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14

Determination of Limb Hemodynamics During Rhythmical Muscle Contractions Assessed by Doppler Ultrasound

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1. Introduction

Ultrasound Doppler instruments may provide valuable measurements of hemodynamic changes with high temporal resolution. In the clinical setting, Doppler velocity profiles may be useful for the evaluation of cardiac function, valvular disease and major conduit vascular disease. Furthermore, arterial stiffness and limb conduit arterial vessel dilatation following cuff-ischemia as measured by 2-dimentional echo imaging and Doppler ultrasound method are useful as surrogate parameters in atherosclerosis and hypertension.

Lifestyle related diseases such as obesity, hypertension, hyperlipidemia, and diabetes mellitus may be prevented by physical activity. Furthermore, a decreased amount of physical fitness or long term bed rest may lead to reduction of exercise tolerance (maximum oxygen consumption) due to cardiovascular-, respiratory- and muscle-dysfunction.

Circulatory changes following physical activity may also be used for studying cardiovascular regulation. For instance, cardiac output increases with increasing exercise intensity along with enhanced skeletal muscle vasodilatation and muscle pumping in the exercising muscle. Perfusion in the active muscle is furthermore one indicator of oxygen delivery to the muscles. Therefore, non-invasive Doppler measurements of blood velocity and flow in the feeding conduit arteries to working skeletal muscle may give us valuable information of the hemodynamic response in peripheral upper or lower limbs.

2. Exercise model

Determinations of blood flow to contractile muscles are the most important focus of the present chapter. Precise and stable measurements in conduit arteries assessed by Doppler ultrasound may be performed during exercise. Whole body exercise methods such as

walking and running on a treadmill would be even better for the investigation of exercising leg blood flow. However, methods do not easily allow measurement of upper- and lower-limb blood flow using Doppler ultrasound in these models as motion artifacts are present. There is also difficulty in fixing the ultrasound Doppler probe. Local lower limb muscle blood flow may be measured using the one-legged, dynamic knee-extensor exercise model described by Andersen and Saltin (1985). This exercise model allows stable measurements of femoral arterial blood velocity using Doppler ultrasound (Figure 1). Therefore, all hemodynamic data described in this chapter are from dynamic knee-extension exercise.
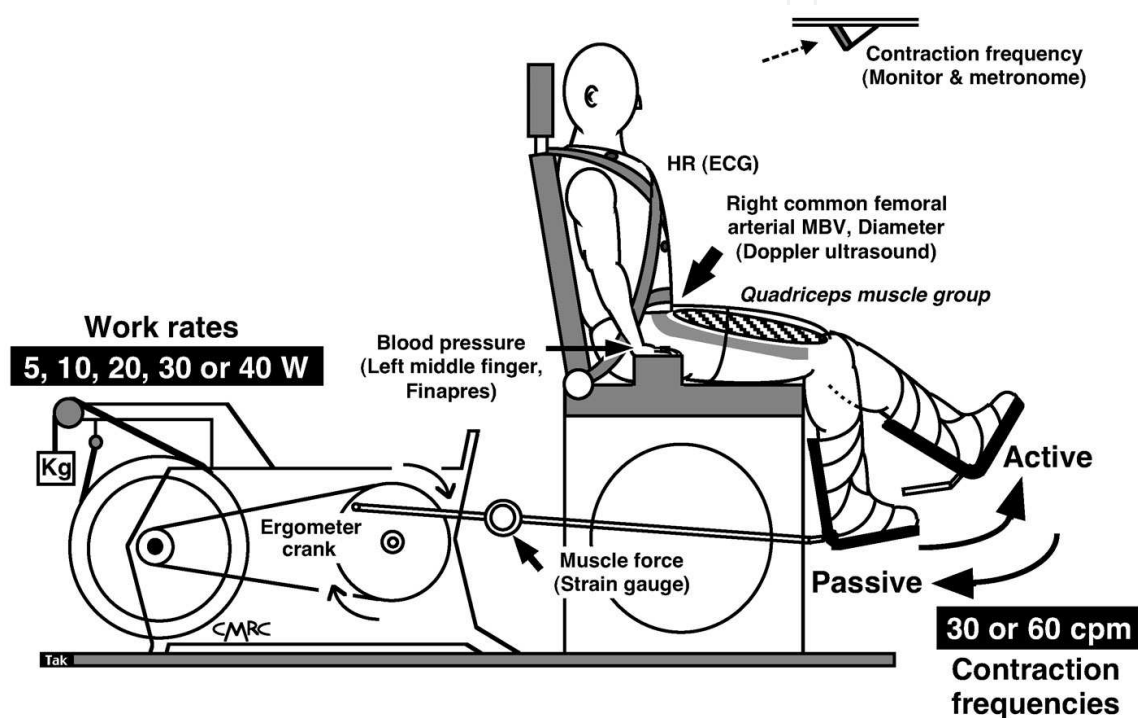

Fig. 1. Rhythmic thigh muscle contractions (one-legged dynamic knee extensor) model. Modified figure, reprinted with permission from Osada, T. & Rådegran, G. (2005). *Japanese Journal of Physiology,* Vol. 55, No. 1, pp. 19-28. by The Physiological Society of Japan.

3. Participants

All healthy male volunteers were familiarized with the above mentioned one-legged, dynamic knee-extensor exercise model before starting the experiments (Andersen et al., 1985; Andersen & Saltin, 1985). They trained at 30 and 60 contractions per minute at external target work rates of 10, 20, 30 and 40 W. Their hamstring muscles were allowed to fully relax, so that only the knee extensor musles performed the work. The subject's thigh was positioned horizontally with the knee joint bent. The lower leg moved up to ~ 60° angle from the bent knee joint. All subjects provided written informed consent to participate in the study, which was approved by the Ethical Committees of Copenhagen and Frederiksberg (KF-01-013/96). The study adhered to the principles of the Helsinki Declaration.

4. Hemodynamic measurements

4.1 Doppler instrumentation

The measurements were performed using 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). The imaging frequency was 7.5 MHz and the Doppler frequencies varied between 4.0 and 6.0 MHz (high-pulsed repetition frequency mode, 4 - 36 kHz). Blood velocity was measured with the probe at the lowest possible insonation angle and always < 60° (Gill, 1985). The mean value of the insonation angle was ~ 50°, which remained constant throughout the experiments for each individual. The probe position was stable and the sample volume was precisely positioned in the center of the vessel and adjusted to cover the diameter width of the vessel.

4.2 Measurement of blood velocity, vessel diameter and blood flow in femoral artery

The measurements of blood velocity and blood flow in the femoral artery using Doppler ultrasound has previously been validated and shown to produce accurate absolute values both at rest and during leg exercise such as rhythmical thigh muscle contractions (Hughson et al., 1997; Osada, 2004; Rådegran, 1997; Shoemaker et al., 1994; Walløe & Wesche, 1988). Compared with thermodilution, the high temporal resolution of Doppler ultrasound additionally enables continuous measurement of blood velocity throughout the kicking cycle during exercise (Osada & Rådegran, 2002; Rådegran, 1997; Rådegran & Saltin, 1998; Roberg et al., 1997; Shoemaker et al., 1994; Walløe & Wesche, 1988).

The angle-corrected, time and space-averaged, and amplitude-weighted mean (V_{mean}) and maximum (V_{max}; outer envelope) blood velocities, respectively, were measured. V_{mean} was defined by averaging the mean blood velocity trace (Osada & Rådegran, 2002; Rådegran, 1997). V_{max} was defined as the maximum outer envelope (Leyk et al., 1992; Osada et al., 1999; Osada et al., 2003). The V_{max} obtained in the present study was expressed as the blood velocity measured at the center of the vessel. Each blood velocity parameter was measured in relation to the blood pressure curve. The site of blood velocity and vessel diameter measurements in the femoral artery was distal to the inguinal ligament but above the bifurcation into the branch of the superficial and deep femoral artery. This location minimizes turbulence from the femoral bifurcation and the influence of blood flow from the inguinal region. In addition, the arterial diameter is not affected by the contractions and relaxations at this site located proximal to the muscle.

The blood velocity measurements were performed for approximately 2 to 3 min, when steady-state had been reached after 3 min of one-legged, dynamic knee extensor (Osada, 2004; Rådegran & Saltin, 1998), as previously described (Rådegran, 1997). The systolic and diastolic diameters of the femoral artery were measured on a monitor relative to the ECG at rest. The mean vessel diameter was calculated in relation to the temporal duration of the blood pressure curve as; [(systolic vessel diameter value × 1/3) + (diastolic vessel diameter value × 2/3)] (Rådegran, 1997). The diameters were measured under perpendicular insonation at rest before exercise. The value of the vessel diameter at rest (pre-exercise) was used to calculate femoral arterial blood flow during rest and during one-legged, dynamic knee extensor, since the diameter does not significantly vary between rest and steady-state exercise (Hughson et al., 1997; Isnard et al., 1996; Leyk et al.,

1992; MacDonald et al., 1998; Osada et al., 1999; Rådegran, 1997). Steady-state leg blood flow was calculated by multiplying the cross-sectional area [Area = $\pi \times$ (vessel diameter/2)^2] of the femoral artery, with the angle corrected, time and space-averaged, and amplitude (signal intensity) weighted mean-blood velocity, where blood flow = mean-blood velocity $\times$ cross sectional area.

5. Validation of blood flow during incremental one-legged, dynamic knee-extensor as measured by Doppler ultrasound

In previous reports, central and peripheral hemodynamic measurements have been demonstrated using the thermodilution technique for cardiac output and limb blood flow during incremental cycling ergometer exercise. However, this invasive technique has the limitation of poor time resolution of blood flow. Several techniques have previously been developed that enable estimates to be made of arterial inflow, venous outflow, and local blood flow within a muscle. Whereas many of the techniques are impaired by different methodological limitations, the indicator methods and the ultrasound Doppler method have both been found to give repeatable measurements of the same magnitude during both rest and dynamic knee extensor exercise (Rådegran, 1997).

The Doppler ultrasound is unique as it allows continuous blood flow measurements non-invasively with a high temporal resolution. With continuous measurements, transitional changes in blood flow can be characterized (Walløe & Wesche, 1988; Wesche, 1986). The technique's precision and accuracy have furthermore been improved by sampling of the blood velocity continuously during dynamic knee extensor exercise (Rådegran, 1997). Doppler ultrasound may therefore be suitable for the investigation of transitional changes of limb hemodynamics in the conduit brachial, femoral and poplitial artery during rest and upper forearm or lower limb exercise.

Temporal blood flow changes due to muscle contractions during dynamic knee extensor exercise have been well described (Osada, 2004). Doppler ultrasound has furthermore been used to examine the hyperaemic response at the onset of exercise (Hughson et al., 1993; Rådegran & Saltin, 1998), steady-state (Osada & Rådegran, 2002) and recovery after exercise (Osada et al., 2003). At the onset of exercise as well as recovery after exercise, variations in blood flow may be influenced by changes in blood pressure, heart rate or strength of muscle contractions.

Furthermore, femoral arterial blood velocity and blood flow increases linearly with incremental target exercise intensities of work rate (workload) during steady-state rhythmic thigh muscle contractions. This implies that an enhanced vasodilatation is elicited, in relation to the increased average muscle force exerted at higher workloads, to meet the elevated metabolic activity.

Figure 2 shows the relationship between limb femoral arterial blood flow and target workload (10, 20, 30 and 40 W) in relation to rhythmic thigh muscle contractions at 60 contractions per minute. In addition, thermodilution blood flow measurements obtained under similar experimental conditions by Andersen and Saltin (1985) are closely related to those obtained by Doppler ultrasound. Thus, blood flow measured by Doppler ultrasound is valid not only at rest but also during incremental one-legged dynamic knee extensor exercise.
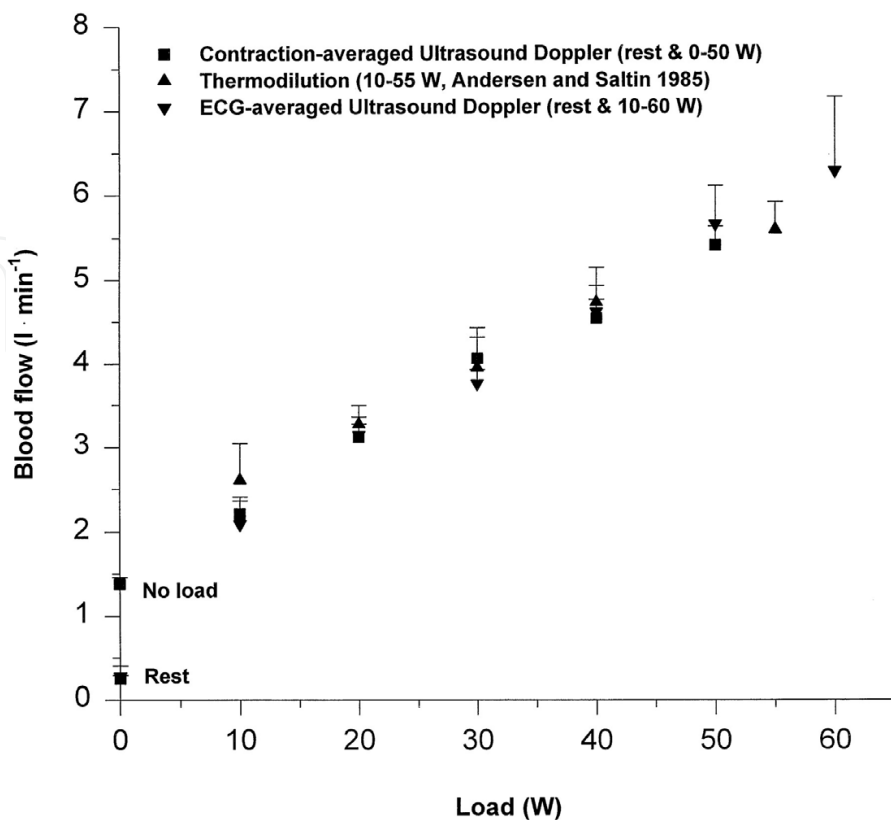
Fig. 2. Blood flow during incremental one-legged dynamic knee extensor exercise, estimated from mean blood velocity and averaged on a beat-by-beat basis or continuously measured and analyzed in relation to muscle contraction cycle. Reprinted with permission from Rådegran, G. (1997), *Journal of Applied Physiology*, Vol. 83, No. 4, pp. 1383-1388, by The American Physiological Society.

6. Variation of blood velocity and blood flow modified by muscle contraction-relaxation cycles

It is well known that conduit arterial blood velocity and blood flow is markedly influenced by intramuscular pressure, as well as the superimposed influence of the arterial blood pressure. The high intramuscular pressure during muscle contractions may consequently temporarily reduce or even reverse the blood velocity, depending on the relationship between the intramuscular- and arterial blood pressure. The major extent of the blood velocity and flow consequently occurs during the muscle relaxation phase. Figure 3 shows that mean blood velocity increased to its highest value at the systolic blood pressure phase during muscle relaxation, and significantly decreased to its lowest value at the diastolic blood pressure phase during muscle contraction. Mean blood velocity showed an intermediate value at the systolic blood pressure phase during muscle contraction and at the diastolic blood pressure phase during muscle relaxation, respectively. The blood velocity curve was furthermore retrograde in the diastolic blood pressure phase during muscle contraction. The figure below shows the contribution to the magnitude of the physiological variability in blood velocity and flow by the contraction-relaxation-induced variations in

muscle force, and consequently the intramuscular pressure variations, along with the superimposed influence of the blood pressure as well as the tonic influence of the state of vasodilatation. Moreover, the exercise blood velocity and blood flow fluctuations due to muscle contractions and relaxations, with the superimposed influence of the cardiac cycle, are described in Figure 4.
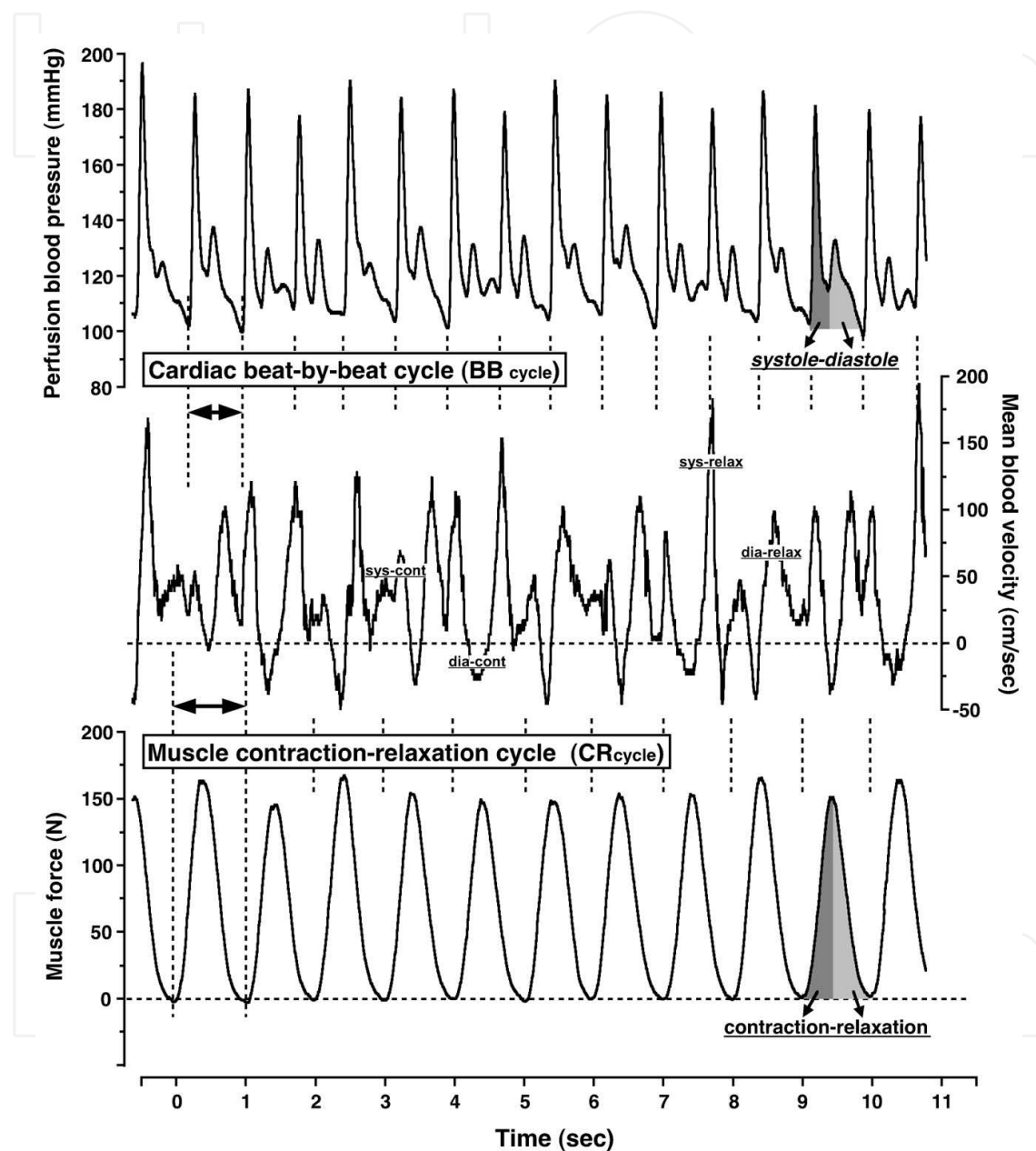

Fig. 3. Continuous recording of mean blood velocity, blood pressure and muscle force during steady-state one-legged dynamic knee extensor at 20 W and 60 contractions per minute in one subject: sys, systole; dia, diastole; cont, muscle contraction; relax, muscle relaxation. Modified figure, reprinted with permission from Osada, T. & Rådegran, G. (2006). *Journal of Sports Medicine and Physical Fitness*, Vol. 46 No. 4, pp. 590-597, by Edizioni Minerva Medica.
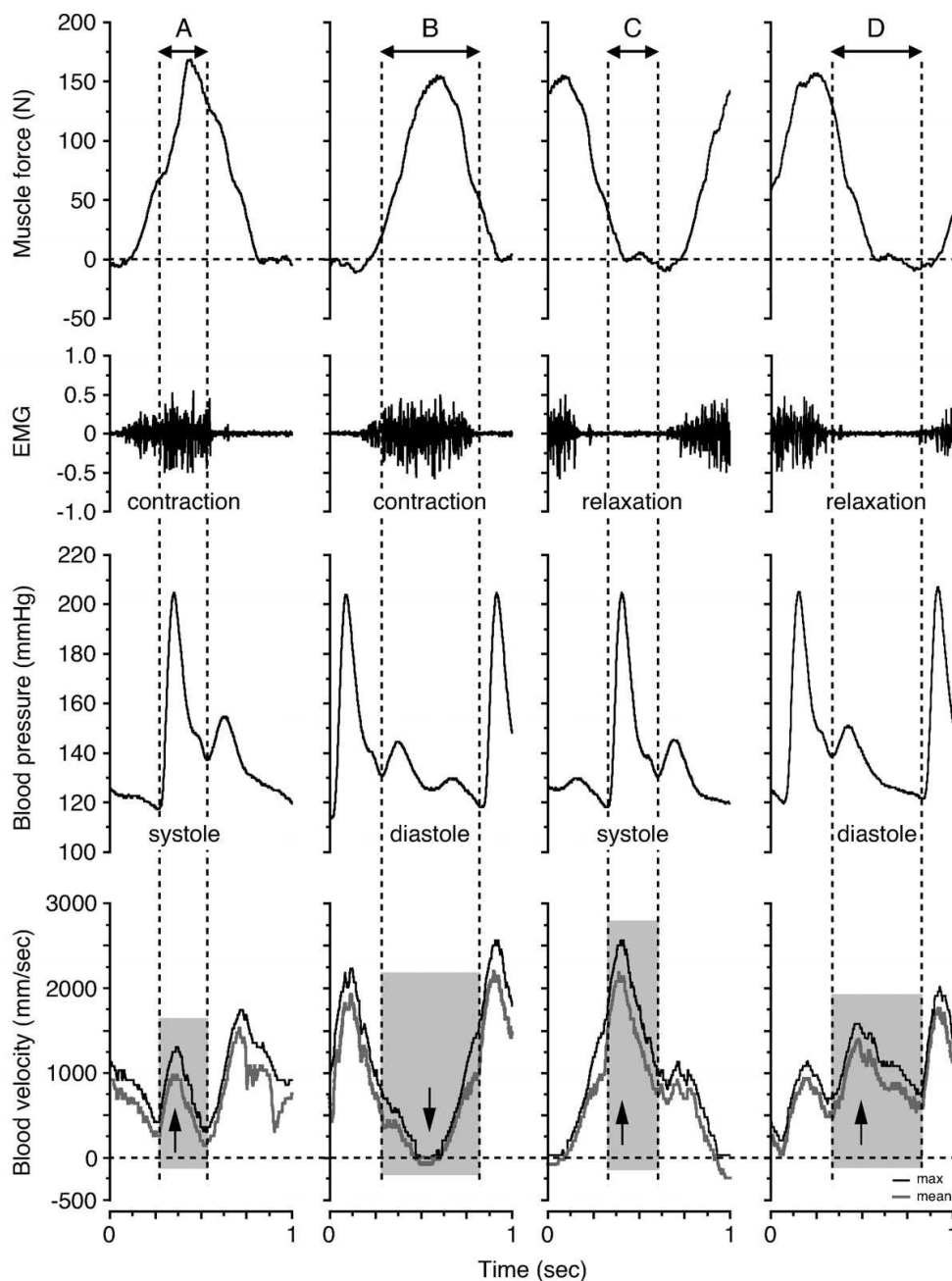
Fig. 4. Flow profile in the blood velocity (V_{max} and V_{mean}) in the limb femoral artery for the cardiosystolic and cardiodiastolic phases during the muscle contraction and muscle relaxation phases of one-legged dynamic knee-extensor exercise at 20 W and 60 contractions per minute. Reprinted with permission from Osada, T. & Rådegran, G. (2006), *Journal of Physiological Science*, Vol. 56, No. 3, pp. 195-203, by The Physiological Society of Japan.

As seen in Figure 4, blood velocities (V_{max} and V_{mean}) during the cardiosystolic and cardiodiastolic phases were measured continuously in parallel with the blood pressure curve during the muscle contraction and muscle relaxation phases determined from the electromyography (EMG) and the muscle force curve. Blood velocity fluctuated in relation to the state of vasodilatation and the muscle contraction-relaxation duty cycles, indicated by the oscillations in muscle force.

Four variations (A–D) in the coupling between the blood pressure curve and the state of contraction and relaxation were indicated; (A) the cardiosystolic phase during muscle contraction, (B) the cardiodiastolic phase during muscle contraction, (C) the cardiosystolic phase during muscle relaxation, and (D) the cardiodiastolic phase during muscle relaxation. V_{max} and V_{mean} were determined as the "average" transient maximum (outer envelope) and mean (amplitude-weighted, time-and-spatial averaged) angle-corrected blood velocity values, respectively, for the cardiosystolic and cardiodiastolic phases. The formation of the blood velocity profile and flow was modified by the intramuscular pressure, as indicated by the muscle force curve, and the superimposed influence of the blood pressure in relation to the cardiosystolic and cardiodiastolic phases. The arrow down ($\downarrow$) and up ($\uparrow$) indicates the influence on the blood velocity and flow, depending on the magnitude of, and temporal relation between, the intramuscular pressure and the arterial blood pressure, respectively.
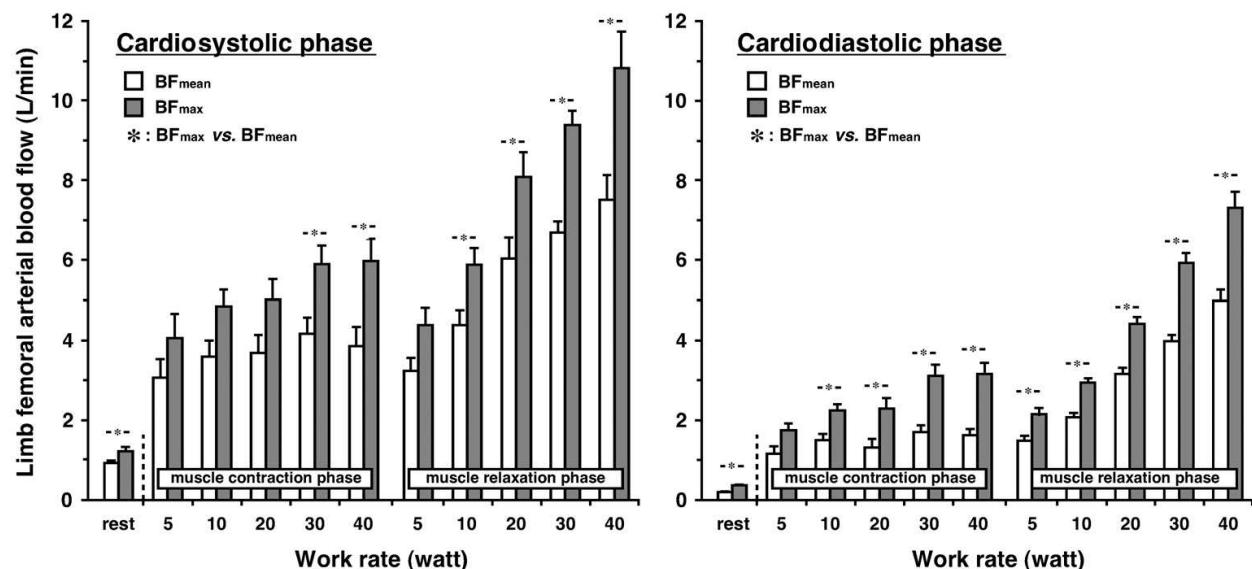

Fig. 5. Blood flow for the cardiosystolic and cardiodiastolic phases during the muscle contraction and muscle relaxation phases of one-legged dynamic knee-extensor exercise at 60 contractions per minute, *$P < 0.05$. Reprinted with permission from Osada, T. & Rådegran, G. (2006), *Journal of Physiological Science*, Vol. 56, No. 3, pp. 195-203, by The Physiological Society of Japan.

There is furthermore information available regarding differences in blood flow variations induced by muscle contractions and muscle relaxations at incremental exercise intensities at 5, 10, 20, 30 and 40 W (Fig. 5). These data clearly demonstrated that blood flow in the femoral artery increases during muscle relaxation, rather than muscle contraction. Also, there is a large difference in blood flow magnitude between cardiosystole and cardiodiastole at incremental exercise intensities. In consequence, the average exercise blood flow value may be over- or under-estimated if the time for measurement is restricted to only a single phase among the muscle contraction and muscle relaxation phases and the cardiosystolic and cardiodiastolic phases. This evidence of blood flow response due to muscle mechanical factor and cardiac pumping cycle is a clear physiological phenomenon, but the blood flow response in relation to the variations in the force of muscle contractions ($\approx$ intramuscular pressure, workload) should be further clarified.

Of further interest is whether sudden changes in workload and rhythm may exert influence upon the magnitude of blood flow. Such information may be useful for the temporal evaluation of exercising blood flow during consecutive rhythmic muscle contractions with large variation due to poor exercise performance.

7. Changes in blood flow due to spontaneous changes of workload

Limb femoral arterial blood flow during steady-state rhythmic thigh muscle contractions increases linearly with incremental target workloads (work rates) (Hughson et al., 1996; 1997; Osada & Rådegran, 2002; Rådegran, 1997; Shoemaker et al., 1994; Tschakovsky et al., 2006). This implies that enhanced vasodilatation is elicited in relation to the increased average muscle force, exerted at higher workloads, to meet the elevated metabolic activity (Fig. 2). However, these blood flow values are a mean of steady-state exercising blood flow measurements, and temporary muscle contraction-induced blood flow variations may therefore be conveyed in the mean average blood flow value (Fig. 2, 4 and 5). For human voluntary exercise, it is of value to consider how variations in repeated muscle contractions at target muscle strength (muscle force) directly influence exercise blood flow in conduit arteries. Therefore, we've investigated whether sudden physiological and spontaneous changes in exercise rhythm, and consequently workload, temporarily alter blood flow to the working muscle.
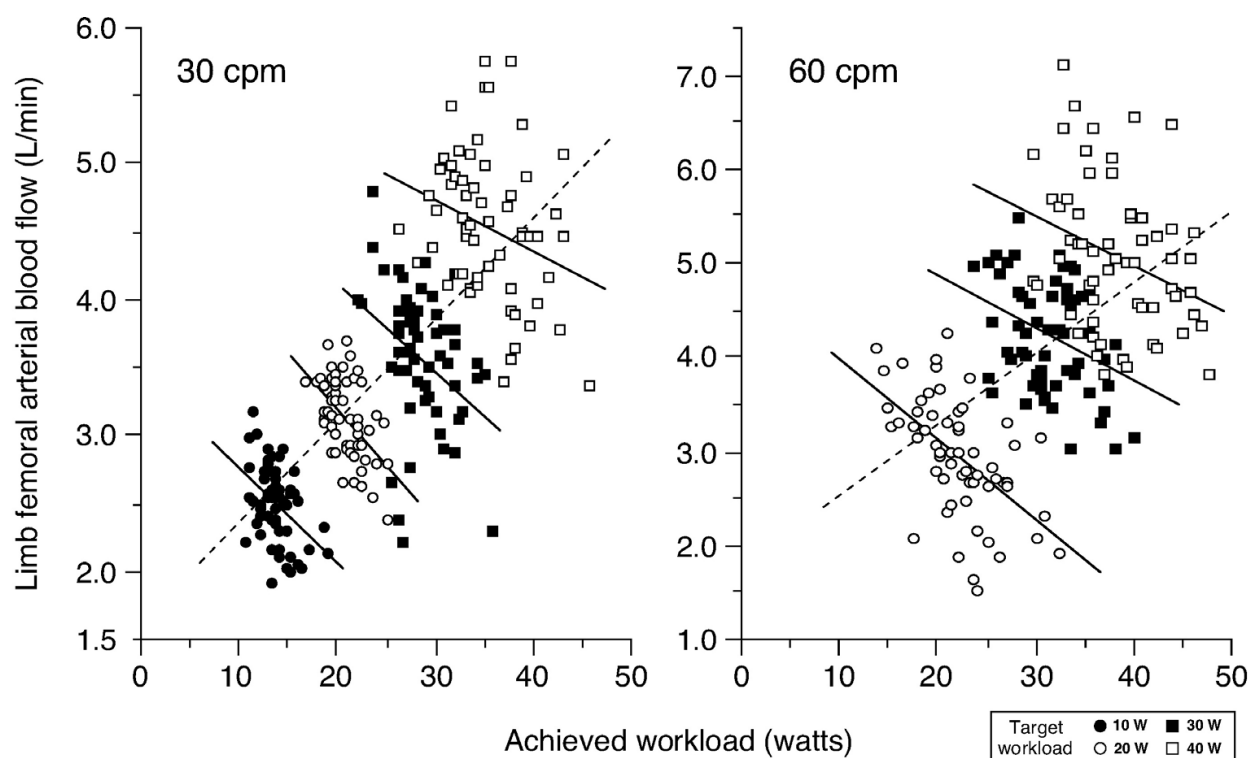

Fig. 6. Relationship between steady-state limb femoral arterial blood flow and the achieved workload during incremental one-legged dynamic knee-extensor exercise at 30 or 60 contractions per minute during a minute measurement in one subject (60 samplings at each workload). Modified figure, reprinted with permission from Osada, T. & Rådegran, G. (2009), *Clinical Physiology and Functional Imaging*, Vol. 29, No. 4, pp. 277-292, by John Wiley & Sons Ltd.

The results showed that limb femoral arterial blood flow increased positively and linearly (dotted line) with increasing target workload. However, limb blood flow was inversely and linearly related (solid line) to the actually achieved workload, when measured over 60 consecutive contraction-relaxation cycle bouts for each target intensity at 30 and 60 contractions per minute, respectively (Figure 6).

Thus any sudden spontaneous increase or decrease in the achieved workload transiently altered the relationship between limb femoral arterial blood flow and the achieved workload. The influence upon the magnitude of limb femoral arterial blood flow, due to fluctuations in the achieved workload from the target workload was similar at target workload sessions of 30 and 60 contractions per minute, respectively. These findings indicate that a transient sudden increase in the workload during rhythmic muscle contractions more rapidly impedes limb femoral arterial blood flow, than that vasodilatation may be elicited to restore the intensity related steady-state limb blood flow response, in relation to the average metabolic activity. This evidence in human applied science may contribute to the evaluation of exercise hemodynamics for rhythmic, dynamic-isotonic exercise training, leading to exercise prescriptions (muscle contraction frequency or muscle contraction intensity) for healthy participants as well as for patients, requiring additional physical activity in the rehabilitation or clinical setting.

8. Conclusion

Measuring exercise blood velocity and blood flow in working skeletal muscle assessed by Doppler ultrasound can be performed, however there are still limitations with regards to the exercise model, target vessel, muscle contraction-intensity and –frequencies, in upper or lower limbs.

9. Acknowledgment

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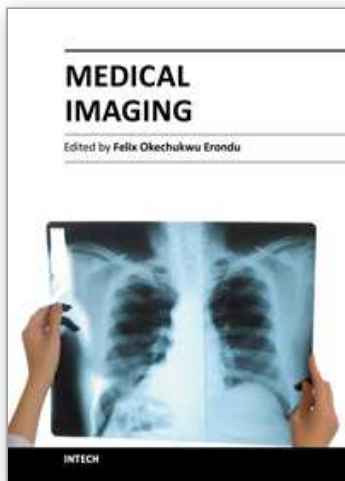
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Edited by Dr. Okechukwu Felix Erondu

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