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Blood flow dynamics in the limb conduit artery during dynamic knee extensor exercise assessed by continuous Doppler ultrasound measurements

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Abstract Exercising muscle blood flow (BF) may be an indicator of oxygen supply change allowing increased muscle energy metabolism through the circulatory response between central and peripheral hemodynamics. During exercise an increase in cardiac output may represent the interplay of alterations in both blood pressure and vascular conductance. Dynamic muscle contractions lead to an increase in cardiac output and promote venous return at the onset of exercise, and concurrently lead to enhanced muscle vasodilatation (and thus increased muscle BF) due to metabolites, neurological responses and/or other mechanisms, causing exercise hyperaemia. Doppler ultrasound can non-invasively detect with high resolution the temporal pulsatile blood velocity profiles in the conduit artery at rest as well as during muscle contractions. Based on this technique, it has been shown that alterations in the physiological blood velocity profile related to cardiac systole-diastole and fluctuations in the beat-by-beat blood velocity profile are due to rapid changes in the blood velocity profile concurrent with muscle contraction and/or relaxation during exercise (dynamic/static) or respiratory cycle, in different states (muscle contraction time/frequency or workload), or of any other type of vasodilatation/vasoconstriction. Muscle contraction-induced alterations in the blood velocity profile may be due in general to the magnitude of intramuscular pressure variation (mechanical factors) and the superimposed influence of perfusion pressure variation (pulsatile hemodynamic factors). This review therefore focuses on methodological considerations for muscle contraction-induced blood velocity/flow variability in the leg conduit artery, which in turn influences the magnitude of exercising BF during dynamic knee extensor exercise.

Keywords : leg blood flow, muscle contractions, dynamic knee extensor exercise, exercising blood flow fluctuations, Doppler ultrasound

Background

Non-invasive measurements using Doppler ultrasound with high temporal resolution can provide valuable information on the dynamics of major conduit arterial vessels such as magnitude of blood flow (BF), beat-by-beat blood velocity profiles and change over time in patients with intravascular diseases such as arteriosclerosis or endothelial dysfunction¹⁻³. Furthermore, the evaluation of peripheral BF feeding the limb muscles, not only at rest but also during exercise, could provide information on oxygen delivery related to active muscles useful in the prevention of cardiovascular disease through exercise training or therapy. During exercise, the increase in limb oxygen uptake (calculated as the product of exercising arterial BF and the arterio-venous oxygen difference to the exercising

limb) is directly proportional to the work performed in relation to the interplay between cardiovascular regulation and muscle energy metabolism. Furthermore, leg peak muscle oxygen uptake is closely related to the vessel diameter of the femoral artery, which may vary based on the needed oxygen supply of the dependent muscle mass⁴. Increased BF magnitude in the working muscle may play a key physiological role. Characterization of its alterations may give us unique insight of mechanisms of vasodilatation in working muscles; and its relation to changes in heart rate, stroke volume, blood pressure and exercising/non-exercising vascular conductance, based on various muscle contraction times (duration), frequency⁵ or muscle contraction workload⁶.

The above-mentioned physiological events are coordinated in a holistic manner via intrinsic regulation regarding endothelial-related factors, autacoid substances, metabolic hyperaemia⁷ and/or myogenic response⁸.

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Similarly, they are also adjusted by extrinsic regulation such as autonomic nerve activity, other signals/reflexes with central command⁹⁻¹¹⁾, or exercise pressor reflexes¹²⁾, with mechanical muscle contraction/accumulated metabolite products against metabolic vasodilatation. Therefore, determination of limb BF dynamics (potential oxygen supply) feeding the contracting muscles can contribute to the understanding of factors limiting work capacity, including, for instance, muscle metabolism, substance utilization and vasodilatation/vasoconstriction in exercising muscle via circulatory and/or neurological regulation.

Using Doppler ultrasound provides valid blood velocity/flow values in the forearm or lower limb conduit artery feeding the muscle group not only at rest but also during dynamic (isotonic) or static (isometric) muscle contractions such as with the exercise model of forearm handgrip, lower limb knee extensor or plantar flexion exercise. In general, muscle contraction-induced variability in the blood velocity may be largely influenced by the magnitude of intramuscular pressure variation and the superimposed influence of perfusion pressure variation. Therefore, we must consider methodologically how variability in the blood velocity influences the magnitude in exercising BF during incremental workloads. Regarding the hemodynamics in the leg conduit femoral artery during dynamic knee extensor exercise, this review presents concepts that clarify 1) Validation of BF during exercise, 2) Changes in BF due to incremental workloads at different muscle contraction frequencies, 3) Physiological variability/fluctuations in the magnitude of blood velocity/BF due to the muscle contraction and relaxation phases, and 4) Changes in BF due to spontaneous changes in workload at target intensity.

Exercise model of dynamic knee extensor

Previous studies reported exercising leg BF measured by the invasive thermodilution technique, in relation to

the one-legged, dynamic knee-extensor exercise model described by Andersen et al.^{13,14)}. This exercise model allows stable measurements of femoral arterial blood velocity using Doppler ultrasound, even though the usual walking/running models do not easily allow BF measurement in the leg conduit artery, due to the difficulty of fixing the scan probe to the hip joint^{15,16)}. Consequently, the workload is confined to the quadriceps muscle group, which may potentially indicate the activation of the large thigh muscle group (Fig. 1). Previous research on the determination of leg BF in relation to dynamic knee extensor has contributed to the investigation of the circulatory response relationship between central and peripheral hemodynamics^{13,14)}.

BF in the femoral artery measured by Doppler ultrasound

Comprehensive unilateral leg BF may be evaluated as the flow rate (ml per minute) of the pulsatile inflow in the femoral artery¹⁷⁾, assuming no anatomical malformation in leg circulation due to collateral vessels between the iliac and lower site in bifurcation of the femoral artery. The measurement site of the blood velocity and vessel diameter of the femoral artery was distal to the inguinal ligament, but above the bifurcation into the branches of the superficial and deep femoral arteries (Fig. 2). This location minimizes turbulence from the femoral bifurcation and the influence of blood velocity from the inguinal region. Furthermore, the changes of the vessel diameter of the conduit artery in the target location are mostly unaffected by muscle contractions and relaxations¹⁵⁾.

The data in this review were measured by a Doppler ultrasound instrument (Model CFM 800, Vingmed Sound, Horten, Norway) equipped with an annular phased array transducer (Vingmed Sound) probe (11.5-mm diameter) with an imaging frequency of 7.5 MHz and Doppler frequencies which varied between 4.0 and 6.0 MHz. Blood

Fig. 1 One-legged dynamic knee extensor exercise as a model of rhythmic thigh muscle contractions. Only the large thigh muscle group is active in forward kicking.

Target area for leg BF measurement

Fig. 2 Target area for leg BF measurement. The target area (circled) in the blood velocity/BF measurements was above the bifurcation to the superficial and deep femoral arteries of the common femoral artery (FA) in two-dimensional vascular images in longitudinal view. BF: blood flow, D: vessel diameter, v : blood velocity.

velocity was measured with a probe at the lowest possible insonation angle, always $< 60^\circ$. The scanning probe position was stable and the sample volume was precisely positioned in the center of the vessel and adjusted to cover the width of the vessel diameter. The angle-corrected, time and space-averaged and amplitude (signal intensity) weighted mean-blood velocity is defined herein as “mean blood velocity”. The BF in the femoral artery was calculated by multiplying the cross-sectional area [$\text{Area} = \pi \times (\text{vessel diameter}/2)^2$] and mean blood velocity¹⁵.

Assessment and validation of net-BF during exercise

The blood velocity profile in the conduit artery during exercise and/or recovery may change due to the type of muscle contraction (the exercise model likely static or dynamic), muscle contraction force/time/frequency (only once or repeated) or exercise time (onset, steady-state, or exhaustion level) influencing the workload (exercise intensity)¹⁸⁻²⁴. These factors may affect the temporal amplitude and/or magnitude of the blood velocity, and thus BF, during exercise or recovery; for instance, there may be a large difference in blood velocity and thus BF magnitude between sustained isometric muscle contraction and relaxation (post-contraction) (see Fig. 3 left side),

Fig. 3 Difference in temporal blood velocity (femoral artery) alterations due to muscle contraction and relaxation between static and dynamic knee extensor exercise.

There is a large difference in magnitude in blood velocity profile between isometric muscle contraction and relaxation (post-contraction) during isometric exercise. Blood velocity was restricted during 10 sec of continuous isometric muscle contraction, but increased rapidly at the onset of muscle relaxation (or recovery after the end of muscle contraction) (left side). Blood velocity shows a large fluctuation following 1 sec of dynamic muscle contraction-relaxation duty cycle (right side). The figure is drawn from our original unpublished data.

or between swift muscle contraction and relaxation in a duty cycle during dynamic muscle contractions (see Fig. 3 right side). Alternatively, there may be an enhancement of the muscle BF magnitude immediately after the end of muscle contraction/exercise (post contraction reactive hyperaemia) at a high rather than low workload.

As seen in Fig. 3, there was a large difference in magnitude/dynamics of consecutive beat-by-beat blood velocity profile between 10s-isometric muscle contraction and 10s-muscle relaxation (post-contraction). However, the blood velocity profile during the isometric muscle contractions showed a clear, consecutive cardiac beat-by-beat systolic and diastolic blood velocity profile due to the sustained muscle contractions with stable intramuscular pressure. Therefore, for evaluation of exercising BF, measurement of the dynamics in single/consecutive blood velocity profile values determined by cardiac beat-by-beat cycle(s) may be suitable physiologically to use in the calculation of BF that is dependent on the cardiac cycle during isometric muscle contraction or muscle relaxation. In regard to the dynamic muscle contractions for BF measurement, there was a large fluctuation in blood velocity profile likely representing the unclear cardiac systolic-diastolic profile in single beat due to swift dynamic muscle contractions and relaxations (Fig. 3 right side). Thus, the validation of temporal BF during repeated swift rhythmic and dynamic muscle contractions measured by Doppler ultrasound is required to compare the validity of previous assessment methods.

Several other techniques for muscle BF have previously been developed that enable estimates to be made of arterial inflow, venous outflow, and local BF within a muscle²⁵⁻²⁹. Leg BF has previously been measured using the thermodilution technique for dynamic knee-extensor exercise^{13,14}. However, this invasive technique has the limitation of not being able to detect the beat-by-beat blood velocity profile for temporal duration in real time. Whereas many of the techniques are impaired by different methodological limitations, the indicator thermodilution and the Doppler ultrasound methods have both been found to give repeatable measurements of the same magnitude during both rest and dynamic knee extensor exercise⁶. Furthermore, the thermodilution measurements obtained under similar experimental conditions by Andersen et al.^{13,14} are similar ($P < 0.02$, $r > 0.985$) to those obtained by Doppler ultrasound in Fig. 4. Thus, BF measured by Doppler ultrasound is valid not only at rest, but also during incremental one-legged dynamic knee extensor exercise^{6,15}.

Net-BF during incremental workload at different muscle contraction frequencies

BF increases linearly with an increase in workload at the rate of 60 contractions per minute (cpm), as reported by Rådegran¹⁵ (Fig. 4). This potentially indicates that the

increase in BF may depend upon an increase in workload, even if the duration of the muscle contraction interval is as short as 0.5 s for muscle contraction and 0.5 s for muscle relaxation. Furthermore, the high intramuscular pressure during muscle contraction may temporarily reduce blood velocity/BF, meaning the major extent of blood velocity/BF consequently occurs during the muscle relaxation phase^{15,30,31}. There have been other reports of muscle BF varying with different muscle contraction frequencies; for example, BF in the femoral artery was found to increase during incremental workload increases of 30, 60 and 90 cpm⁶) as shown in Fig. 5. In particular, there was no reduction of BF at the high contraction frequency of 90 cpm.

Meanwhile, the lower leg BF determined by strain gauge plethysmography during plantar flexion exercise increased, as contraction frequency increased, and reached a peak at 60-80 cpm with an exercise workload of one-third maximum voluntary contraction. However, at a high contraction frequency of 100 cpm, the lower leg BF decreased significantly compared to the value recorded at 60 cpm at the point of either 2 min exercise or exhaustion. The shorter duration of both muscle contraction and relaxation intervals during higher muscle contraction frequency may potentially restrict lower leg BF during plantar flexion, which may limit the flow perfusion to the working muscles³². This difference in BF response between knee extension and plantar flexion may be due to

Fig. 4 Validation of blood flow measured by non-invasive Doppler ultrasound from rest to dynamic knee extensor exercise.

There is a close correlation ($P < 0.02$, $r > 0.985$) in blood flow values during exercise between the thermodilution and Doppler ultrasound methods. The determination of blood flow was evaluated by samplings, through the muscle contraction-relaxation duty cycle and ECG cardiac beat-by-beat cycle. Figure adapted from Ref.¹⁵, reproduced with permission from The American Physiological Society.

Fig. 5 Influence on BF by muscle mechanical factors obtained by altering the muscle contraction frequency (for instance, 30, 60 and 90 cpm) during exercise at different workloads.

BF was positively linearly related to the workload at each contraction frequency. A positive linear correlation ($P = 0.08$, $r = 0.92$ at 30 cpm; $P < 0.05$, $r = 0.98$ at 60 cpm; and $P < 0.05$, $r = 0.97$ at 90 cpm) was observed between BF and the workload during exercise. Abbreviations are as follows: BF: blood flow, WL: workload, cpm: contractions per minute. Figure adapted from Ref.⁶⁾, reproduced with permission from The American Physiological Society.

the type of muscle contraction, as well as the size of muscle mass between the thigh muscle and lower leg muscles.

Physiological variability/fluctuations in the magnitude of blood velocity/BF due to muscle contraction and relaxation phases

Continuous recordings of hemodynamic parameters during repeated dynamic muscle contractions can clearly determine the magnitude of the physiological variability in blood velocity through the contraction-relaxation-induced variations in muscle force, and consequently the intramuscular pressure variations, along with the additional influence of blood pressure, as well as the tonic influence of the state of vasodilatation^{15,16,33)}. The high intramuscular pressure during muscle contractions may temporarily reduce or even reverse blood velocity, depending on the relationship between the intramuscular- and arterial blood pressure. The major extent of the blood velocity (resulting in BF) consequently occurs during the muscle relaxation phase^{16,31,34)}. Blood velocity fluctuates in relation to the state of vasodilatation and the muscle contraction-relaxation duty cycles, indicated by the oscillations in muscle

force (Fig. 6A).

The specific four variations in the relationship between the blood pressure curve and the state of muscle contraction and relaxation are indicated in Fig. 6A: the systolic phase during muscle contraction (CS), the diastolic phase during muscle contraction (CD), the systolic phase during muscle relaxation (RS), and the diastolic phase during muscle relaxation (RD). Formation of the blood velocity profile was influenced by intramuscular pressure as indicated by the muscle force curve, with the additional influence of blood pressure in relation to the systolic and diastolic phases. In particular, the magnitude of the tip point of blood velocity profile for a single muscle contraction or muscle relaxation with single systolic or diastolic phase was found to be closely related to the point in a peak systolic- or second peak-diastolic blood pressure curve throughout the muscle contraction and muscle relaxation phases as determined by muscle force curve and/or the electromyography signals (not indicated in figure) (see ↑ or ↓ in Fig. 6A). The magnitude of the BF value is plotted against the above-mentioned four variations in Fig. 6B. A strong positive linear correlation was found between BF and workload ($P < 0.01$; r value, 0.972-0.999) for both the systolic and diastolic phases during the muscle relaxation phase, but not during the muscle contraction phase (Fig. 6B). These results demonstrate that the majority of blood velocity/BF occurs during the muscle relaxation phase compared to muscle contraction phase. Moreover, BF variations/fluctuations may be influenced by the interplay between muscle contraction and cardiac cycles.

Muscle contraction-induced blood velocity/BF variability evaluated by sampling number of muscle contraction and relaxation duty cycle

As stated above, blood velocity/BF variation during steady-state exercise is predominantly related to oscillations in intra-muscular pressure due to muscle contraction and the influence of superimposed waves on the cardiac cycle and arterial perfusion pressure. During steady-state exercise with rhythmic muscle contractions at 60 cpm (0.5 s muscle contraction / 0.5 s relaxation), it was found that BF variability was smaller for 15 s ~ to ~ 60 s (longer periods) than shorter periods, such as 3 s, as determined by averaging net-BF measurements over time periods¹⁵⁾. This difference in BF variability may depend on the sampling duration, which may potentially include the various magnitudes in fragments of the exercising blood velocity profile (Fig. 6A). Thus, it was necessary to demonstrate that the methodological concept for measurement of steady-state net-BF value requires the determination whether or not the blood velocity variability induced by the muscle contraction-relaxation cycle (CRcycle) will be reduced by sampling further CRcycles (with longer sampling duration) and thus less variability of voluntary muscle contraction force, blood pressure and heart rate.

Fig. 6 Blood velocity profile in relation to muscle force, blood pressure and four variations of specific BF during exercise.

A. Continuous recording of blood velocity, blood pressure and muscle force during steady-state exercise at 20 W and 60 cpm. The four variations of the specific phase were indicated as CS: Systole during muscle contraction, CD: Diastole during muscle contraction, RS: Systole during muscle relaxation and RD: Diastole during muscle relaxation. In general, the blood velocity increased to its highest value at RS, and significantly decreased to its lowest value at CD. The blood velocity showed an intermediate value at CS and RD, respectively. The blood velocity curve was furthermore retrograde in the CD. The up (↑) and down (↓) arrows indicate the influence of the blood velocity, depending on the magnitude of, and temporal relation between, the muscle force curve ($\approx$ intramuscular pressure), and blood pressure (cardiac cycle) curve, respectively. During exercise, peak thigh muscle force was seen over or under the target workload. The difference (either higher or lower) in achieved workload versus target workload (shown with $\updownarrow$) may influence BF values in Fig. 10.

B. Both systolic and diastolic BF at rest and during muscle contraction or relaxation phase at 5–40 W and 60 cpm. There is a clear increase ($P < 0.01$) in BF with an increase of workload during the muscle relaxation phase. However, there is less change in BF among workloads during the muscle contraction phase. Abbreviations are as follows: Blood flow: BF, CONT: muscle contraction, RELAX: muscle relaxation, S: systole, D: diastole, W: watts, cpm: contractions per minute. Values are means $\pm$ SE. Figure adapted from Refs.^{31,33}, reproduced with permission from Edizioni Minerva Medica and The Physiological Society of Japan.

For investigation of BF variation regarding samplings for muscle contraction cycles, the magnitude of muscle contraction-relaxation-induced variability in the blood velocity between “consecutive muscle contraction cycles” during steady-state exercise has been examined at different workloads (10, 20, 30 and 40 watts) and muscle contraction frequencies (30 and 60 cpm). BF variability was determined for seven consecutive cycles (1-, 2-, 5-, 10-, 15-, 20- and 30-cycles) for cardiac beat-by-beat cycles (BBcycles) at rest, and for CRcycles during steady-state

exercise at 10, 20, 30 and 40 watts, and for 30 and 60 cpm, as illustrated by Fig. 7 (simultaneous recordings of hemodynamic parameters at 20 watts and 60 cpm). Determination of BF variability will potentially help us understand the difference in the evaluation of the BF value by muscle contraction and relaxation duty cycles between a single cycle and 30 cycles. Furthermore, it was determined that the sampling duration of the CRcycle had the smallest variability required to obtain accurate steady-state net-BF measurements during repeated rhythmic

Fig. 7 Analysis of blood velocity values for consecutive muscle contraction-relaxation cycles (20 W and 60 cpm). Blood velocity was measured at steady-state in relation to the muscle force curve for 1-, 2-, 5-, 10-, 15-, 20-, and 30-consecutive contraction-relaxation cycles (CRcycles). Measurements were guided by the force curve from the initial phase of activity which represents the initial contraction point ($\downarrow$: Start of measurement). The letters indicated in the blood velocity profile are expressed in Fig. 6A. W: watts, cpm: contractions per minute. Figure adapted from Ref.¹⁶, reproduced with permission from Wolters Kluwer Health.

muscle exercise.

Fig. 8 represents the coefficients of variation for BF variability for seven consecutive cycles. Single BBcycle-induced BF variability was almost 15% at rest. The BF variability at rest measured for 1- and 2-BBcycles (14%) were higher ($P < 0.05$) than for 30-BBcycles (9.7%), however the BF variability for 5-, 10-, 15-, 20- and 30-BBcycles was similar. At the same time, single CRcycle-induced BF variability had a range from 11.4-13.2% at 30 cpm and from 13.3-18.1% at 60 cpm between 10 and 40 watts. During exercise at both 30 and 60 cpm, the BF variability was significantly higher ($P < 0.05$) for 1- and 2- than for 30-CRcycles, although BF variability did not differ between the incremental workloads for either 30 or 60 cpm at each sampling for CRcycles. Consecutively, the longer 30-cycles measurements represented a stable condition with minimal BF variations (4.1-4.3% at 30 cpm and from 4.5-5.8% at 60 cpm) both at rest and during steady-state exercise. It was shown that the BF variability declined exponentially to a stable level from 1- to 30-BBcycles at rest, and from 1- to 30-CRcycles during exercise as shown in Fig. 8. For steady-state rhythmic muscle contractions in the present exercise model, the findings potentially indicate that BF variability is markedly reduced with a longer sampling measurement, at least 10 CRcycles, which may be appropriate for the determination of net-BF with less influence of BF fluctuations. Results with longer sampling measurement had coefficients of variation of approximately 5%¹⁶⁾ (see ↔ in Fig. 8). Furthermore, the single-CRcycle induced BF variability was similar ($P = ns$) among workloads (exercise intensities) in Figs. 8 and 9C, but significant variation was seen between contraction frequencies at lower workloads (below 15-CRcycles at 10 watts and below 5-CRcycles at 20 watts in Fig. 8).

The higher coefficients of variation in peak muscle force were generated at 60 cpm ($P < 0.05$, $r = 0.962$) rather than at 30 cpm ($P = ns$), which may imply a somewhat larger influence of peak intra-muscular pressure on BF variation (Fig. 9A). Moreover, the BF variability for CRcycle may potentially be more closely related ($P < 0.05$, $r = 0.986$) to mean muscle force (mean intra-muscular pressure) variation at higher contraction frequencies as 60 cpm compared to 30 cpm (Fig. 9B). Thus, a possible explanation for the higher BF variability in lower workloads at 60 cpm compared to 30 cpm may be the larger coefficients of variation in peak/mean muscle force, which in turn represent the intra-muscular pressure variation playing the dominant role in BF variation response with the blood pressure wave superimposed on top.

The above-mentioned methodological consideration of sampling numbers for CRcycles may be one acceptable procedure for the determination of valid net-BF values in such situations as rhythmic and dynamic muscle exercise. However, another exercise model such as intermittent muscle contractions (for instance, when the ratio between

Fig. 8 BF variability determined by consecutive muscle contraction-relaxation cycles.

The BF variability (coefficients of variation in BF) was determined in seven consecutive cycles (1-, 2-, 5-, 10-, 15-, 20- and 30-cycles) for cardiac beat-by-beat cycles (BBcycles) at rest, and for consecutive muscle contraction-relaxation cycles (CRcycles) during steady-state exercise at 10-40 W and for 30 and 60 cpm. BF variability declined exponentially to a stable level from 1- to 30-BBcycles at rest, and from 1- to 30-CRcycles during exercise. BF variability was markedly reduced with a longer sampling measurement of at least 10-CRcycles giving results within approximately 5% (see ↔). BF variability was higher ($\$P < 0.05$) at 60 cpm compared to 30 cpm in lower workloads (10 and 20 W) and compared across 30-BBcycles ($\#P < 0.05$) or 30-CRcycles ($*P < 0.05$ at 30 cpm, $\dagger P < 0.05$ at 60 cpm). Abbreviations are as follows: Blood flow: BF, W: watts, cpm: contractions per minute. Values are means $\pm$ SE. Figure adapted from Ref.¹⁶⁾, reproduced with permission from Wolters Kluwer Health.

Fig. 9 Factors for BF variability during exercise.

A/B. Relationship between BF variability and peak muscle force variability (A) as well as mean muscle force variability (B) determined for single-muscle contraction-relaxation cycle (1-CRcycle). BF variability was positively and linearly related to the peak muscle force variability ($P < 0.05$, $r = 0.962$) as well as mean muscle force variability ($P < 0.05$, $r = 0.986$) at 60 cpm, but not at 30 cpm.

C/D. Comparison in BF variability between 1-CRcycle and cardiac beat-by-beat cycles (1-BBcycle) at different workloads (C) and its relationship to the duration of a cycle (D) during exercise at 30 and 40 W. There was a significant ($\dagger P < 0.01$) difference in the BF variability between the 1-BBcycle and 1-CRcycle at 30 and 40 W, although BF variability was similar at each workload when determined for 1-CRcycle. BF variability determined for 1-BBcycle was significantly ($*P < 0.05$) higher at 40 W compared to 10 W. As BF variability was closely ($P < 0.01$) related to the duration of 1-BBcycle, but not 1-CRcycle, the duration of the 1-BBcycle may be assumed to influence the variation in BF. The variability was expressed as coefficients of variation. Abbreviations are as follows: Blood flow: BF, W: watts, cpm: contractions per minute. Values are means $\pm$ SE. Figure adapted from Refs.^{16,33}, reproduced with permission from Wolters Kluwer Health and Edizioni Minerva Medica.

muscle contraction and relaxation interval is not equal, or both intervals are long) may cause differences in the magnitude of muscle contraction-relaxation induced BF variability. Though the magnitude of BF variability may differ among exercise models, data regarding its variability and impact on assessment of steady-state exercising BF can be provided by the non-invasive Doppler method.

Comparison of BF variability between muscle contraction-relaxation duty cycle and cardiac beat-by-beat cycle during exercise

The (net-)blood velocity measurement determined by BBcycle(s) may be a physiologically suitable procedure to evaluate BF (BF in a beat or net-BF in multiple beats) in the non-muscle contraction or isometric muscle con-

traction state, since it potentially includes the BF variability in relation to fluctuations in the cardiac beat-by-beat cycle. Since the net-blood velocity value determined by CRcycle and BBcycle may not be similar visually as illustrated in relation to the blood pressure curve and muscle force curve during dynamic knee extensor exercise (see * ↔ in Fig. 6A), the comparison of BF variability

(the coefficients of variation) between each BBcycle and each CRcycle may provide more valid data for the determination of net-BF values during steady-state exercise at different workloads. Fig. 9C shows similar BF variability among workloads during dynamic muscle contractions at 60 cpm as seen in the determination of CRcycles³³). However, larger variations in BF with a range of 18-29% were

Fig. 10 Alterations in BF in relation to achieved workload during exercise.

A. Relationship between BF and the achieved workload during steady-state exercise at 30 or 60 cpm in one subject.

BF was inversely and linearly related ($P < 0.05$; r value, -0.545 to -0.281) to the actual achieved workload (60 samplings) at 30 and 60 cpm, respectively, for each target workload (solid line). Furthermore, the linear relationship was positive ($P < 0.0001$) between BF ($r = 0.752$ for 30 cpm; $r = 0.551$ for 60 cpm) and the target workloads (10-40 W for 30 cpm and 20-40 W for 60 cpm, dotted line).

B. Changes in BF related to fluctuations in the achieved workload around the target workload. A negative linear relationship ($P < 0.01$; r value, -0.962 to -0.769) was found between changes in BF (Δ BF) and variation in the achieved workload compared to mean (target) workload (Δ Workload) at 30 and 60 cpm, respectively. The basal BF value at the mean (or target) workload was measured as the average of the plotted BF values defined by the horizontal standard line at the BF value ("zero"). Abbreviations are as follows: Blood flow: BF, WL: workload, W: watts, cpm: contractions per minute. Figure adapted from Ref.³⁵, reproduced with permission from John Wiley & Sons Ltd.

observed with an increase in workload and a shorter duration of the 1-BBcycle (Figs. 9C and 9D). On the other hand, the time duration of the 1-CRcycle was constant (≈ 1 sec corresponding to 60 cpm) for the incremental workload, resulting in a smaller BF variability of approximately 15% as shown in Fig. 9C. In contrast, the BF variability for the BBcycle measurement may be closely related to the duration of the BBcycle (corresponding to heart rate) with the additional influence of variation in blood pressure and intramuscular pressure.

Changes in BF determined by muscle contraction and relaxation duty cycle due to spontaneous changes in workload

BF in the femoral artery during steady-state rhythmic dynamic knee extensor exercise increases linearly with incremental target workloads^{6,15,31,34}. This may imply that enhanced vasodilatation is elicited in relation to the increased average muscle force, exerted at a higher workload, to meet the elevated metabolic activity (Fig. 5). However, since these BF values are a mean of steady-state exercising BF measurements, temporary muscle contraction-induced BF variation may therefore be conveyed in the mean BF value.

For human voluntary exercise, it is of value to consider how variation in repeated muscle contractions at target workload (muscle force) directly influences exercising BF in the conduit artery (see $\uparrow$ in Fig. 6A). Therefore, we have investigated whether sudden physiological and spontaneous changes in workload temporarily alter BF to the working muscle³⁵. Fig. 10A shows that net-BF increased positively and linearly ($P < 0.0001$) with increasing target workload (dotted lines in the figure). However, BF at each CRcycle was inversely and linearly ($P < 0.05$) related to the actual achieved workload (solid line in Fig. 10A) when measured over bouts of 60 consecutive muscle contraction and relaxation duty cycles for each target workload at 30 and 60 cpm, respectively. Thus, any sudden spontaneous increase or decrease in the achieved workload transiently altered the relationship between BF and the achieved workload. A higher achieved workload (a higher muscular force) reduced BF, while a lower achieved workload (a lower muscular force) increased BF. At both 30 and 60 cpm, changes in BF (BF at achieved workload minus BF at target workload) were within 0.6 and -0.6 l/min from baseline at the target workload in all sessions. Furthermore, changes in BF ranged between 0.2 and -0.2 l/min from baseline when the achieved workload was increased or decreased by ~ 2 W.

It may be concluded that the influence on the magnitude of changes in BF due to changes in workload (achieved workload minus target workload) was similar (a negative linear correlation, more similarity with the slope in the regression line at 60 cpm than at 30 cpm) at target workload sessions of 30 and 60 cpm, respectively (solid lines

in Fig. 10B). This may indicate that during steady-state repeated rhythmic exercise a sudden transient increase in the workload during rhythmic muscle contractions more rapidly impedes BF, and that vasodilatation resulting from intrinsic and extrinsic controls may be comprehensively elicited to restore the intensity-related steady-state BF response, in relation to the average metabolic activity representing target workload (Figs. 5 and 10).

Summary

This review focuses on the methodological process for determining BF in the femoral artery during rhythmic muscle exercise using non-invasive measurement with Doppler ultrasound. The BF dynamics in relation to working muscles measured by Doppler ultrasound are frequently used for advanced research areas of study, including muscle pumping and substance-induced vasodilatation/vasoconstriction in contracting muscles with coordination of circulatory and neurological response, among others, which may be intricately related to an increase in the contracting muscle BF. Primarily, the capability to detect blood velocity/vessel diameter with high temporal resolution in real time during exercise, as well as at rest, is an advantage for the investigation of circulatory regulation, as is the determination of an indicator for oxygen transport to the working muscles. It was considered that evaluation of BF during muscle contractions likely to cause exercise hyperaemia, as measured by Doppler ultrasound, may potentially include internal factors representing a physiological difference in the blood velocity profile between cardiac systole and diastole in the muscle contraction-relaxation duty cycle. Moreover, external factors representing repeated voluntary muscle contraction force may also influence the magnitude of BF fluctuation due to spontaneous changes in muscle contraction workload. The content in this review has partially been discussed in our previous review articles^{36,37}.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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