at rest only (Williams & Lind, 1979; Benjamin *et al.* 1995).

Indicator methods: overview

The indicator methods require steady-state conditions which must not be altered by injection of the indicator (Lassen *et al.* 1983). Different types of indicators have been used such as intravascular indicators (e.g. indo-cyanine green (ICG) dye, Evans-blue, labelled microspheres and labelled albumin), freely-diffusable indicators (e.g. ^{133}Xe, heat, N_2O and H_2 gas) that pass through the cell membranes, as well as small hydrophilic extracellular indicators (e.g. sucrose, EDTA and inulin) which distribute in the interstitial space via passage through pores in the capillary walls.

A distinction is made depending on whether the measurements are based on dilution (e.g. thermodilution and dye dilution) or perfusion (e.g. ^{133}Xe clearance) detection procedures. In order for the indicators to be indicative of blood flow they must not be retained or be metabolized. The indicators must also be fully mixed with the blood. Specifically for the dilution method based on heat transfer, a thorough mixing must occur before reaching the nearby sampling point. For dye dilution the 'mixing' and binding to

a carrier must occur before it spreads into the capillary network. The perfusion method estimating capillary flow (by external detection of the wash-out of an injected tracer) is addressed under local blood-flow measurements.

Indicator methods: indo-cyanine green dye

Principle. Dye dilution is based on the assumption that the indicator concentration in blood is flow-dependent (Lassen *et al.* 1983).

Practicalities. The ICG dye procedure was initially developed to measure cardiac output (Stewart, 1897, 1921), but according to the same principles may be used for limb blood-flow measurements. Blood flow may be measured by infusion of dye of known concentration at a specific rate and site upstream in a vessel, followed by determination of the dye concentration (using a photo-densitometer) in blood samples withdrawn after thorough mixing at a point downstream. Blood flow $= j_{in,\infty}/C_{out,\infty}$, where $j_{in,\infty}$ represents the steady-state infusion rate (mg/min) and $C_{out,\infty}$ is the steady-state outlet concentration of the dye. This equation is valid if it is assumed that there is no re-circulation, which may give a constant rising dye curve (Lassen *et al.* 1983). However, correction for re-circulation can be made where there is bilateral symmetry, by subtracting the signal in the outlet of a non-infused limb from that for the limb within which blood flow is to be determined. A prerequisite, however, is that both limbs have the same mean transit time, and thus are at the same state of activity. Re-circulation is further minimized by using intravascular indicators such as ICG

that quickly reach a steady-state, and indicators that re-circulate minimally, as well as by infusion upstream close to the mixing site and sampling downstream close to this site. With regard to leg blood-flow measurements during intense bicycle exercise, two different approaches have been used: infusing the dye either in the femoral artery or in the distal part of the femoral vein and sampling the blood in the proximal part of the femoral vein at the level of the inguinal ligament, where uniform mixing could be ensured (Jorfeldt & Wahren, 1971; Wahren & Jorfeldt, 1973).

Strength and weaknesses. Continuous ICG infusion gives reliable measurements at rest when infused for an extended period of time, i.e. approximately 3 min (Jorfeldt & Wahren, 1971). It also gives accurate intermittent steady-state measurements during incremental exercise up to peak effort when infused for approximately 1 min and 20 s (Jorfeldt & Wahren, 1971). Furthermore, it is not restricted to a specific type of exercise mode. However, the technique is invasive, requiring dye infusion. It is also slightly more troublesome to perform than thermodilution as it involves sampling and handling of blood which may have to be re-infused in order to avoid a blood loss. The equipment is relatively inexpensive, but the running expenses of ICG are high.

Indicator methods: thermodilution

Principle. Thermodilution blood-flow measurements are based on the thermodynamic heat balance relationship (Ganz & Swan, 1974), knowing the specific heats of blood, the infusion solution and their mixture, as well as the masses of blood and the infusion solution. Blood flow is determined by measuring the temperature change in a vessel during simultaneous infusion of cold saline (9 g NaCl/l). The temperature deflection is proportional to the temperature and rate of saline infusion, as well as the flow rate of blood.

Practicalities. A thermistor and a catheter are inserted into a peripheral vessel in the proximal and either proximal or distal direction respectively. Improved mixing may be achieved by infusing the saline against the bloodstream. The blood flow (ml/min) is calculated as blood flow $= (V_I \times (S_I \times C_I) / (S_B \times C_B)) \times (((T_B - T_I) / (T_B - T_M)) - 1)$, after measuring the blood temperature T_B before saline infusion, the temperature of the infusate T_I during infusion, the temperature of the blood and saline mixture T_M during the steady-state infusion, and calculating the resulting temperature deflection ($\Delta T = T_B - T_I$). V_I is the infusion rate (ml/min) of the infusate, S_i (1·005) and S_B (1·045) are the specific gravities of the infusate and blood respectively, and C_I (4·17 kJ/g per °) and C_B (3·51 kJ/g per °) are the specific heats of the infusate and blood respectively.

The thermodilution technique, originally developed for cardiac output measurements (Fegler, 1954), was adopted in the early 1960s for peripheral blood-flow measurements (Fronek & Ganz, 1960; Ganz *et al.* 1964). The method has subsequently been refined in a series of studies (Sørlie & Myhre, 1977; Jorfeldt *et al.* 1978; Andersen & Saltin, 1985), in which the latter authors optimized the technique for measurements of venous outflow from an isolated muscle group. As *in vitro* studies have shown that the saline may be slightly warmed during the passage through the external tubing and catheter (Gonzales *et al.* 1998), accuracy can be improved by measuring the temperature of the infusate. At rest bolus injections are often used. To calculate flow the mean temperature change is integrated over the time period of the effect. The infusion rate is determined from the volume injected over the time period of its effect. However, since the boluses only reflect a short period of time, and since blood flow is affected by many factors such as the cardiac cycle, blood pressure, breathing patterns, slight movements and 'vasalva' manoeuvres, a rigid control has to be undertaken to minimize the variability. Continuous infusion is also commonly used at rest, but care has to be taken to prevent cooling of the tissue. Moreover, since blood flow during exercise is markedly affected by the variations in intramuscular pressure, mathematical integration of the curve should be performed during as many contraction–relaxation cycles as possible, i.e. for approximately 10 s. In order to eliminate the dependence on when the saline meets the blood, i.e. during a contraction or relaxation or both, continuous infusion is required rather than bolus injection. By continuously monitoring the blood and infusate temperature, attempts have also been made to perform rapid measurements. However, the theoretical basis for indicator techniques is that the measurements are performed during steady-state conditions.

Strength and weaknesses. Heat is the only indicator for which re-circulation can be fully ignored. Thus, with no re-circulation problems this method allows for more frequent measurements than ICG infusion. Using continuous saline infusion, measurements can be performed at submaximal exercise intensities as well as at peak effort, independent of the exercise mode, as it is unaffected by movement artifacts. However, there has to be rigid control of the volume and frequency of saline infusion to avoid tissue cooling, haemodilution and too large a saline load. Moreover, with continuous infusion at rest, cooling of the surrounding tissue may be problematic. However, resting measurements are still of sufficient quality to be used as a baseline for comparison with exercise responses (Andersen & Saltin, 1985). Although the technique is invasive, it is easy to use. Also, the equipment is inexpensive and running costs are low.

Ultrasound Doppler

Principle. The measurements are based on determining the Doppler frequency shift (Δf) that occurs as transmitted sound waves of a specific frequency f are reflected by the erythrocytes (Gill, 1979, 1985). The Δf contains information about both the magnitude and the direction of the flow. The direction is encoded such that Δf is positive or negative depending on whether the blood is moving towards or away from the direction of the transmitted sound waves, respectively. Information about the full parabolic velocity profile is obtained by sampling over the width of the artery. The velocity of blood (v) is calculated from the equation $\Delta f = 2 \times f \times v \times \cos\Theta / c$, where Θ is the angle of insonation (direction of the transmitted sound wave beam towards the site of measurement) and c is the velocity of sound in tissue (approximately 1540 m/s).

Practicalities. Ultrasound Doppler was introduced for medical purposes in the 1950s (Keidel, 1950; Wild & Neal, 1951; Howry & Bliss, 1952; Edler & Hertz, 1954; Satamura & Kaneko, 1960), but improvements were needed before estimates of blood flow could be performed during exercise (Wesche, 1986; Walløe & Wesche, 1988). An ultrasound Doppler with a transducer possessing the capacity to both determine the arterial diameter and blood flow velocity is needed. Blood flow (litres/min) $= 6 \times 10^4 \times v_{mean} \times A$, is thus calculated by multiplying the cross-sectional area of the artery (A) with the angle corrected, time and space averaged, and amplitude (signal intensity) weighted mean blood velocity (v_{mean}; m/s). The constant 6×10^4 converts m/s to litres/min.

Measurement of and correction for the insonation angle are important in obtaining accurate flow estimates. Insonation angles below $60°$ are recommended for the velocity measurements, since the lower the insonation angle the less error may be induced in the flow estimate. Moreover, the pulsed-wave Doppler mode allows for measurement at the specific depths required, over the width of the vessel diameter and parabolic velocity profile. However, the arterial diameter measurements should be obtained under perpendicular insonation. Furthermore, the best image resolution is obtained in the axial direction of the transmitted sound beam. Since the diameter of the artery oscillates with each pulsation, a diameter ($D_{(systole/3)+(diastole \times 2/3)}$) based on the relative time periods of the systolic (1/3) and diastolic (2/3) blood pressure phases may be used to determine the cross-sectional area ($A = \pi r^2$, where r is the radius; $2r = D_{(systole/3)+(diastole \times 2/3)}$); Rådegran, 1997). However, the fluctuation in the size of the arterial diameters is very small (Eriksen, 1992). The fluctuations between systole and diastole in the femoral artery are actually of the same magnitude as the spatial resolution (Rådegran, 1997), and are a minimal problem in relation to the accuracy of the flow measurements. The spatial resolution corresponds to half the spatial wavelength, i.e. approximately 0·1 mm; spatial wavelength $= c/f$, where c is the velocity of sound in soft tissue (1540 m/s) with an imaging frequency f of 7·5 MHz (Chapman, 1990). Moreover, the larger the vessel, the lower the impact any diameter measurement error will have on the flow estimate (Gill, 1985). As the size of the femoral artery does not alter during dynamic knee-extensor exercise compared with rest (Rådegran, 1997), femoral artery blood flow during exercise can be based on the diameter at rest, eliminating measurement errors potentially introduced by frequently altering the insonation angle.

Strength and weaknesses. The ultrasound Doppler is unique as it allows continuous blood-flow measurements with a high temporal resolution. As the precision and accuracy of the technique have been improved by sampling the blood velocity continuously (Rådegran, 1997) instead of averaging the velocity in relation to each cardiac cycle (Wesche, 1986; Walløe & Wesche, 1988), it is now possible to differentiate between physiological and methodological variations, as well as detect rapid changes in flow induced by exercise, different metabolic states, or any other type of vasodilatation. With a well-trained operator, the day-to-day variability is also minimal (Shoemaker *et al*. 1996). Thus,

the ultrasound Doppler is currently the most sensitive technique for blood-flow measurement at rest, at onset of and during submaximal exercise, as well as post exercise. However, to avoid insonation failures, the exercise mode must be such that the limb and the artery are in a fixed position, as for instance during knee-extensor and specific types of forearm exercise. However, the technique is expensive, technically advanced and time-consuming to learn.

Magnetic resonance velocity imaging

Principle. Phase-contrast magnetic resonance velocity imaging, is based on the principle that the hydrogen nuclei in blood moving through a magnetic-field gradient accumulate a phase shift proportional to their velocity (Firmin *et al*. 1987; Hundley *et al*. 1996).

Practicalities. Blood flow is calculated by integrating the blood velocity over the cross-sectional area of the vessel (Hundley *et al*. 1996). Blood-flow (ml/min) $= V_{mean} \times A \times 60$, where V_{mean} (cm/s) is the mean velocity and A (cm^2) is the cross-sectional area of the vessel (both determined using magnetic resonance imaging), and the factor 60 converts s/min to ml/min.

Strength and weaknesses. Magnetic resonance velocity imaging has recently evolved as an unique possibility for determining local blood flow in deep single vessels (Firmin *et al*. 1987; Enzmann *et al*. 1993; Zananiri *et al*. 1993; Li *et al*. 1995; McCauley *et al*. 1995; Hundley *et al*. 1996). The technique is non-invasive and does not require injection of any tracer molecule. An advantage over ultrasound Doppler is that the angle of insonation does not have to be determined (Zananiri *et al*. 1993). An advantage over PET is that no isotopes are needed (Vallé *et al*. 1998). The temporal and spatial resolution has also been suggested to be slightly better than that with PET (Vallé *et al*. 1998). Magnetic resonance imaging equipment is also more readily available than PET (Vallé *et al*. 1998). However, the equipment and running costs are very high. The apparatus and imaging procedures are also cumbersome, limiting the type and intensity of exercise that can be studied.

Local blood flow measurements

Isotope clearance

Principle. The method is based on the principle that the clearance of a molecule or ion is purely flow-dependent (Kety, 1951).

Practicalities. The tracer is administered by intramuscular injection. With external observation of its clearance rate depicted as a wash-out curve (Lassen *et al*. 1964), a measure of muscle perfusion or capillary flow is obtained. A fast wash-out rate results when exercise is started. Muscle blood flow is calculated from the slope of the wash-out curve. Blood-flow (ml/min per 100 g) $= 100 \times \lambda_m \times k_m$, where λ_m is the partition coefficient for muscle and k_m is the rate-constant (Lassen *et al*. 1983).

Strength and weaknesses. The isotope-clearance technique has been utilized to measure blood flow during dynamic exercise (Tønnesen, 1964; Grimby *et al*. 1967;

Bonde-Petersen *et al.* 1975). Different tracers have been used. As the lipid cell membranes impose no barrier to ^{133}Xe, the tissue distribution of the tracer was improved by injecting the lipophilic ^{133}Xe instead of the hydrophilic ^{24}Na (Lassen *et al.* 1964). ^{133}Xe also maintains equilibrium between tissue and venous effluent better than ^{24}Na at high flow rates (Lassen *et al.* 1983), which is required by the wash-out technique. However, the method was found to be non-linear and to underestimate blood flow (Clausen & Lassen, 1971; Bonde-Petersen *et al.* 1975; Cerretelli *et al.* 1984; Rowell, 1993). As indicated in previous reviews (Lassen *et al.* 1983; Rowell, 1993), this underestimation could be due to the fact that the measurements were affected by: different solubility constants of muscle, connective tissue and fat; exchange of isotopes between arterioles and veins; dissociation of labelled moieties with diffusion limiting clearance; injection trauma producing local hyperaemia (most pronounced at low flow rates); site of injection in the muscle. Thus, one reason for the levelling off in peak muscle blood flow may be the different solubility of tracers in various muscle components. The partition coefficient in skeletal muscle is 0·7 ml/g, which is much lower than that in other tissues such as fat. The amount of adipose cells and connective tissue surrounding the vessels and separating the muscle cells may therefore reduce the wash-out rate and underestimate flow. Furthermore, correct values for blood flow at rest are hard to obtain due to shunting by diffusion and re-circulation (Tønnesen & Sejrsen, 1970; Lassen *et al.* 1983). Calculation of blood flow from the constant shallow slope of the wash-out curve may also underestimate blood flow by approximately 50 %. Furthermore, because of an initial injection artifact there must be an interval of approximately 10 min before measurements are performed. The type of mathematical analysis utilized to compute blood flow may also influence the results. Thus, in addition to the requirement for injection of a radioactive molecule, there are many theoretical limitations. However, estimates of relative changes in blood flow may be of value (Tønnesen & Sejrsen, 1970; Lassen *et al.* 1983), but the equipment is expensive and running costs are relatively high.

Microdialysis ethanol outflow : inflow

Principle. The technique is based on measurement of the transport of molecules across the semi-permeable membrane of a microdialysis fibre inserted into the muscle. By perfusing the fibre with an infusate containing ethanol (i.e. a tracer suggested to be metabolically inert in the muscle), and measuring the ethanol concentration in the outflowing dialysate, the loss of ethanol is suggested to be proportional to the local muscle blood flow surrounding the fibre (Hickner *et al.* 1991).

Practicalities. The microdialysis ethanol outflow : inflow value (ethanol$_{dialysate}$: ethanol$_{infusate}$) is therefore suggested to represent relative changes in local muscle blood flow (Hickner *et al.* 1991). As the dialysate concentration is dependent on the perfusion rate through the fibres, an accurate perfusion pump is needed. The microdialysis fibres are also very delicate and must be inserted carefully into the muscle. Some microdialysis fibres may also be damaged during exercise due to the contractions.

Also, the collected samples must be properly handled as ethanol easily evaporates.

The method has been used in attempts to estimate changes in local blood flow in skeletal muscle of animals and human subjects (Hickner *et al.* 1992, 1994*a*,*b*, 1995; Rosdahl *et al.* 1993; Wallgren *et al.* 1995). However, when the ethanol-removal technique was established it was specifically validated against ^{133}Xe clearance (Hickner *et al.* 1994*a*), which is known to be non-linear and to underestimate muscle blood flow. Furthermore, there was no investigation of whether the changes in ethanol removal during muscular contractions only represented changes in muscle blood flow, or whether it reflected other factors causing alterations in the molecular transport across the membrane. By determining the relative loss of labelled molecules across the membrane, it was recently found that arterial infusion of the vasodilator adenosine in the absence of muscle contractions did not change either the ethanol outflow : inflow value or the transport across the membrane (Rådegran *et al.* 1998). However, both factors were altered by muscle contractions (Rådegran *et al.* 1998). Thus, other factors may have a larger influence on the ethanol outflow : inflow than local blood flow.

Strength and weaknesses. In essence, microdialysis has evolved as an unique tool to measure local interstitial release of metabolites and vasodilators in relation to local blood flow. Although the technique is extensively used with pharmacological interventions, further studies are still needed to elucidate the different factors that may alter the transport across the membrane, as well as to define the direct sensitivity of the method in detecting changes in flow. Further investigation is also necessary to evaluate whether tracers other than ethanol may be used (Stallknecht *et al.* 1999). Moreover, although the equipment used is relatively inexpensive, the costs of the microdialysis probes for human use are high.

Near i.r. spectroscopy

Principle. In analogy with ICG regional blood flow measurements, where a photo-densitometer is used to detect the dye concentration, the NIRS–ICG method is based on detecting the light attenuation that is induced in the muscle during infusion of ICG (Boushel *et al.* 1997).

Practicalities. NIRS allows measurements to be made of concentration changes of oxyhaemoglobin, deoxyhaemoglobin and total haemoglobin in local muscle regions. Changes in these concentrations were previously used as a measure of local muscle blood flow (Edwards *et al.* 1993; De Blasi *et al.* 1994). However, as blood flow and delivery of O_2 to the muscle may be independent of the O_2 consumption the NIRS–ICG procedure is a better concept for estimating local blood flow. Muscle perfusion is determined using a combination of NIRS, venous infusion of ICG and arterial concentration measurements of ICG (Boushel *et al.* 1997). Following the venous infusion of ICG, the tracer is rapidly bound to plasma proteins, with albumin as the predominant carrier. The quantified values of the rapid component of ICG accumulation in the muscle are thus taken to represent capillary blood flow. This value is based on the fact that the early component is thought to

represent the period before blood containing ICG has reached the venous side, whereby only the arterial concentration is required as an input function for the calculation. The light-emitting and -receiving optodes are placed several mm apart. With an algorithm (Hamamatsu Photonics K.K., Hamamatsu, Japan) using the extinction coefficients for ICG at 775, 826, 859, and 910 nm, the change in ICG concentration is determined. Muscle blood flow (ml/min per 100 g) is then calculated according to the formula, blood flow $= k \times (ICG_{mu})(t)/_O\!\int^t (ICG_a)$ dt, where ICG_a (mg/l) is the integrated arterial ICG concentration and ICG_{mu} is the ICG accumulated in the muscle. The constant k includes the density of the muscle (1·04 g/ml) and the molecular mass of ICG.

Strength and weaknesses. The technique is unique, as it allows an estimate to be made of local muscle oxygenation in relation to local perfusion. However, further methodological work is needed to verify how the NIRS–ICG blood flow measurements correspond to regional arterial inflow and venous outflow. Although the NIRS technique is non-invasive, the method requires invasive ICG infusion and arterial blood sampling. Also, the equipment and ICG are relatively expensive.

Positron emission tomography

Principle. PET is based on imaging of biochemical processes *in vivo* after infusion of 'natural' substrates labelled with short-lived positron-emitting isotopes, and detection of γ rays resulting from the interactions between positrons and electrons (Raitakari, 1996).

Practicalities. Tracers such as ^{82}Rb, ^{13}NH$_3$, and H$_2$^{15}O have been used for blood flow measurements (Herscovitch *et al.* 1983; Raichle *et al.* 1983; Choi *et al.* 1993; Muzik *et al.* 1993; Raitakari, 1996; Raitakari *et al.* 1996). However, the ideal tracer for measurement of blood flow should be freely diffusable and inert with respect to changes in tissue metabolism (Raitakari, 1996), which is the case for H$_2$^{15}O. Furthermore, its half-life of approximately 2 min allows repetitive measurements. Rb, which is a K$^+$ analogue, is not ideal as it may be affected by substances that influence the membrane transport of K$^+$. ^{13}NH$_3$ is not appropriate in skeletal muscle because it may be dependent on the tissue metabolism.

The muscle blood flow (ml/min per 100 g) may be modelled according to the autoradiographic or the steady-state method (Raitakari, 1996). The autoradiographic method allows numerical calculation of flow according to the equation: $_0\!\int^T C(t)dt = {}_0\!\int^T BF_{PET} \times C_a(t) * \exp(-BF_{PET} \times t/p)dt$, where BF_{PET} is the blood flow. The righthand side of the equation is calculated from the decay-corrected arterial time *v.* radioactivity curve $C_a(t)$ for different flow levels, assuming a constant value for the partition coefficient (p), and * denotes the convolution integral (Raitakari, 1996). The lefthand side corresponds to the pixel-by-pixel solved integral of the decay-corrected tissue time *v.* radioactivity curve. T corresponds to the integration time. The steady-state method is based on continuous administration of H$_2$^{15}O, where the tissue radioactivity concentration reaches an equilibrium when the constant administration of radioactivity is in balance with the wash-out and tracer decay. Blood flow is calculated as $\lambda/((C_{a\text{-}avg}/C_{t\text{-}avg}) - (1/p))$, where λ denotes the decay constant of ^{15}O, p is the partition coefficient of water in muscle tissue, and $C_{a\text{-}avg}$ and $C_{t\text{-}avg}$ are the average radioactivity concentrations in blood and tissue respectively, during steady-state condition.

Strength and weaknesses. PET allows direct quantification of muscle blood flow without contamination from other tissue compartments. Furthermore, it is unique in allowing spatial and temporal quantification of the distribution of a label. The link between local metabolic activity and blood flow, as well as local blood flow distribution, may therefore be studied. However, PET requires injection of positron-emitting isotopes. The equipment is expensive and running costs are also extremely high. The potential for many researchers to use the technique is therefore limited. Moreover, the apparatus, imaging, and data retrieval procedure restrict the type of exercise mode that can be studied.

Laser Doppler

Principle. Laser Doppler is based on the same principle as ultrasound Doppler, but instead of ultrasonic waves, divergent (non-collimated) continuous-wave (non-pulsed) monochromatic light is used.

Practicalities. The laser light is applied via an optic fibre inserted through the subcutaneous tissue into the muscle. The fibre allows bi-directional travel of light, and thereby detection of the Doppler shift in the light scattered by the moving erythrocytes. The magnitude and frequency distribution of this Doppler shift is a collective property related to both the number and velocity of the erythrocytes. The Doppler shift is used as a relative estimate of changes in flow, expressed in arbitrary flux units. Fig. 4 shows the blood flow response to a bolus injection of adenosine.

Strength and weaknesses. Traditionally, laser Doppler has been used to estimate changes in skin blood flow (Holloway & Watkins, 1977). However, the technique could have potential for use in the study of relative changes in local muscle blood flow induced by pharmacological interventions. The sensitivity of the technique, with respect to the minimal vasodilatation and vasoconstriction that can be registered, still remains to be investigated. Whereas laser Doppler has already been used to estimate changes in human muscle blood flow during isometric contractions (Jensen *et al.* 1995), the technique needs to be evaluated during dynamic contractions. It is likely, however, that movement artifacts during exercise may be problematic. Furthermore, an absolute quantitative measure of blood flow will not be possible, as there are no means of estimating the vessel diameters from which the laser Doppler signal originate. The equipment is relatively expensive, but the running costs are low.

Discussion

Accurate measurements of blood flow are of particular importance both at rest and during exercise, since blood flow is used to estimate the substrate fluxes, energy turnover and metabolic rate of skeletal muscle. As long as the period
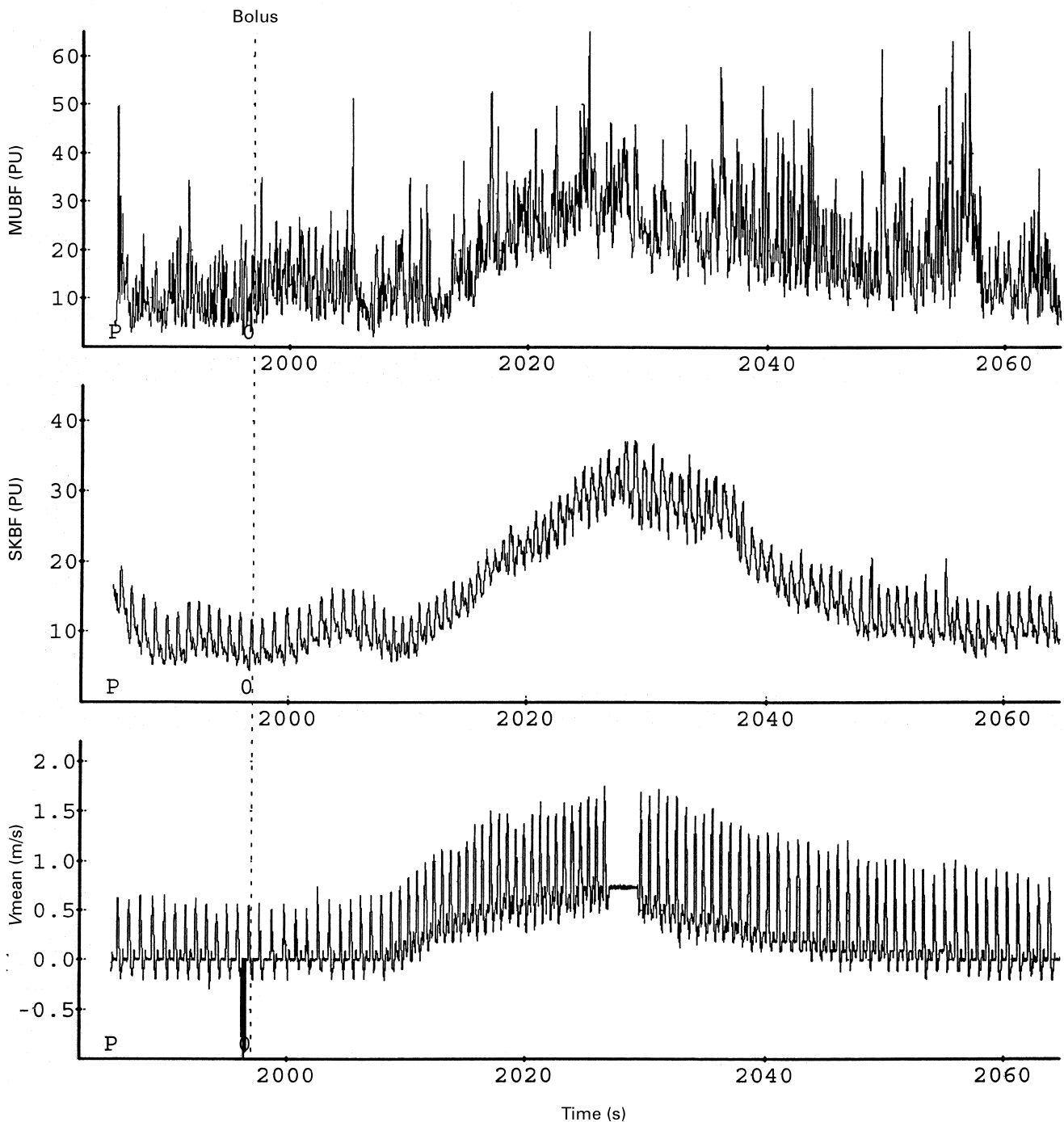
Fig. 4. Femoral artery blood velocity (*V*mean, representing arterial inflow; ultrasound Doppler), skin blood flow (SKBF; laser Doppler), and muscle blood flow (MUBF; laser Doppler) during a bolus injection (2·5 mg) of adenosine into the femoral artery of a representative human subject at rest (¦). Femoral artery blood flow, SKBF and MUBF transiently increased by a peak factor of approximately 11·5, 3·5 and 2·9 respectively. PU are arbitrary perfusion units, as measured using a PeriFlux 4001 laser Doppler (Perimed, Järfälla, Sweden) operating at a maximal emission of 780 nm.

of the blood-flow oscillations is short in relation to the time period of metabolic measurements, the mean value for blood flow is acceptable for the theoretical assumptions of steady-state. As skeletal muscle blood flow dramatically increases from rest to exercise, and since the flow markedly fluctuates with the intramuscular pressure variations during exercise, with the cardiac cycle and arterial blood pressure as a super-

imposed modulator, the method used must not only be sensitive, but also allow for measurement during both contraction and relaxation phases. In addition, as blood flow in recovery post exercise decreases as the metabolic influence ceases, and since blood flow during exercise is not only affected by metabolic vasodilatation but also by the effects of muscle mechanical factors, post-exercise

measurements are not representative of exercise. Moreover, measurements of arterial inflow to or venous outflow from a limb have generally been taken to reflect muscle blood flow. This assumption is reasonable when comparing changes in blood flow from rest to during muscle contraction. In order to minimize the contribution of non-active tissue compartments, exercise modes such as the knee-extensor model have been developed where the circulation to the lower leg is excluded by a cuff around the calf (Andersen & Saltin, 1985).

In addition to the technical limitations of each method, the focus of each metabolic study and the type of interventions performed determine which technique may be suitable. The electromagnetic flow meters are too invasive for use in healthy human subjects. Moreover, due to occlusion and motion artifacts, plethysmography is not suitable for measurements during exercise, but is limited to intermittent measurements at rest. The isotope-clearance method also has limited application for accurate measurements of skeletal muscle blood flow during exercise. The two best techniques for measurements at rest are ultrasound Doppler and continuous ICG dye infusion. In addition to the advantage of being non-invasive, ultrasound Doppler also has a better temporal resolution, which allows detection of normal physiological variations. Thus, transitional states, such as onset of and recovery from exercise, are also characterized best by continuous ultrasound Doppler measurements. However, the exercise mode must be such that the artery is in a fixed position. Ultrasound Doppler, thermodilution and ICG dye dilution are comparable options during steady-state submaximal exercise. However, thermodilution and ICG dye dilution are preferable at peak effort and during exercise with marked limb movements. Furthermore, thermodilution is slightly easier to use than ICG dye dilution, and is not affected by recirculation.

Thus, currently, ultrasound Doppler, thermodilution and ICG dye infusion seem to be the three best complementing techniques for regional blood-flow measurements in human subjects during various modes of exercise. Magnetic resonance velocity imaging offers the unique possibility of determining the blood flow in deep single vessels. For local blood-flow measurements in relation to metabolic activity, PET seems to be the most promising tool for the future.

Acknowledgements

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