BLOOD FLOW KINETICS AT THE ONSET OF MODERATE EXERCISE IN THE ELDERLY

by

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Abstract. The age-related effects on the time course of the rapid increase in blood flow at the onset of exercise are largely unknown. In addition, whether this time course is altered with training in older people is also unclear. It appears that this dearth of information was partly due to the lack of non-invasive methods of measurements that could present high temporal resolution and could be used during the exercise. Another difficulty that might also have contributed to this lack of information was that the mechanisms involved in blood flow kinetics regulation in humans are not well understood. It is also not clear how these mechanisms are affected by age or physical training. In order to address the questions about whether blood flow kinetics were altered by aging or physical activity, three studies were developed. In all three studies, forearm mean blood velocity and mean arterial perfusion pressure were measured on a beat-by-beat basis, using pulsed Doppler ultrasound and a plethysmographic finger cuff, respectively. This allowed calculation of forearm vascular conductance also on a beat-by-beat basis. The diameter of the brachial artery was measured at several points in time and during exercise. Since the exercise mean diameter was not different from the resting values, it was assumed that mean blood velocity kinetics represented the blood flow kinetics.

The results showed that forearm blood flow and vascular conductance kinetics were not impaired with age. Regarding the effects of increased physical activity on blood flow kinetics, the results were more difficult to interpret. While a longitudinal study showed improved blood flow and vascular conductance kinetics in response to a training protocol, a cross-sectional study showed only a tendency for 20% faster kinetics ($p>0.05$), in the very active older people compared to their less active counterparts. Since the kinetics values presented a large variability with overlap between the groups, it is possible that the small sample size might have

compromised significant differences.

The findings of the three studies are confined to dynamic contractions of a small muscle mass with moderate workload.

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Dedication

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"High above the hushed crowd, Rex tried to remain focused. Still, he couldn't shake one nagging thought: He was an old dog and this was a new trick".

"The Far Side" by Gary Larson

Table of Contents

List of Tables

Chapter III

Chapter IV

Chapter V

List of Illustrations

Chapter III

Chapter IV

Chapter V

Appendix I

CHAPTER I

INTRODUCTION

Functional hyperaemia refers to the physiological feature of increasing blood flow and it is designed to meet the requirements of an increased metabolic demand. The energy required to produce mechanical work is provided almost instantaneously. However, blood flow which includes O_2 delivery, takes longer to reach the optimal level for the mechanical work produced. This situation can be thought as a "loose" mechanism of control. Despite its efficacy, the energy production through anaerobic means, is limited and metabolites tend to be accumulated with practical consequences in terms of discomfort associated with physical exertion.

The control mechanisms responsible for blood flow adaptation in response to augmented levels of physical activity have been the topic of considerable research, particularly over the past few years. The importance and complexity of this topic (demanding an interdisciplinary approach) motivated the American College of Sports Medicine to promote the premier interdisciplinary conference on regulation of oxidative metabolism and blood flow in skeletal muscle (ACSM, 1995). It is clear that, at the onset of exercise, there are some mechanisms which facilitate blood flow and others which are limiting. The balance of these mechanisms contribute to regulation of blood flow kinetics and are examined in the next sections.

In addition to the mechanical effects of the skeletal muscle acting as a pump by "milking" the vessels (Sherrif, 1993; Shoemaker, 1996), vasodilation appears to occur very fast. This vasodilation is proposed to occur due to the action of neural, hormonal and metabolic factors (Gorman, 1991; Rowell, 1993).

On the other hand, deleterious changes occurring with age, can impair blood flow to working muscles. Studies on changes in blood flow regulation with aging have also been a subject of intense scientific debate. Studies have shown that older people present: 1) lower maximal heart rate; however, the rate of change in heart rate in response to exercise seems not to be affected (Babcock et al., 1994; Chilibeck et al., 1995); 2) lower maximal limb blood flow (Saltin, 1986); however, blood flow response to submaximal exercise seems to be largely unaffected (Jasperse et al., 1994); 3) a moderate narrowing of resistance vessels (Folkow and Svanborg, 1993); however, there is no direct evidence of that; 4) increased level of norepinephrine (Silverman and Mazzeo, 1996; Taylor et al., 1992) and faster plasma norepinephrine kinetics (Veith et al., 1986). These changes could provide higher vasoconstrictor stimuli; however, age-related changes in α_1 receptors density and/or responsiveness have not been shown (Sun and Narayunan, 1993); 5) increased stiffness of central and conduit arteries (Nichols and O'Rourke, 1990), decreased responsiveness to vasodilator agents (Luscher and Vanhoute, 1990), decreased production of nitric oxide (Folkow and Svanborg, 1993), and decreased production of prostacyclin (Tokunaga et al., 1991) would have negative effects on vascular conductance; 6) reduced capillary network shown in some (Parizkova, 1970; Coggan et al, 1992; Chilibeck et al., 1995) but not all studies could also compromise blood flow; 7) a decreased oxidative capacity reported only in a few studies (Essen-Gustavsson and Borges, 1986; Coggan et al., 1992) would potentially accelerate blood flow as a result of premature accumulation of metabolites. Although inconclusive in many aspects, it seems that most of the suggested age-related changes point to a decline in blood flow regulation.

The large majority of studies on age-related changes in blood flow have used steady-state

measurements, and there is need for studies looking at the transient phases of blood flow adjustment to exercise.

Some studies have demonstrated age-related changes in blood flow in response to a training stimulus (Sinoway, 1987; Green et al, 1994; Martin et al., 1990). However, studies on blood flow kinetics in response to training in older people are rare in the literature.

In summary, it is clear that blood flow kinetics related to age and physical activity warrants further investigation. This investigation will benefit greatly from the significant research that has been ongoing related to mechanisms of blood flow control at the onset of exercise (University of Waterloo) and $\dot{V}O_2$ kinetics in the elderly (The University of Western Ontario).

AIM OF THE THESIS

Based on the brief summary noted above, there seems to be compelling rationale leading to potential changes in blood flow regulation with aging and physical training. In addition, there is a specific need for studies examining the relation between blood flow kinetics and physical fitness in older people. Therefore, it was proposed that three studies be initiated to focus on the following three questions:

. Study 1. Is there an age-related impairment in blood flow kinetics?

. Study 2. Do physically very active older people present faster blood flow kinetics than their less active peers?

. Study 3. Does relatively short-term endurance training improve blood flow kinetics in older people?

METHODOLOGICAL CONSIDERATIONS

Exercise Protocol. A handgrip exercise protocol with a moderate workload was used during all the studies. This protocol is described in detail in study # 1 (Chapter III). Therefore, the results of the studies are limited to dynamic contractions of a small muscle mass performing a moderate level of work.

There were several reasons for chosing the handgrip exercise model. The most important of them are: 1) It has previously been demonstrated that handgrip exercise can be performed without producing significant motion at the site of measurement (brachial artery close to the antecubital fossa); 2) It minimizes the involvement of central circulation so that peripheral factors can be emphasized; 3) It allows several repetitions necessary to reduce the extensive variation in blood flow values due to muscular contraction and relaxation phases.

Measurement Techniques. Blood flow has been measured by means of several direct and indirect techniques. These techniques are presented in more detail in the literature review (Chapter II). The use of different techniques might be a limitation to compare results across the studies.

In the studies comprising this thesis, pulsed Doppler ultrasound was used to measure mean blood velocity (MBV) on a beat-by-beat basis. Although this technique presents a few limitations (see Study # 1, under methods) it was already demonstrated to be accurate and repeatable across different days (Shoemaker, 1996).

Aiming to calculate blood flow (mean blood velocity x cross-sectional area)Brachial artery diameter was assessed using an Echo-Doppler system operated in brightness-mode (B-

Mode) to calculate blood flow (mean blood velocity x cross-sectional area). The average values of 5 measurements at different times during rest and 5 during exercise, were used to represent the mean resting and exercise brachial diameter. In addition, these measurements were taken only during the first of the five trials of the exercise protocol . Consequently, diameter measurement was limited in comparison with an extremely ideal situation where it would be assessed on a beat-by-beat basis simultaneously with blood velocity. The diameter determination is described in more detail in Study # 1 (Chapter III).

Participants

One major difficulty in carrying out these studies was the recruitment of participants. Despite sending letters, directly talking to groups of elderly, and posters and notes in church bulletins, only a small number of volunteers agreed to participate in the study. This was particularly acute for study # 3, which involved several long-lasting visits to the lab in addition to daily exercise training for 30 days.

A significant fraction of the volunteers did not meet the criteria for inclusion and could not participate. The reasons for exclusion were high blood pressure, medication (β-blockers; diuretics; anti-inflammatory drugs, including aspirin; and occurrence of premature heart beats, probably due to ventricular arrhythmias). It was observed that premature heart beats interfered with forearm blood flow and blood pressure on a beat-by-beat basis , which could represent a confounding factor. However, it was judged that this would be an interesting area for future investigation, and a case study was reported and included as Appendix I of this thesis. Interestingly, a very recent study (Mayuga et al., 1996) from the Baltimore Longitudinal Study

of Aging, focused on the increase in exercise-induced arrhythmias with age. They reported that age independently predicted exercise-induced ventricular arrhythmias (EIVA).

GENERAL DISCUSSION AND OVERALL SUMMARY

Results from study # 1 (chapter III) suggested that there was no age-related impairment in blood flow kinetics at the onset of dynamic contractions of small muscle mass. Based on the extensive literature pointing to age-related changes (Lakatta, 1993; Folkow and Svanborg, 1993; Luscher and Vanhoute, 1990; Nichols and O'Rourke, 1990) that could potentially impair blood flow, the results of this study were unexpected. Possible explanations for the lack of difference in blood flow and vascular conductance kinetics between young and older participants might be associated with: a) increased kinetics (rate of appearance) of norepinephrine in the older subjects(Veith et al., 1986) which could provide more adequate blood redistribution through vasoconstriction of non-active parts of the body; however it is unlikely because the exercise model used should have minimized this factor and, in addition, it has to be assumed that the density and responsiveness of α_1 receptors is unchanged with age; b) for unknown reason, the lower relative workload performed by the young group might have influenced the results; c) a lower muscle oxidative capacity in the older subjects (Essen-Gustavsson and Borges, 1986; Coggan et al., 1992), might have allowed an increased rate of metabolite formation, which in turn could accelerate blood flow by increased vasodilation; however, most of the studies have shown no age-related impairment in oxidative capacity (Roger and Evans, 1993; Larsson and Karlsson, 1978; Larsson et al., 1978; Orlander et al., 1978; Grimby et al., 1982; Borges and Essen-Gustavsson, 1989) and; 4) the moderate workload used in the exercise protocol was not enough to challenge the system to a point where possible changes could be observed.

If changes in blood flow kinetics are associated with changes in $\dot{V}O_2$ kinetics, as

demonstrated by Hughson (1995), then the lack of an age-related impairment in blood flow kinetics in study # 1 (chapter III) is in line with the results reported by Killabakh et al. (1995) i.e., no age-related changes in $\dot{V}O_2$ kinetics. They also used a small muscle mass exercise model.

The results of the two studies on forearm blood flow and vascular conductance kinetics related to the physical activity status(chapters IV and V) were somewhat controversial. The results of study # 2 (chapter IV), failed to show any difference between very active and normally active older people, while study # 3 (chapter V) demonstrated a small but significant improvement in blood flow and vascular conductance kinetics. Other studies on the subject are also controversial. For example, Shoemaker et al., (1996) showed faster blood flow kinetics in response to ten days of training in young people, while McCully (1991) showed no effect of endurance training on phosphocreatine (PCr) rate of recovery in older people which might have included no change in blood flow kinetics.

One possible explanation for the apparent controversial results between study # 2 (normally active vs. hockey players) and study # 3 (training) can be related to the training principle of specificity. The very active older people had habitually been using their forearm muscles to handle the stick, however, the exercise pattern, velocity of contraction and intensity were not as specific to the training protocol as the training exercise used in study # 3.

Transfer of training effects of the oxygen transport system seems to be associated with the specificity of the exercise. In order to utilize the aerobic potential in an optimal way in a given activity, one must train in that activity (Astrand and Rodahl, 1986). For example, a treadmill test is not a good predictor of performance in other types of activity (Holmer and Astrand, 1972; Magel et al., 1975).

The more a training exercise simulates the target performance, the greater the transfer of learning and coordination (Rutherford and Jones, 1986, cited in Sale, 1987). This effect is supposed to include reduced activation of antagonists and then, it will be easier to activate the agonists fully (Sale, 1987). A second aspect of specificity of training is to have the training exercises performed at velocities similar to those of the target task. This specificity may reflect the much different motor unit firing rate and firing patterns that occur in fast versus slow contractions and the influence that discharge patterns may have on muscle contractile properties (Sale, 1987).

It is important to mention that very active older people showed a tendency to present 20% faster blood flow and vascular conductance kinetics compared to their less active counterparts ($p>0.05$). It was noted that there was a large inter-individual variability in the values of MBV kinetics and the range of the values in the two groups overlapped each other. Therefore, the small sample size could not be ruled out as a factor that might have compromised any significant difference in blood flow and vascular conductance kinetics between the two groups.

The total gains (rest to exercise values) in forearm blood velocity and vascular conductance were not different ($p>0.05$) between the groups or condition in all three studies. This means that the calculated kinetics were not influenced by different amplitude of changes.

Except for heart rate in the young group (chapter III) and brachial artery diameter in all three studies (chapters III-V), which remained unchanged ($p>0.05$), the remaining variables (forearm mean blood velocity, blood flow, mean arterial perfusion pressure and vascular conductance) increased significantly from rest to exercise ($p\leq 0.05$).

Despite no statistical significance ($p > 0.05$), the brachial artery tended to be larger in

older participants compared to younger ones and it was consistent across the three studies (chapters III-V). Studies on age-related changes in brachial artery diameter are inconclusive (Boutouyrie et al., 1992; Kawasaki et al.,1987; Safar et al., 1981) but, the consistent trend across the three studies in this thesis pointing to a larger brachial artery in the elderly seems to warrant further investigation.

Another interesting finding of this study, which also appears to warrant further investigation, was a higher resting forearm blood flow in the elderly than in the young. Similar findings were also reported in rats (Tymil et al., 1992) and in some studies in humans (Safar et al., 1981; Foley et al., 1993), but not in all (Jasperse et al., 1994). Moreover, the causes for a higher resting blood flow in the elderly, if it exists, have not yet been established.

It was also demonstrated that the occurrence of premature heart beats immediately affected blood flow and blood pressure in limb muscles (Appendix I). Therefore, when assessing data on a beat-by-beat basis in older people, care should be taken about the presence of these ventricular or even supra-ventricular arrythymias. Furthermore, exercise-induced ventricular arrythymias (EIVA) are reported to increase with age (Mayuga et al., 1996). This subject also seems to warrant further investigation.

In summary, the three studies in this thesis demonstrated:

1. Blood flow kinetics and vascular conductance kinetics are not impaired with age;

2. Increased physical activity tends to improve blood flow and vascular conductance kinetics in older people. However, these findings should be taken with caution. Although the longitudinal study (study # 3, Chapter V) has shown improvement in forearm blood flow and vascular

conductance kinetics that reached statistical significance ($p<0.05$), results from the cross-sectional study (Chapter IV) suggest only a tendency for improvement. Therefore, further investigation is necessary to better elucidate this question.

Finally, the findings of the studies included in this thesis are limited to dynamic contractions of a small muscle mass with a moderate workload.

FUTURE DIRECTIONS

1. Design a study using higher workloads in the exercise model in order to verify whether the lack of age-related differences in blood flow and vascular conductance kinetics also holds when the physiological system operates closer to its limits.

2. Study age-related implications on blood flow and vascular kinetics in a larger muscle mass. One example would be to assess these variables during exercise using a "kick" ergometer (knee extension and flexion). This kind of experiment would provide some light on the role of blood flow kinetics on the slower $\dot{V}O_2$ kinetics found in older people during cycling exercise (Babcock et al., 1994; Kowalchuk et al., 1995).

3. Develop studies to evaluate age-related effects on the mechanisms which are thought to control blood flow kinetics. For example, despite the fact that the muscle pump has been implicated in blood flow kinetics, there appear to be no studies which have directly addressed the question in the elderly.

4. In order to better elucidate the role played by higher levels of physical activity in older people, the sample sizes need to be increased substantially (a power test would be helpful for this determination).

5. Use an endurance training protocol that includes progressive workloads (based on a maximal endurance test), to identify how blood flow and vascular conductance respond to changes in training stimulus.

6. Age-related changes in brachial artery diameter need to be clarified.

7. Impairment in blood flow to skeletal muscle has been implicated as a potential factor in

"sarcopenia" with age (McCully et al., 1995). However, the results in study # 1(Chapter III) showed higher resting blood flow in the elderly compared to young people. Other studies, but not all, have reported the same result. Therefore, it is proposed that future studies address this topic.

8. Finally, the implications of atrial/ventricular arrhythmias to exercising muscles need to be addressed in specific studies.

CHAPTER II

Literature Review

This chapter is a brief review which aimed to complement what was already included in the studies comprising this thesis.

Techniques of Blood Flow Measurements

Blood flow through skeletal muscle can be measured through a variety of techniques. This part of the review will briefly describe these techniques.

Direct measurement of blood flow can be done using intravital microscopy, plethysmography, radioactive microspheres, ^{133}Xenon washout, thermodilution, pulsed Doppler ultrasound, color-Doppler imaging (echo-Doppler), and magnetic resonance imaging/angiography (MRI/MRA). Indirect measurement of blood flow can be done using the arm-to-ankle pressure index , 31-P magnetic resonance spectroscopy (MRS)-recovery rate of PCr, and near-infrared spectroscopy (NIRS)-recovery rate of O_2 saturation (McCully et al.,1995). Laser Doppler, based on the shift frequency of light scattering moving red blood cells is another technique (Tymil et al., 1992).

Intravital microscopy measures the red blood cell velocity in small vessels including the capillaries, in various in vitro and in situ preparations and it is probably the most direct measurement of blood flow (McCully et al., 1995).

Plethysmography involves inflating a pressure cuff to occlude blood flow in the veins without impeding blood flow in the arteries. Blood flow is measured as the accumulation of fluid in the tissue, originally measured as a change in limb volume, but now more commonly measured as a change in limb cross-sectional area. Plethysmography is one of the earliest

developed methods to estimate blood flow, and is perhaps still the gold standard for research measurements. The limitation in this method is that it can only be used under resting or hyperaemic conditions.

The most successful method of quantifying skeletal muscle blood flow in exercising animals has been the radiolabelled microsphere technique. Microspheres (usually 15 μm) are injected into the left atrium or left ventricle and their distribution to any and all muscles and even parts of a muscle (e.g. red versus white fibers) can be determined to derive blood flow (Rowell, 1993). Unfortunately, the invasive nature of this technique precludes its use in humans.

The ^{133}Xenon (^{133}Xe) clearance method (^{133}Xenon washout) uses this radiolabelled tracer to estimate blood flow. ^{133}Xe is normally injected into the muscle and the rate of its clearance is taken as a reflection of the rate of blood flow (Folkow et al., 1971). ^{133}Xenon washout and other isotope clearances are affected by the direct exchange of isotopes between arterioles and veins, by differential solubility of labelled substances in different structural components of the muscle, and finally by injection trauma, which alters local blood flow. Further, the chances of injecting the indicator into a collection of fibers with a representative population of high and low intrinsic rates of blood flow are virtually nonexistent. The most serious criticism of isotope-clearance techniques is that the derived flows to active muscle are far too low to account for the increase in oxygen uptake (McCully and Posner, 1995).

Probably the most successful invasive technique to measure skeletal muscle blood flow during exercise in humans is thermodilution. Constant infusion of dye or ice-cold saline through a catheter designed to provide adequate mixing can be used. However, some assumptions have to be made in terms of exercise being restricted to a specific muscle or muscle group, and that

these experimental conditions do not significantly raise skin blood flow in the limb. When skin blood flow is increased, the muscle arterio-venous O_2 difference [(a-v)O $_2$diff.] will be underestimated because the skin extracts little oxygen. In this situation, calculated muscle $\dot{V}O_2$ and mechanical efficiency would be correct, but the muscle blood flow will be overestimated (Rowell, 1993).

The most recent techniques to measure skeletal muscle blood flow include the bidirectional pulsed Doppler (also called bidirectional Doppler-ultrasound velocimetry), the Echo-Doppler, Magnetic Resonance Imaging(MRI) and near-infrared spectroscopy (NIRS).

The combination of bidirectional pulsed Doppler ultrasound and echo-Doppler techniques provide non-invasive measurement of real time blood velocity and vessel cross-sectional area(Mc Cully and Posner, 1995; Rowell, 1993). Until recently these techniques have been used during rest or post-exercise because they are sensitive to limb movement. However, this limitation has been overcome when the blood velocity profile and cross-sectional area are measured by positioning the probe in conduit arteries close to the exercising muscle (brachial artery close to the antecubital fossa for forearm exercise and common femoral artery close to the inguinal ligament for knee extension exercise) and care is taken to avoid movement at the site of probe positioning (Shoemaker et al., 1994; Tschakovsky et al., 1995; Hughson et al., 1995; McDonald et al., 1995).

The pulsed Doppler is very sensitive to changes in blood velocity and its validity to measure blood velocity has already been demonstrated during both rest and exercise with a number of other accepted methods of flow measurement (Guldvog et al., 1980; Levy et al.,1979; Zaniriri et al., 1993). In regard to the bidirectional pulsed Doppler, the following assumptions

and limitations apply: a) the main source of error is the angle of insonation (Gill, 1985). Usually it is assumed that the artery runs parallel to the skin, and then a placement of a flat probe with a built-in ultrasound transducer maintains the angle in relation to the skin; b) errors due to the operator, improper alignment of the ultrasound beam with the artery, and Doppler signal processing and frequency estimation can be reduced by averaging four repeated trials into one data set and by using both auditory and visual feedback of the Doppler signal . Both brachial artery blood velocity measured by pulsed Doppler and diameter measured by echo-Doppler, during rest and exercise, have shown high reproducibility across different test days (Shoemaker, 1996). When measurements of diameter are not possible, calibration between blood velocity and blood flow through plethysmography (Tschakovsky et al., 1995; Van Leuven, 1992) may offer a good estimation of blood flow on a beat-by-beat basis.

The vessel wall can be imaged through echo-Doppler using B-mode technique. In this technique, the reflected echo pulse is recorded as a dot which is brighter in proportion to the intensity of the reflected energy, that is brightness modulation (B-mode). (Fronek, 1989).

Aging and blood flow

Physical activity involves adaptative processes to meet the energy requirement. These processes include the rate of adjustment in blood flow to supply the exercising muscles. Structural and functional alterations associated with aging could potentially impair this adaptative process with practical implications in day-to-day living. Therefore, this part of the review will highlight : 1) observed differences in the adaptative response of older subjects; 2) potential mechanisms involved in the hyperaemia of exercise; and, 3) effects of aging on

structural and functional vascular response with exercise.

Onset of exercise in older people

In cycling exercise, the $\dot{V}O_2$ kinetics have been shown to be slower in the older compared to younger subjects regardless of whether the exercise intensity is expressed in relative (Babcock et al., 1994; Kowalchuk et al., 1995) or absolute terms (Kowalchuk et al., 1995) up to 80% of the ventilatory threshold. However, this appears not to be the case for moderate-intensity plantar flexion exercise (Chilibek et al., 1995). One possible explanation for the apparently controversial results may be that the plantar flexion is a movement continuously performed throughout the lifespan while the level of activity of muscles involved in cycling may decrease with age. That is, the level of activity and not the aging process itself may be the cause of slower $\dot{V}O_2$ kinetics with age.

The $\dot{V}O_2$ kinetics have normally been used to represent those circulatory, respiratory and muscular adaptations at the onset of physical effort. In this field, Hughson and other researchers in his laboratory at the Department of Kinesiology, University of Waterloo, have pointed towards the rate of increase in tissue O_2 consumption being limited by the ability of O_2 transport to the working muscles (Hughson and Morrissey, 1982; Hughson, 1990; Hughson et al, 1995; Tschakovsky, 1993.). Conversely, other studies have proposed that $\dot{V}O_2$ kinetics at the onset of exercise, appear to be limited by the rate of O_2 utilization within the muscle (Barstow et al., 1990; Whipp, 1980). Therefore, the current state-of-the-art in the area points towards O_2 delivery and/or O_2 utilization within the muscle as limiting factors to $\dot{V}O_2$ kinetics.

If $\dot{V}O_2$ kinetics are limited by O_2 transport, then the kinetics of blood flow to the

working muscle represents a potential contributor to the $\dot{V}O_2$ kinetics at the onset of exercise. To date, blood flow kinetics(BF kinetics) appear to be correlated to $\dot{V}O_2$ kinetics; that is, faster $\dot{V}O_2$ kinetics are parallelled by faster BF kinetics. Hughson et al. (1995) observed slower brachial artery blood flow associated with slower $\dot{V}O_2$ kinetics in response to a step change of workload in handgrip exercise when the arm was supported above the heart level compared to an arm position below the heart level.

Exercise hyperaemia

The blood flow to dynamically active tissues is under the control of neurohumoral, metabolic and mechanical regulators (Rowell, 1993; Laughlin et al., 1996). This blood flow can be regulated centrally by altering Cardiac Output (HR x SV) and blood pressure or locally by increasing vascular conductance in the exercising muscle. It appears that central circulation regulation of blood flow during moderate dynamic contractions of small muscle mass is negligible and, therefore, vascular conductance of the exercising muscle is the key variable in functional hyperaemia.

Neural control involves the somatic and autonomic nervous system. The somatic nervous system causes release of acetylcholine through the motor neurons, which might initiate vasodilation. Acetylcholine may cause dilation of the arterioles of striated muscle by activating muscarinic receptors on endothelial cells(Segal and Kurjiaka, 1995). The autonomic nervous system includes the parasympathetic (PNS) and sympathetic(SNS) nervous system which regulate heart rate (variations of vagal tone and NE and E release), contractility of the myocardium (NE and E), and vascular tone (NE and E). It has also been proposed that an as yet

undiscovered vasodilator neurotransmitter liberated in a feed forward fashion or by muscle contraction can also be responsible for the exercise hyperaemia (Gorman, 1991; Honig, 1979).

The metabolic control involves substances released into the interstitial space during contractions, in addition to a decreased PO_2. These substances include CO_2, adenosine, lactate, P_i and K^+ (Gorman, 1991; Rowell, 1993) which can also generate hyperosmolarity -a condition that can play some role in the initiation of exercise hyperaemia (Rowell, 1993). The metabolites have a vasodilator effect that usually predominates over the sympathetic vasoconstriction , in part by interfering with NE release at sympathetic nerve endings (Segal, 1992). This metabolic control is affected by O_2 availability provided by blood flow and/or the metabolic oxidative capacity of the muscle cells.

The mechanical regulators involve the myogenic response, muscle pump and substances released by endothelial cells in response to the shear stress (Rowell, 1993). Originally, *myogenic response* meant the active contraction of vascular smooth muscle elicited by an increase in transmural pressure. Although the definitive mechanisms are still unknown, two possible mechanisms have been proposed: 1) activation of the stretch-sensitive Ca^{2+} channels in the plasma membrane and 2) the existence of a tension sensor (Holstein-Rathlou and Marsh, 1994). The myogenic response due to a reduction in transmural pressure, through compression of the arterioles, is vasodilation (Rowell, 1993). The *muscle pump* facilitates an increase in muscle blood flow by temporarily increasing the perfusion pressure gradient across a muscle. The muscle contraction drives blood from the venous system toward the heart. Immediately upon muscle relaxation, the pressure in the venules is reduced, perhaps to zero or below (Rowell, 1993; Laughlin ,1987) so that the perfusion pressure gradient across the muscle capillary bed has

increased resulting in a transiently marked increase in blood flow. Repeated contractions in combination with vasodilation can account for a large increase in blood flow. The compression of arterioles by the exercising muscles would reduce transmural pressure which may (Segal, 1994) or may not (Tschakovsky et al., 1996) induce myogenic vasodilation which further increases blood flow. An increase in *shear stress* on the vessel wall can stimulate the production of nitric oxide and prostaglandins which also are thought to mediate vasodilation (Rowell, 1993).

Functional hyperaemia related to exercise refers to the process that increases blood flow to the working muscle in response to increased metabolic demand. Its onset is within 1 to 2 seconds from the beginning of exercise, and it is too fast to be explained by the flow-dependent or metabolic -dependent vasodilation which takes several seconds. It has been proposed by Segal et al. (1989a; 1989b; 1992; 1994; 1995) that the onset of functional hyperaemia involves ascending vasodilation of the arterial tree and its control shifts from the terminal arterioles (8 to 15 μm) which control capillary perfusion, to the intermediate arterioles (15 to 60 μm; controls flow distribution) and into proximal arterioles (60 to 100 μm) and feed arteries (100 to 500 μm) controlling flow magnitude. The terminal arteriole vasodilatory stimulus can be conducted to proximal branches in less than 1 sec. The hypothesis of "conducted vasodilation" elaborated by Segal et al. (1992) proposes that vasodilation in the arteriolar network is transmitted cell-to-cell (endothelial-to-endothelial cells and/or smooth-to-smooth muscle cells and/or endothelial-to-smooth muscle cells) in response to activity in surrounding tissues. One possibility is that the electrical signals during depolarization or polarization of one cell may travel to other cells through gap junctions between the cells.

In summary, the rapid onset of functional hyperaemia can be elicited either/or by the muscle pump, myogenic response, and cell-to-cell communication (conducted vasodilation), with flow-induced and metabolic dilation contributing thereafter (Segal, 1992; Segal,1994). Furthermore, putative output of intrinsic neurons inside the wall of small arteries may also play a role in the early onset of functional hyperaemia (Gorman, 1991; Honig, 1979; Rowell, 1993).

It is noteworthy to mention that functional hyperaemia control is expressed within the limits of vessel structural and mechanical properties, such as distensibility of the vessel wall, vessel lumen and the density of the capillary network.

Structural and Functional Vascular Response with Aging

The aging process, and/or related decrease in the level of physical activity, might impair blood flow adaptations by altering the structure and contractility of vessel walls and mechanisms related to vasoactivation.

In terms of structure, the two main aging features related to blood flow are the changes in diameter and wall thickness of the vessels. These changes seem to be spread throughout systemic arteries. The increased wall thickness is consistent with the increase in collagen, ground substance and calcium and a decline in elastin (Lakatta, 1993).

Arterial degeneration with age appears to be a consequence of repeated high pulsatile stresses over long periods of time. This degeneration is not seen in experimental animals over the normal age span. It is also not seen in other large blood vessels (veins and pulmonary arteries). Therefore, this description appears to fit into the theory of aging called *Wear and Tear* . The hypothesis can be stated as follows: The fatiguing effects of cyclic stress cause fracture of

the load-bearing elastin fibers. These fractures lead to progressive dilation of the vessels with transfer of stress from elastin to collagen fibers. This process leads to attempts at remodeling by cellular elements of the wall (Nichols and O'Rourke, 1990).

Vessel diameter appears to be bigger with aging in central and peripheral capacitance arteries (Lakata, 1993; Nichols, 1987; Roach, 1959). It has also been observed that age-related adaptations in the arterial tree present different characteristics; that is, increase in diameter predominates in central arteries while wall thickening is prevalent in more distal arteries (Learoyd and Taylor, 1966). The mechanisms for this difference in regional adaptation between central and peripheral vasculature are unknown. Following the same direction, it has also been suggested that there is moderate structural narrowing of the resistance vessels which is thought to lead to a reduced regional flow capacity (Folkow and Svanborg, 1993). For example, even in elderly well-trained athletes, the maximal limb flow is ~10-15 % lower than in the young athletes (Saltin, 1986). Furthermore, an age-related adaptive narrowing of systemic resistance vessels may additionally serve to match the gradual reduction in maximal cardiac performance, thereby maintaining a reasonable balance between heart and vessels in terms of their respective maximal capacity (Folkow and Svanborg, 1993). The increased thickness of small artery walls, caused by hypertrophy and/or hyperplasia and/or redistribution of cell mass may provide structural "advantage" for contractile strength of the muscle in response to vasoconstrictor agents. That is, the smooth muscle cell contracting in a thin-walled vessel exerts less force than one contracting in the outer part of a thick wall (Schwartz, 1996). Therefore, the effect of vasoconstrictor stimuli on a thicker vessel is amplified when compared to a thin vessel wall.

Functionally, changes in structural properties of the wall vessels lead to arterial stiffness.

Arterial stiffness has been inferred by:a) increased elastic modulus (measure of strain at a constant stress) e.g. from 10 kg/cm^2 at age 20 years to 42.5 kg/cm^2 at age 85 years at a stress of 100 mmHg in the aorta (Lakatta, 1993); b) increased pulse wave velocity e.g. about 20% faster from age 10 years to age 50 years in the brachial to radial system and femoral to anterior tibial system; c) decreased wave amplification between central and peripheral arteries which leads to the prediction that brachial artery and aortic systolic and diastolic pressure should be similar at the age of 65 years (Nichols and O'Rourke, 1990).

The age-related changes in elastic modulus present opposite results in central (elastic) and peripheral (muscular) arteries. That is, it increases in the central arteries and decreases in peripheral arteries. The suggested explanation refers to the fact that the increased thickness in peripheral arteries is greater than in more central arteries and this additional thickness is responsible for the reduction of the stress per single element of the vessel wall. (Learoyd and Taylor, 1966).

Pulse wave velocity increases with age throughout the systemic arterial tree. However, the increase in velocity is greater in central than in peripheral arteries (Nichols and O'Rourke, 1990).

Pressure wave amplification is attributed principally to peripheral wave reflection at arterial/arteriolar junctions, and with elastic non-uniformity between central and peripheral arteries contributing as well. Relative loss of elastic non-uniformity (similar aortic and peripheral wave velocities) and increased wave velocity (more pronounced centrally) seem to be the factors involved in decreased amplification with aging (Nichols and O'Rourke, 1990).

In addition to arterial stiffness, decrease in arterial dilation may also be due to an

increased vascular smooth muscle (VSM) contractile tonus (Lakatta,1993; Carroll et al., 1991). This would suggest that the aged vessel wall either becomes hyperreactive to vasoconstrictor stimuli (Luscher and Vanhoute, 1990) and/or hyporeactive to vasodilator agents (Lüscher and Vanhoute, 1990; Van Brummelen, 1981;) . Higher levels of plasma NE at rest and in response to exercise (Silverman and Mazzeo, 1996; Lakatta,1993; Taylor el al., 1992) can also play a role in increased VSM contractile tonus with aging . The responsiveness to vasodilator agents seems to decrease progressively with aging. It has been observed that relaxation in response to nitrovasodilators decreases with age in intact arteries. However, in preparations where the endothelium is removed, the response to vasodilators (papaverine and nitrovasodilators) is unaltered with age. From these observations it has been suggested that endothelium may be impaired to release relaxing factor (s) and/or it may produce a factor(s) which attenuates the effect of soluble guanylate cyclase (Lüscher and Vanhoute, 1990). The ability of the aging endothelium cells ability to respond with the release of vasodilator nitric oxide declines with age (Folkow and Svanborg, 1993).

The capillary network is normally expressed in terms of capillary density (capillaries per mm^2), capillary-to-fiber ratio (number of capillaries/number of fibers) and capillary in contact with each fiber. Although the results on age-related changes of capillary network are controversial and inconclusive (Rogers and Evans, 1993), some of them have shown age-related declines in capillary density (Chilibeck et al., 1995; Coggan et al., 1992), capillary-to-fiber ratio (Parizkova,1970; Coggan et al., 1992) and capillary in contact with each fiber (Coggan et al., 1992) , even in muscle that is frequently recruited during common daily activities. Even so, it is likely that the reduction in muscle capillarization in the older subjects was the result of lower

levels of activity, compared with young subjects, that put less of a demand on their muscles.

Most of the available evidence, particularly from Scandinavian studies have consistently shown no reduction in muscle oxidative capacity with aging (Rogers and Evans, 1993; Chilibeck et al., 1995). A few other studies (Essen-Gustavsson and Borges, 1986; Coggan et al., 1992) showed that sedentary older people present lower oxidative capacity, which was concluded from lower muscle oxidative enzymatic activities. The age-related decline in oxidative capacity found in these later studies is in agreement with the decline in O_2 uptake capacity of aged muscle measured in vitro (Meredith et al. ,1989). The enzymes usually evaluated to determine muscle oxidative capacity have been citrate synthase (CS), succinate dehydrogenase (SDH), and β-hydroxyacyl-CoA dehydrogenase (β-HAD). If age-related decline in oxidative enzymatic activity can be assumed, then $\dot{V}O_2$ kinetics might slow down and, with exercise, more vasodilator metabolites (ADP, P_i, La^- and H^+) could be formed. This particular factor would then contribute to a stronger drive for metabolic-dependent vasodilation in the older compared to younger people.

In summary, blood flow to exercising muscle may be locally altered with aging because of resistance vessels narrowing, increase in artery stiffness, increased sympathetic nervous activity, decreased responsiveness to or production of vasodilator agents, increased responsiveness to vasoconstrictor stimuli, decline in capillary network and decline in muscle oxidative capacity. Except for a potential stronger drive for metabolic-dependent vasodilation, which may have some effect on the later part of the on-transient blood flow kinetics curve at the onset of exercise, all other age-related vascular adaptations appear to contribute to an impairment in blood flow kinetics. Therefore, the main purpose of this study was to test the hypothesis that blood flow kinetics will be impaired with aging in response to exercise.

CHAPTER III

STUDY # 1

FOREARM BLOOD FLOW KINETICS IN RESPONSE TO HANDGRIP DYNAMIC CONTRACTIONS IN YOUNG AND OLD PEOPLE.

Abstract. The hypothesis that blood flow kinetics, at the onset of exercise, is slowed in older people compared to young people was tested in 6 young (mean age 28.8±9.7 years) and 5 old (mean age 72.0 ± 6.4 years) men who were non-smokers, normotensives, without history of cardiovascular disease and normally active. Diameter of the brachial artery (DM) was assessed by echo-Doppler while beat-by-beat mean blood velocity (MBV) and mean arterial perfusion pressure (MPP) were assessed by pulsed Doppler and a Finapres finger cuff, respectively. To assess the kinetics of the response, each participant's data were fitted by a two-component exponential curve. This procedure provided information on the mean response time (MRT) which represented the time when 63% of the response was achieved. The measurements were taken at rest and during 5 min of exercise, with the participants in the supine position. The exercise consisted of repeated handgrip contractions by lifting and lowering a 4.4 kg weight, using a 1 sec/2sec work-to-rest ratio with the arm supported above the heart level. The DM (4.2 mm and 4.7 mm, in the young and old group, respectively) was not significantly different between the groups nor between rest and exercise. Therefore, the kinetics of blood flow were represented by the MBV kinetics. The young group increased MBV from 7.9 cm/sec at rest to 22.6 cm/sec during exercise while the old group showed an increase from 9.1 to 22.9 cm/sec, with no significant inter-group differences . The difference in MBV- MRT between the two groups (young = 23.39 ± 9.12 s and old = 26.65± 8.72 s), was not statistically significant. Forearm vascular conductance index (FVCI), calculated by the quotient between MBV and MPP, increased from 0.10 ± 0.01 to 0.26 ± 0.01 cm . (s . mmHg)$^{-1}$ in the young and from 0.12 to ± 0.03 to 0.25 ± 0.07 cm . (s . mmHg)$^{-1}$ in the older group, with no significant difference between the

two groups. The difference in FVCI -mean response times (20.11 ± 7.34 s in the young group and 24.4 ± 8.24s in the old group) was not statistically significant. These data did not support the hypothesis that blood flow adaptation to exercising muscle is slowed with aging.

Introduction

In day-to-day living, we constantly go from one level of physical activity to another. This challenge must be met as efficiently as possible to minimize the alterations in metabolic state within the active skeletal muscles. Thus, on going from seated rest to normal walking, the cardiovascular system must contribute by rapidly supplying greater blood flow, with consequent increases in O_2 supply and removal of waste products of metabolism. The muscle makes appropriate adaptation for the utilization of the O_2 in the production of energy. Yet, in older subjects, one or more of these adaptative processes could be impaired. For example, at the onset of cycling exercise, the $\dot{V}O_2$ kinetics have been shown to be slower in the older compared to younger subjects regardless of whether the exercise intensity is expressed in relative (Babcock et al., 1994; Kowalchuk et al., 1995) or absolute terms (Kowalchuk et al., 1995) up to 80% of the ventilatory threshold.

The $\dot{V}O_2$ kinetics have been associated with blood flow adaptation at the onset of exercise; that is, faster $\dot{V}O_2$ kinetics is correlated with faster blood flow kinetics (Hughson et al., 1995). Accordingly, a slower blood flow kinetics may slow down the $\dot{V}O_2$ adaptation at the onset of exercise which in turn, can lead to a greater increase in lactate production, H^+ and decreased P Cr. Therefore, a practical implication of slower blood flow, is that the exercise might result in a higher perceived rate of exertion. This sensation of higher rate of exertion, in turn, may influence one to decrease the level of spontaneous physical activity and in doing so, the fitness level would decrease. Consequently, the $\dot{V}O_2$ kinetics would tend to be progressively slower and a vicious cycle is then established. A process like this might contribute to a decreased

physical activity with aging.

In spite of ongoing controversy in this area, the aging process has frequently been reported to be associated with structural and functional alterations that may influence the rate of adjustment of blood flow to exercising muscles at the onset of exercise. These alterations include the narrowing of resistance vessels (Folkow and Svanborg, 1993), an increase in the stiffness of arteries (Lakatta, 1993; Folkow and Svanborg, 1993; Nichols and O'Rourke, 1990), increased vascular smooth muscle contractile tonus (Lakatta, 1993), increased sympathetic nervous activity (Silverman et al., 1996; Taylor et al., 1992), decreased responsiveness to or production of vasodilator agents (Folkow and Svanborg, 1993; Lüscher and Vanhoutte, 1990; Van Brummelen et al., 1981), structural "advantage" provided by thicker vessel walls (greater force exerted by smooth muscle cell) in response to vasoconstrictor stimuli (Schwartz, 1996), decline in one or more measurements of the capillary network (Chilibeck et al., 1995; Parizkova,1970; Coggan et al., 1992) and decline in muscle oxidative capacity (Essen-Gustavsson and Borges, 1986; Meredith et al. ,1989; Coggan et al. 1992). Taken together, these findings appear to suggest that blood flow kinetics might be slowed at the onset of exercise in older people. Therefore, the main purpose of this study was to test the hypothesis that blood flow kinetics at the onset of exercise is slowed with aging.

Method

Subjects.

Six young (28.8 ± 9.7 years) and five old (age 72.0 ± 6.4 years), healthy and normally active males participated in the study. They were all normotensives (resting systolic and diastolic pressure lower than 140 and 90 mmHg, respectively). None of them reported a medical history of cardiovascular disease or was under medication known to interfere with blood flow control as assessed through a medical screening form. Seven older men volunteered to participate in the study but two of them did not meet the criteria for inclusion and they were excluded. The first one presented high and unstable blood pressure and abnormalities in the ECG tracing and the second one revealed an abnormal variation in heart rate and a few premature heart beats. The data collected from the second volunteer are presented as interesting findings in Appendix I of this thesis. Informed consent was obtained from the participants and the study was approved by the Office of Human Research and Animal Care of the University of Waterloo.

Experimental Protocol.

Participants were instructed to consume no caffeine on the days they reported for the study, and to refrain from eating for at least 2 h prior to the experiment. The experiments were performed at a laboratory temperature of ~ 20° C. Dynamic handgrip contractions were performed while in supine position (reclined comfortably on their back). The exercising arm was supported on a platform that elevated it at an upward angle of 50° relative to the horizontal and extended to a handgrip device (note Figure 4.2 in study # 2). The participant then lifted and lowered a weight of 4.4kg, through a vertical distance of 5cm, at a frequency of 20

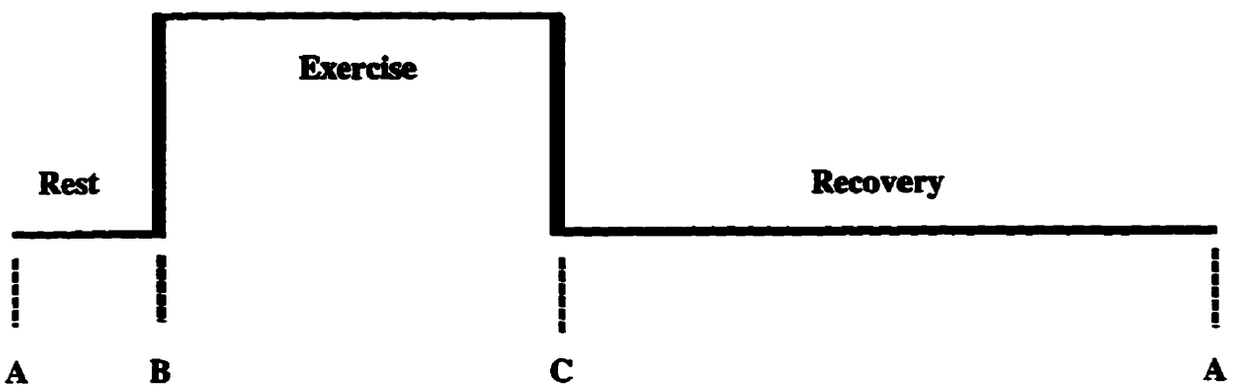
contractions/min, using a pulley system. The resultant work and power output were 4.32J and 1.44 w respectively. The frequency (1s contraction by 2s pause between contractions) during the exercise was monitored through equipment which provided visual and auditory signals. The participant was given 30 min. rest before starting data collection. A schematic illustration of the testing protocol is presented in Figure 3.1.

Measurements.

Maximal Voluntary Isometric Handgrip Contraction. The maximal voluntary handgrip contractions (MVIC) were assessed through a strain-gauge load cell (Linear Variable Differential Transformer - Daytronil model 3230 P) attached to a handgrip device. The participant performed three maximal voluntary contractions with a rest period between them. Since these measurements normally presented small variations between the three trials, mainly due to motivation of the participants and position of the fingers, the mean of them was thought to better represent the participant's forearm MVIC.

Stroke Volume Using Impedance Cardiography. Stroke volume (SV)was monitored beat by beat with impedance cardiography (304-B Minnesota Impedance Cardiograph). The electrical signals were transmitted and received by 4 pair of electrodes. Two pairs were applied to the neck skin and the other two pairs were applied to the lateral midline of the chest according to standard placement (Du Quesnay et al., 1987). The raw impedance data were smoothed by using a five-heart beat moving-average procedure, after which a software program marked the rate of change of the impedance signal (Muzzi et al, 1985; Shoemaker et al., 1994). SV for each beat was calculated according to the equation of Kubicek et al. (1966).

Figure 3.1. Schematic representation of the testing protocol sequence after the participants have been instrumented and have had 30 minutes rest in the supine position.



Legend:

A = Data collection started (1 minute before the exercise onset); B = Exercise started; EXERCISE = handgrip contractions for 5 minutes; C= Data collection and exercise stopped; RECOVERY = rest for 10 minutes or longer until baseline values were recovered .

The sequence from A to A was repeated 5 times. The first trial was designed for diameter measurements while the remaining four trials were analysed for HR, MPP, SV and MBV on a beat-by-beat basis.

Heart Rate. Heart rate was continuously monitored on a beat-by-beat basis with an electrocardiogram through 3 spot electrodes on the skin.

Mean Arterial Perfusion Pressure (MPP): A photoplethysmograph finger blood pressure cuff (Ohmeda 2300, Finapres) was applied on the middle finger of the contralateral hand. The hand was rested at the same level as the ultrasound probe on the exercising arm.

Blood Velocity. Measurements of MBV were taken similarly to the method described by Tschakovsky et al. (1995). The Doppler equipment (model 500 V, Multigon Industries, Mt. Vernon, NY) operated in pulsed wave mode was used. After determination of brachial artery position by palpation (~ 2cm proximal to the antecubital fossa) a 4-MHZ flat probe was manipulated over the brachial artery until optimal auditory and visual signals were obtained and then the probe was taped on the skin. The angle of insonation of the built-in transducer relative to the skin was 45°. It was assumed that the brachial artery ran parallel to the skin surface at the site of probe position. The gate was set at full width (12 mm) to facilitate insonation of the total width of the artery with approximately constant intensity. With this apparatus, it was possible to maintain a clear Doppler signal both at rest and during exercise.

The Doppler ultrasound technique uses an ultrasonic beam at the megahertz level directed at the blood vessel so as to diagonally intersect it. Stationary objects reflect sound back at the same frequency while sound reflected back by moving particles (red blood cells) is shifted in frequency. This frequency shift (f_D; in Hz) is in the audio range and is proportional to the red blood cell velocity such that $V = f_D \cdot c/2f_t \cdot \cos\theta$, where V is the velocity (in cm/sec), f_t is the

transmitted frequency (in Hz), θ is the angle of insonation of the ultrasound beam, and c is the velocity of sound in tissue and/or blood (157000 cm . s^{-1})(Wells, 1977).

The main source of error in MBV determination is the angle of insonation (Gill, 1985; Shoemaker et al., 1994). To reduce the magnitude of this error, the body position during the handgrip trials was fixed. In addition, a flat probe, which maintains the angle of the sound transducer with the skin was used. Further limitations associated with Doppler measurements of blood velocity include random error attributable to the operator, improper alignment of the ultrasound beam with the artery, and Doppler signal processing and frequency estimation. These sources of error were reduced by averaging four repeated trials into one data set (Gill, 1985) and by using both auditory and visual feedback of the Doppler signal (Shoemaker et al., 1994).

The Doppler shift frequency spectra were processed by a quadrature audio demodulator (Micco, 1989) that provided instantaneous MBV in real time allowing collection of MBV in analog-to-digital units. The quadrature audio demodulator also generated the appropriate Doppler shift frequency signals to produce a two-point calibration (0.5 m/s and -0.5 m/s at a 0° probe insonation angle). Beat-by-beat MBV was calculated by integrating the total area under the instantaneous MBV profile, with the marked QRS complex of the electrocardiogram tracing signalling the end of one heartbeat and the beginning of the next (Shoemaker et al., 1994).

Vessel Diameter. An ultrasound imaging (echo-Doppler) and Doppler System (model SSH-140-A, Toshiba Corporation, Japan) operated in Doppler (D) and brightness modulation (B) modes was used. After determination of brachial artery position by palpation proximal to the antecubital fossa, a 7.5 -MHZ hand-held probe, comprising an array of transmitting and receiving

transducers, was manipulated over the brachial artery until optimal auditory and visual signals were obtained. The vessel wall image and blood velocity profiles were video-taped for subsequent analysis. During the analysis, the velocity profiles generated by this system were only used to provide additional feedback about the optimal position of the probe.

Through the echo-Doppler system, the transmitted signal is sent out in periodic pulses and the time of arrival of the echo is proportional to the depth of the echo source. The image is built up in real time by displaying each echo as a dot on the screen. The intensity of the echo is determined by the strength of the echo and its location by the time of arrival. The result is a two-dimensional image of structures along the ultrasound beam.

Data Storage.

The electrocardiogram, finapres, Doppler and impedance cardiography signals, including time, were recorded at a frequency of 200 Hz on a microcomputer data acquisition system. These signals were collected continuously for each 6-min trial. The Echo-Doppler images collected during 1 trial for each participant were stored on VCR tapes.

Data Analysis.

The MBV, HR, SV, MPP data collected on a beat-by-beat basis from four repetitions for each subject were averaged at 1-s intervals to yield a single data set per subject. This method is similar to that described previously to examine breath-by-breath VO_2 (Phillips et al., 1995; Shoemaker et al., 1994) and MBV (Hughson et al.,1995; Shoemaker et al., 1994).

Cardiac output (CO) was calculated by the product of heart rate (HR) and stroke volume (SV). Mean arterial pressure was estimated by adding the MPP plus the difference in mmHg (2 mmHg per inch) due to the vertical distance between the site of measurement and the heart level. Forearm vascular conductance index (FVCI) was calculated by the quotient between MBV and MPP.

The MBV kinetic parameters for the response to the step increase in work rate were described from a two-component exponential model, already used in previous studies of MBV (Tschakovsky, 1993; Hughson et al., 1995). This model is the same used to describe VO_2 kinetics (Cochrane et al., 1992; Hughson et al., 1993; Phillips, 1995). The model contains a baseline (G_0) and two gain terms (G1 and G2) as well as two time delay (TD1 and TD2) and time constant terms (τ1 and τ 2). Therefore the MBV kinetic response was described as function of time by the following equation :

$$MBV(t) = G_0 + G_1 \cdot [1 - e^{-(t-TD1)/\tau 1}] \cdot u_1 + G_2 \cdot [1 - e^{-(t-TD2)/\tau 2}] \cdot u_2 \qquad \textit{Equation 1}$$

where $u_1 = 0$ for $t < TD_1$, $u_1 = 1$ for $t \geq TD_1$, $u_2 = 0$ for $t < TD_2$ and $u_2 = 1$ for $t \geq TD_2$.

The model parameters were determined by least square procedure in which the best fit was established by minimization of the residual sum of squares (Hughson et al., 1988).

The overall time course of the response was represented by the MRT, which was calculated from a weighted sum of TD and τ for each component.

$$MRT = [G_1/(G_1 + G_2)] \cdot (\tau_1 + TD_1) + [G_2/(G_1 + G_2)] \cdot (\tau_2 + TD_2) \qquad \textit{Equation 2}$$

MRT is equivalent in time to the point at which the response passes through ~ 63% of the difference between G_0 and the new steady-state value.

Diameter (Dm) of the brachial artery was assessed by freezing the VCR image at 5 different points in time during the resting period (one for each 10 seconds) and 5 during the exercise period (one for each minute) . For each point in time three measurements were taken and the mean of these values was used to represent the diameter at that point in time, and the mean diameter values for rest and exercise were calculated for each participant.

Figure 3.2 and 3.3 show brachial artery image and how the diameter measurements were taken.

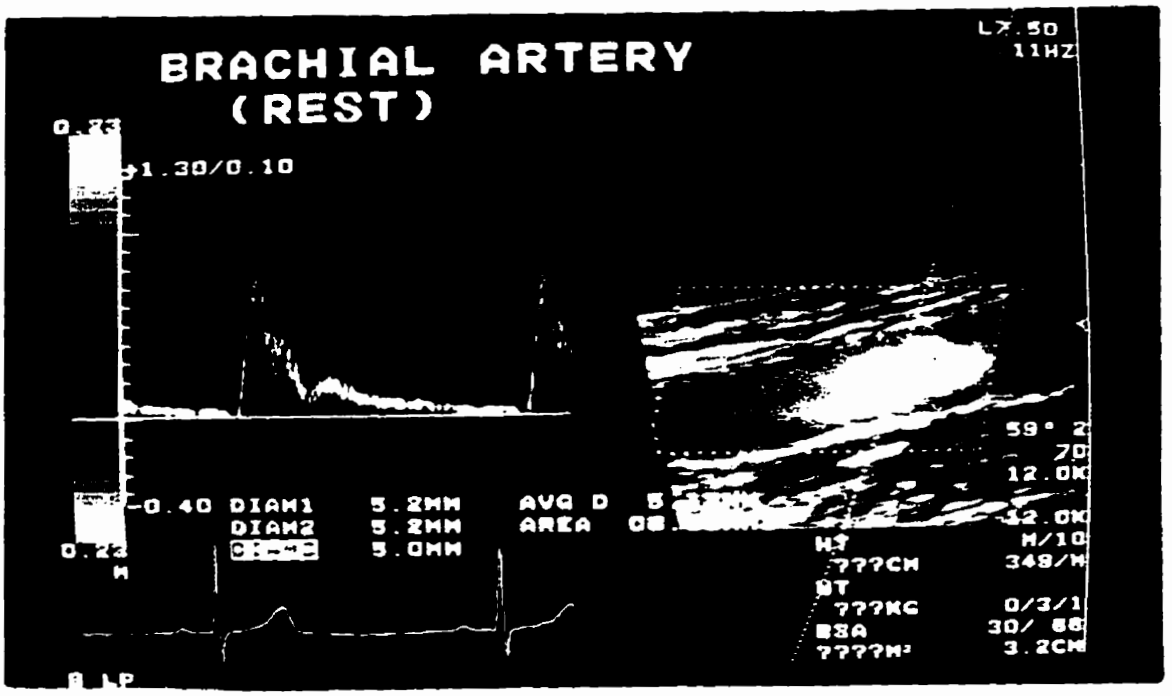
Blood flow in the brachial artery was calculated by the equation used by Chaveau et al., (1985): $FBF = MBV.\pi . d^2/4$ *Equation 3*

where FBF=forearm blood flow ; d=diameter of the artery ; MBV=mean blood velocity; π = a constant (3.1416).

Statistical Analysis

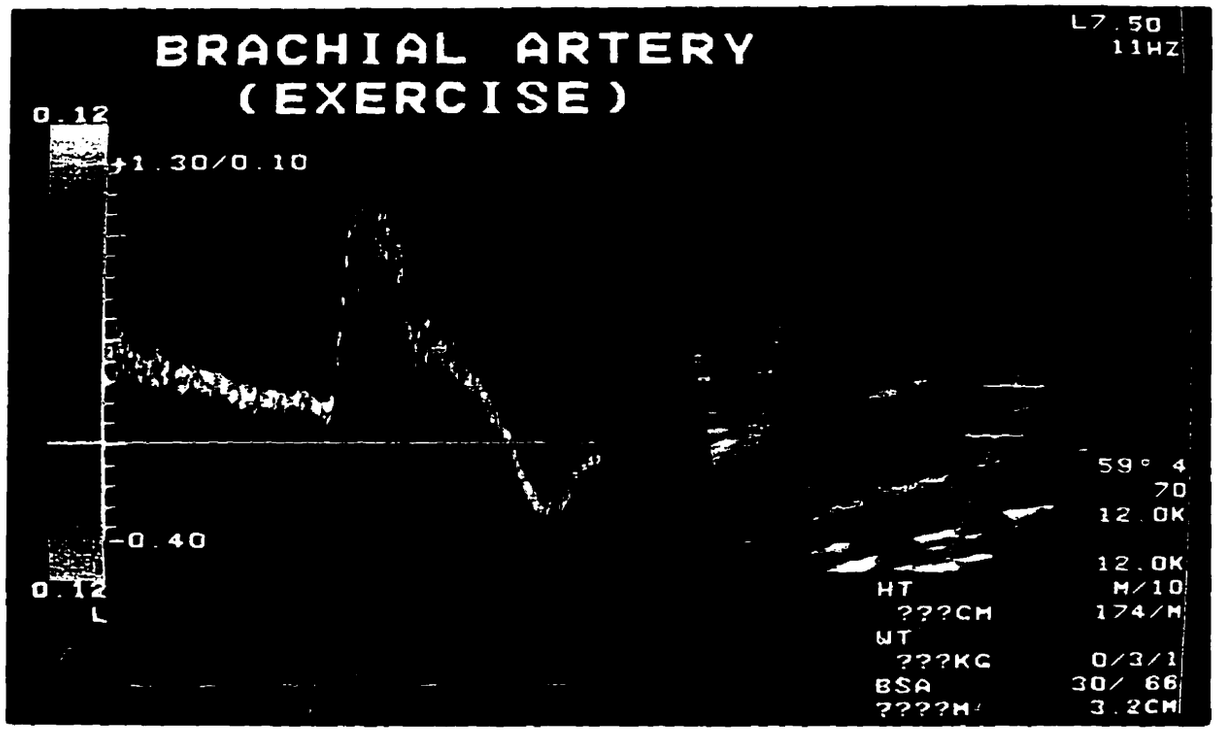
Intra-group differences between rest and exercise means of HR, SV, CO, DM, MBV, FBF, MPP, MAP and FVCI were compared through a paired T-test. Inter-group differences in these variables (except SV and CO) and in the MBV and FVCI kinetic parameters were compared through T-test for independent samples. The level of statistical significance was established at $p \leq 0.05$.

Figure 3.2. Brachial artery diameter assessment (at rest) through ultrasound imaging and Doppler system



Note: A sample of the pulsed Doppler spectral display (top tracing on left side) obtained from the lumen of the brachial artery as imaged by echo Doppler (right side). Three callipers markers were placed on the vessel wall to obtain three measurements [DIAM 1; DIAM 2 and DIAM 3 (bottom on left side)]. The averaged diameter (AVG D) was taken as the diameter for that point in time.

Figure 3.3. Brachial artery image during handgrip dynamic exercise.



Note: The pulsed Doppler spectra display (top tracing on left side) obtained from the lumen of brachial artery (right side) as imaged by echo Doppler. The pulsed Doppler spectra profile shows greater mean blood velocity compared to the rest values showed in the previous figure (Fig. 3.2).

Results

Participants' characteristics.

The mean values in Table 3.1 show that the older compared to the young group was 43.2 years older; 6.0 cm shorter; and 11.6 kg weaker in handgrip MVIC. Therefore the same absolute workload used in the study (4.4 kg.) required an extra 3.6 % MVIC to be moved by the older group.

Table 3.1. Participants' characteristics in the young and older group.

Group	Age (years)	Height (cm)	Weight (kg)	MVIC (kg)	Workload (% of MVIC)
young	28.8 ± 9.7 *	178.8 ± 3.1 *	76.7 ± 6.0	45.6 ± 4.2 *	9.7 ± 0.9 *
older	72.0 ± 6.4	172.8 ± 2.7	79.2 ± 5.1	34.0 ± 6.7	13.3 ± 2.0

Values are means ± SD; n = 6 and 5 for young and old groups, respectively. * means that the differences are statistically significant between the two groups at $p \leq 0.05$; MVIC=maximal voluntary isometric contraction.

Mean Blood Velocity

The difference in mean blood velocity at rest between the young and older group (7.89 ± 1.21 and 9.14 ± 1.66 cm/sec, respectively) was not statistically significant. It increased to 22.6 ± 2.5 in the young and to 22.9 ± 4.6 in the old (286 % increase in the young and 250% in old). The difference between rest and during exercise was significant in both groups. However, there was no significant difference between the groups either at rest or during exercise (Figure 3.4).

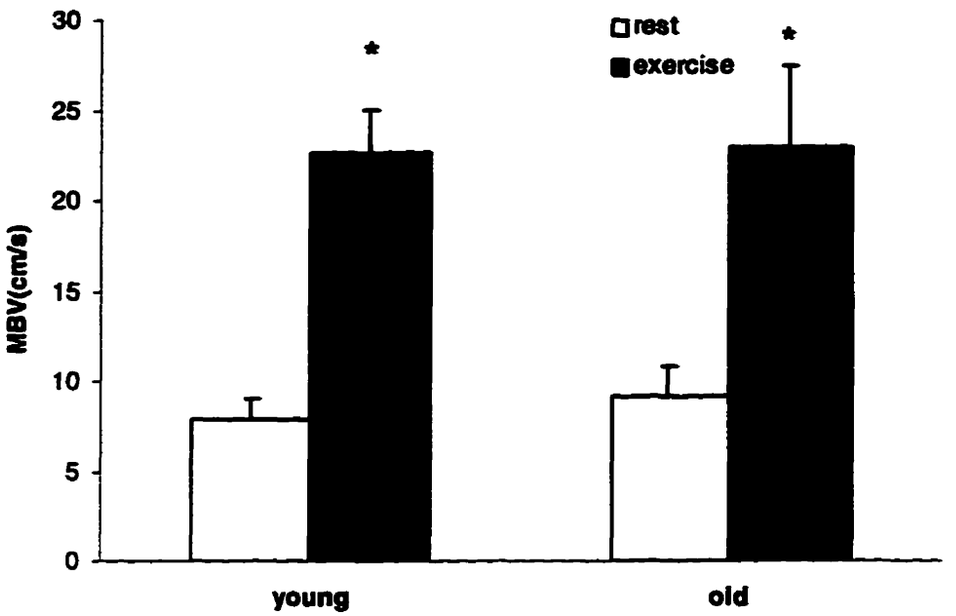


Figure 3.4. Mean blood velocity (MBV) the response to moderate handgrip exercise in young and old men. Values are means ± SD; n=6 in the young group and 5 in for older group tively. *the differences are statistically significant between rest and exercise at $p \leq 0.05$.

The mean values of blood velocity kinetic parameters (Table 3.2 and Figure 3.5) show that the blood velocity mean response time (MBV-MRT) and total gains were not statistically different between the young and older group of participants. More precisely, the only kinetic parameter that showed statistical difference was time delay 1(td1). That is, the beginning of the response to exercise was somewhat slower in the older group. Confounding factors such as the concentration of the participant and reaction time may be involved in the difference found in td1.

Table 3.2. Mean kinetic parameters of Mean Blood Velocity for young and old participants in response to a step change handgrip dynamic exercise.

Group	Young	Old
td1(sec)	0.58 ± 0.32*	2.17 ± 1.26
tau1(sec)	2.30 ± 1.83	4.45 ± 2.18
td2(sec)	16.63±8.90	23.49±5.00
tau2(sec)	26.14±19.28	17.07±10.34
g0 (cm/sec)	7.89±1.21	9.14±1.65
g1 (cm/sec)	7.00±1.48	6.04±2.82
g2 (cm/sec)	7.44±2.71	7.75±1.61
MRT (sec)	23.39±9.12	26.65±8.72
tg (cm/sec)	14.67 ±1.99	13.80 ± 3.22
mse (cm/sec)	2.30±2.03	1.72±1.09

Values are means ± SD; n = 6 and 5 for young and old groups, respectively. td1 = time delay from the onset of exercise to the beginning of the response; tau1 is the time when 63% of the response in the first component of the exponential curve was achieved up to td2; td2 = time delay from the onset of the exercise to the beginning of the second component of the exponential curve; tau2 = time when 63 % of the response in the second component of the exponential curve was achieved; g0 = values at rest; g1= gain obtained during the first component of the exponential curve up to td2; g2 = gain obtained during the second component of the exponential curve; MRT = time taken to reach 63 % of the overall response; tg = total gain obtained; mse = standard error of mean of the curve fit data; * = the mean values between the two groups are statistically different.

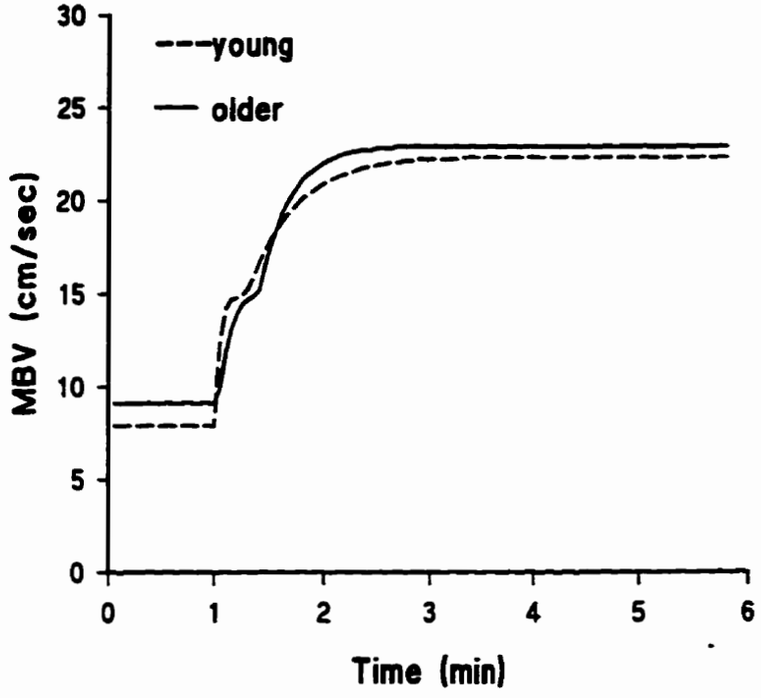


Figure 3.5. Mean Blood velocity (MBV) response to a moderate dynamic handgrip exercise in young and old men. The data were fitted by a two-component exponential model.

Diameter of the brachial artery

The diameter mean values for young (4.19 ± 0.48 and 4.19± 0.32 mm) and for old people (4.66 ± 0.42 and 4.72 ± 0.39 mm) at rest and during exercise, respectively , were not different within each group. When the means of the two groups were compared the older participants showed only a trend (p>0.07) to present larger DM in the brachial artery. Figure 3.6 illustrates the mean diameter values for both young and old groups.

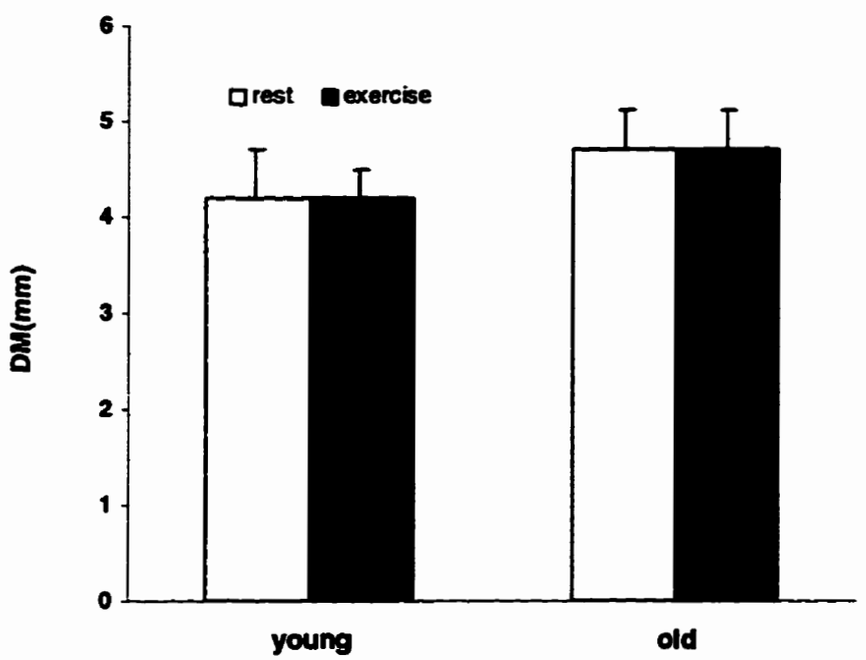


Figure 3. 6. Brachial artery diameter(DM) at rest and during moderate handgrip exercise in young and old people.

Blood Flow

Since the diameter of the brachial artery was not different during rest and exercise, the blood flow values were calculated using the mean diameter for each participant. The resting forearm blood flow was higher ($p \leq 0.05$) in the old group (94.5 ± 22.6 ml/min) compared to the young (64.7 ± 10.6 ml/min). The blood flow increased to 186.8 ± 38.2 ml/min in the young and to 235.8 ± 49.5 ml/min in the older subjects. The calculated net blood flow gain from rest to the steady - state phase during exercise was 122.1 ± 31.4 ml/min in the young and 141.3 ± 30.3 ml/min in the old. However, neither the difference during exercise nor the blood flow total gain was statistically significant between the two groups (Fig.3.7).

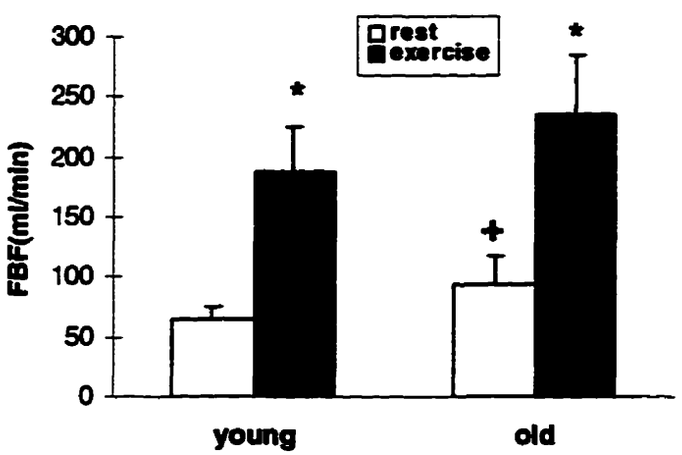


Figure 3. 7. Forearm blood flow (FBF) response to moderate handgrip exercise in young and old men. FBF is expressed in mililiters of blood per minute (ml . min^{-1}). *significant difference between rest and exercise; + significant difference between groups).

Mean Arterial Perfusion Pressure

The mean arterial perfusion pressure was statistically different when the rest values were compared to those at the last minute of exercise. The mean arterial perfusion pressure was almost identical in the young and old group during the rest period. The old people tend to have a higher blood pressure at the end of the exercise period, that is, the increase in blood pressure tends to be greater in the older people compared to younger (Table 3.3; Figure 3.8). However, this difference did not reach statistical significance.

Table 3.3. Mean Arterial Perfusion Pressure for young and old groups during rest and exercise.

Group	BPrest (mmHg)	BPend (mmHg)	% increase
young (n = 5)	78.7 ± 10.1	84.1 ± 9.6 *	7
old (n = 5)	78.4 ± 10.8	88.5 ± 5.8 *	13

Values are means ± SD; n = 6 and 5 for young and old groups, respectively. *= difference is statistically significant compared to resting values; BPrest= blood pressure mean values during 1 minute rest; BPend = blood pressure mean values at the end of exercise period (from 4th to 5th minute of exercise).

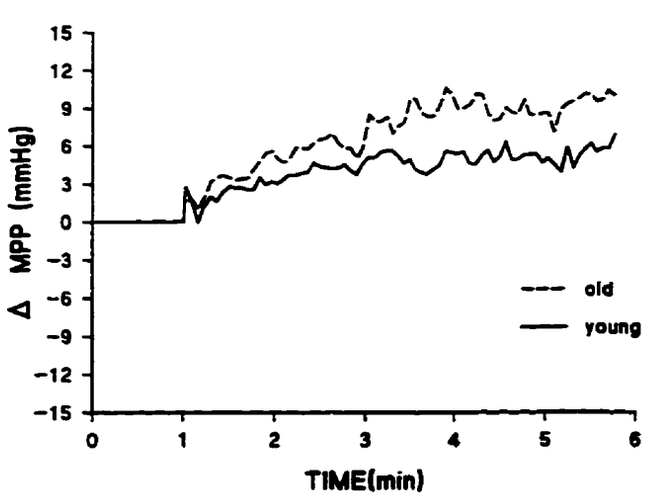


Figure 3.8. Mean arterial perfusion pressure(MPP) in response to moderate handgrip exercise in young and old men. (Beat-by-beat values were averaged for 4sec).

Forearm Vascular Conductance

An index of the forearm vascular conductance (FVCI) was calculated dividing MBV by MPP on a beat-by-beat basis. The resting values of FVCI were 0.10 ± 0.01 and 0.12 ± 0.03 cm (s . mmHg)$^{-1}$ in the young and old group, respectively. During exercise, the FVCI significantly increased to 0.26 ± 0.01 and 0.25 ± 0.07 cm (s . mmHg)$^{-1}$ in the young and in the old respectively. The differences between the two groups were not statistically significant ($p > 0.05$).

The kinetic parameters obtained through a two-component exponential model showed that the values for FVCI-mean response time (FVCI-MRT), tended to be slower in older people (24.4 ± 8.24 seconds) compared to 20.11 ± 7.34 seconds in the young group. However, this difference did not reach statistical significance.

Heart Rate

The heart rate values, either during rest or at the end of the exercise period, were not statistically different between the two groups. The increase in heart rate due to the exercise was not statistically significant in the young group, however the 4.4 bpm increase in the old group was statistically significant ($p<0.05$).

Table 3.4. Mean heart rate for young and old groups during rest and the last minute of exercise.

Group	HRrest (bpm)	HRend (bpm)
young (n = 6)	62.3 ± 12.6	65.9 ± 14.6
old (n = 5)	64.9 ± 10.6	69.3 ± 12.8 *

Values are means ± SD; n = 6 and 5 for young and old groups, respectively. *= difference is statistically significant compared to resting values; HRrest= heart rate mean values during 1 minute rest; HRend = heart rate mean values at the end of exercise period (from 4th to 5th minute of exercise).

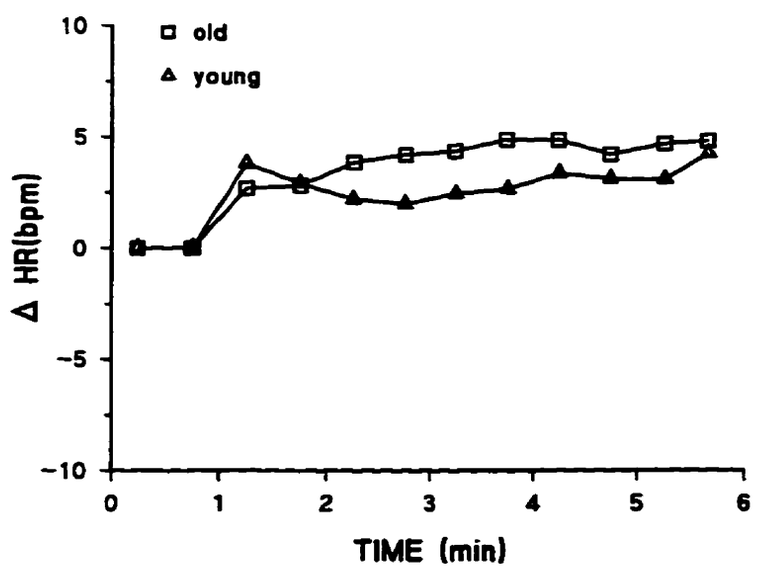


Figure 3.9. Heart rate(HR) changes in response to moderate handgrip exercise in young and old group. Values were averaged for each 30 second period.

Cardiac Output.

Cardiac output was evaluated in the old group to assess the effect of the dynamic moderate handgrip exercise on cardiac function. Stroke volume was similar when the mean resting value (69.6 ± 10.5 ml) was compared to the mean value during exercise (70.4 ± 11.3 ml). (Figure 3.10).

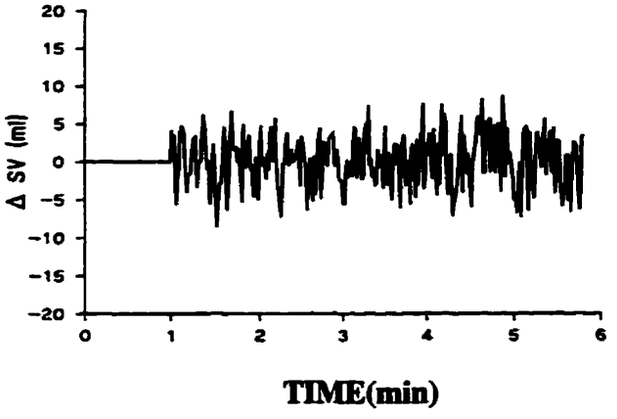


Figure 3.10.. Stroke volume (SV) response to moderate . handgrip exercise in older men. Values are the five-heart beat moving-average for all five subjects.

The mean cardiac output (CO) of 4.68 ± 0.34 L . min^{-1} observed at rest increased approximately 5% during the handgrip exercise to 4.96 ± 0.46 L . min^{-1} . This increase occurred throughout the exercise period (Fig.3.11).

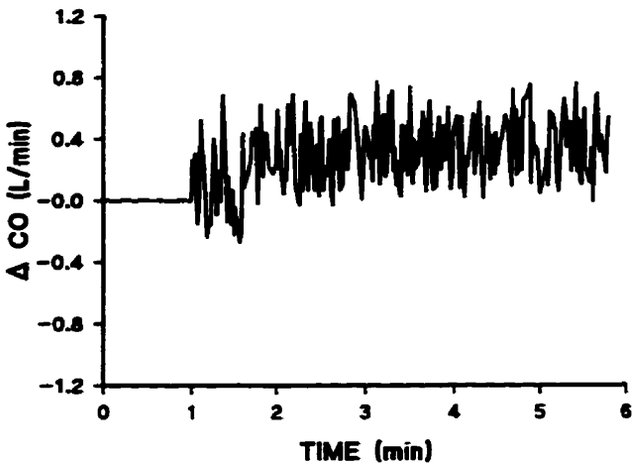


Figure 3.11. Cardiac output changes in response to moderate handgrip exercise in older men. Values are five-heart beat moving-average for all five subjects.

Discussion

In addition to what was already mentioned in the section on methods, it seems useful to include the following observations made during this study: 1) Regarding the measurement of MBV, the "looser" skin of the old people compared to young subjects appeared to impose an additional difficulty for this measurement. Despite the care taken to prevent any movement that could cause motion at the site of the measurement, a few times during the exercise, the Doppler signal started to deteriorate due to skin movement. When this happened, the operator rectified the situation by applying slight pressure with one finger to a specific part of the probe and the good quality of the Doppler signal was promptly recovered. Any small transient fluctuation of the Doppler signal due to skin movement was minimized by the averaging process over four trials; 2) The measurements of blood pressure might have been compromised over the relatively long period of data collection. That is, the finger cuff was kept inflated during the total time of each trial (6min). Cuff deflation between each of the four trials could have minimized this potential limitation.

Regarding the participants' characteristics (Table 3.1), the difference in MVIC between the two groups seems to be the most important difference to be discussed. The present study showed an age-related decline in strength around 25% in the old compared to the young group. This decline is within the range reported in the literature (Larsson, 1987; Roger and Evans, 1993; Skelton, 1994). This strength decline can potentially be attributed either to the loss of muscle mass or to some alteration of the muscle capacity to generate force (because of a reduced activation of motor units and/or a loss of contractile or mechanical properties of the

muscle) or to a combination of these two mechanisms thus making older muscle intrinsically weaker (Rogers and Evans, 1993).

Since we used the same absolute workload in the present study, then the older participants worked at a slightly higher relative workload (3.6% higher compared to the young group). However, the use of same absolute workload for young and old was judged to be advantageous in terms of: 1) the workload represented a moderate intensity of effort that appears to be normally faced during daily activities of both groups; 2) control for potential differences in blood flow that could be due to different absolute workloads. That is, the group performing the lowest absolute workload might not have to raise the blood flow as high as the other group because the absolute $\dot{V}O_2$ required could be lower. Consequently, this different gradient from rest to exercise, between the groups could have an effect on blood flow kinetics.

The present study showed that, in response to moderate dynamic handgrip exercise : 1)brachial artery MBV increased from rest to exercise in both groups, however there was no significant difference between the two groups. The time course of MBV increase in response to exercise, represented by the MBV-MRT, was not different in old compared to young; 2) FVIC increased in both groups, however no significant differences were found at rest, during exercise and in the time course to reach the new FVIC levels during exercise, between the two groups; 3) The brachial artery diameter did not change with exercise and there was no age-related change in diameter; 4) Forearm blood flow (FBF) at rest was higher in older compared to young participants; 5) Mean arterial pressure was not different between young and old, at rest and during exercise. However, the increase in MAP during exercise tended to be larger in old than in young; 6) HR from rest to exercise was significant higher in old but not in young

participants; 7) CO was slightly elevated in older participants.

Regarding the hypotheses of this study, the most important finding was that blood flow kinetics was not impaired with aging. This finding does not support the hypothesis that the time course of adaptation of blood flow kinetics at the onset of exercise is slowed with aging. Comparison of results between the present study and others is somewhat difficult for the following reasons: a) the present study appears to be the first one to use a direct and high temporal resolution method on a beat-by-beat basis to look at changes in age-related blood flow kinetics during exercise; b) Most of the studies done on age-related changes in blood flow have been focusing on steady-state blood flow or maximal capacity measured either with plethysmography (Allwood, 1958; Martin et al., 1990; Martin et al.,1991; Jaspersen et al., 1994; Taylor et al., 1992) and ^{133}Xenon washout (Amery et al., 1969) in humans or microsphere (Irion et al., 1987; Irion et al., 1988; Haidet and Parsons, 1991) and intravital microscopy (Tymil et al., 1992) in animals ; c) Some other studies measuring rate of P Cr (McCully et al.,1991) or O_2 saturation(McCully et al., 1994) recovery after exercise only give indirect evidence regarding blood flow. Results of these previous studies about age-related changes in blood flow kinetics are controversial (McCully and Posner, 1995).

Bearing in mind the above limitations for comparison purposes, the lack of difference between young and old participants in FBF-MRT at the onset of exercise, seen in the present study seems to be: a) in agreement with the results reported by McCully et al. (1995), where the rate of recovery in O_2 saturation did not change with age after ischemia; and this was taken as evidence for no age-related impairment in O_2 delivery. The subjects for this study were healthy older individuals who were exercising regularly; b) different than the results reported also by

McCully et al. (1991), when they observed age-related slowing of the P Cr recovery rate which could suggest impaired O_2 delivery (it appears that the subjects in this study were not exercising regularly as in the former mentioned study); c) not in line with results reported by Tyml et al (1992), using intravital microscopy in rats in which the post-ischemic blood velocity presented a slower return to pre-ischemic levels in the older blood vessels.

Although there is extensive literature suggesting age-related impairment in the parameters that could affect blood flow, the results of the current study demonstrated that the older participants could adjust their blood flow as fast as the young people in response to handgrip exercise. The mechanisms by which this fast response occurs remain to be studied. Meanwhile, some possible explanations are as follows: 1) If older people are impaired in their oxidative capacity (Essen-Gustavsson and Borges, 1986; Coggan et al. 1992), then more metabolites tend be formed at a faster rate which might increase the drive to vasodilation; 2) The exercise model used in the present study involved forearm muscles. These muscles appear to be extensively recruited over the lifespan during daily activities. Consequently, the age-associated decline in physical activity which could impair blood flow adjustment might not apply to this small muscle mass. In this regard, blood flow kinetics may follow the same trend seen in apparent controversial results between the studies on $\dot{V}O_2$ kinetics (Babcock et al.,1994a; Babcock et al, 1994b; Kowalchuk, 1995; Chilibeck, 1995) and in studies on the rate of P Cr or O_2 saturation recovery (McCully et al., 1991; McCully et al.,1995), where age-related changes in the kinetics appeared to be associated with the level of physical activity (training) of the muscles involved in the exercise protocol; 3) The results of the present study could be influenced by the fact that the old group performed a slightly higher relative workload than the young group; 4) The

exercise protocol in this study used only a workload approximately 10% of the MVIC and, at this workload, might not have stressed the system to a point where differences could show up.

In the present study, the kinetics of forearm blood flow (FBF) were represented by the kinetics of mean blood velocity (MBV) because the diameter of the brachial artery did not change from rest to exercise (Fig 3.6). Among the MBV kinetic parameters (Table 3.2 and Fig.3.5), only td1(time delay between the verbal signal to start the exercise and the beginning of blood velocity response) was slower in the older participants. Confounding factors such as level of concentration of the participant, reaction time and adjustment to the rate of rhythmic contraction/relaxation ratio make any interpretation difficult. Furthermore the magnitude of the above parameter appears not to have a significant impact in mean response time (MRT).

The magnitude of change in MBV between the two components of the exponential model (g1 and g2) were similar either inter or intra-groups. However, the kinetics of the first component (tau1) was smaller than the time constant of the second component (tau2). At the muscle level, the first component may be thought to be due to: 1) an immediate vasodilation of the arterioles in response to exercise. The signal is either transmitted neurally through intrinsic neurons of the vasculature wall, and/or propagated cell-to-cell electrically or biochemically, either in response to neural or mechanical stimuli and/or by the myogenic response caused by muscle contraction/relaxation; 2) the muscle pump acting by compression/decompression of the venules; 3) a combined effect of the mechanisms listed previously. The second component is largely influenced by flow-dependent and metabolic-dependent vasodilation which effects take a little longer to take place. Slower tau2 was also reported by Hughson et al. (1995), for young participants using the same exercise protocol. In both groups the MBV-MRT (time to 63% of

steady-state values), used to represent the overall kinetic response, was close to that reported in a previous study (Hughson et al., 1995).

FVCI significantly increased from rest to exercise steady-state in both groups, however it was not different between the two groups either during rest or during exercise. The trend of slowing FVCI kinectics in the old, can be explained by the non-significant slower changes in MBV(Table 3.2) and larger increase in MPP in the older group (Table 3.3 and Fig. 3.8). However, when the FVCI data were fitted by a two-component exponential model, no significant difference showed up between the two groups. Therefore, the present data suggest that the rate of vasodilation adjustment in the resistance vessels, in response to handgrip exercise, is not impaired with aging.

Having discussed the kinetics of adaptation at the onset of handgrip exercise which was the main purpose of the present study, it may be useful to discuss the steady-state values of the related variables measured in the present study. Therefore, the next part of this discussion is devoted to comparing the steady-state values found between young and old people with findings from the other studies.

The absolute MBV values at rest for both groups and the trend to be slightly higher in the old are in agreement with those values reported by Safar et al. (1981). However, the values for the young group were approximately 40% lower when compared to those reported by Anderson and Mark (1989), using Doppler technique. This discrepancy may be explained in terms of two differences between the studies a) Anderson and Mark' study involved men and women. Since women tend to present a smaller vessel diameter than men (Boutouyrie et al., 1992), the blood velocity may be faster in women; and b) the arm was supported at midthoracic level compared

to above heart position in the present study and the hydrostatic pressure might have affected the MBV values. In addition, the values found in the present study and those mentioned previously are by far greater than those reported by Laurent et al. (1988) for the resting blood velocity.

The FVCI increased significantly from rest to exercise in both groups, however the mean values were not different between the groups either during rest or during exercise. These findings are in agreement to those reported by Jasperse et al. (1994) and they are also in line with those reported by Martin et al. (1991) who did not observe any age-related decline in maximal calf conductance in men. These findings suggest that the vasodilatory response is not impaired with age.

The absolute mean values of brachial artery diameter found in the present study for the young people were close to those observed in previous studies (Hughson et al.,1995; Safar et al., 1981); however, they were somewhat smaller than those observed in a study done by Anderson and Mark (1989). One possible explanation for this difference may be due to a different room temperature during the experiment; that is, while the temperature was around 20° C in the present study, it was around 27° C during Anderson and Mark's study. It has been shown that temperature can influence brachial diameter (Whelan et al., 1994). The absolute mean value of brachial artery diameter in the older group was in between the 4.36 ± 0.47 mm reported by Safar et al.(1981) and the 4.9 ± 1.2 mm reported by Kawasaki et al. (1987). These inter-studies differences in Dm may be affected by the difference in participants' age and Dm variability between participants.

Some studies have shown that the diameter of the brachial artery changes under certain stresses (Anderson and Mark, 1989). Under a variety of physiological and pharmacological

conditions, these changes do not exceed 15% and this represents less than a 32% increase in cross-sectional area (London and Safar, 1993). In the present study, no changes were observed in diameter of the brachial artery in the young or old group in response to handgrip exercise. This finding is in agreement with those reported for young people using the same protocol (Hughson et al.,1995) and those reported for the femoral artery in young and older people during leg exercise at several intensities up to 80% of the peak power output (Hopman et al.,1995). Therefore, these findings support the idea that blood flow is increased in peripheral conduit arteries, in response to moderate exercise intensity, by solely increasing blood velocity while the cross-sectional area remained unchanged. This increase in blood velocity may be explained by vasodilation in the downstream arterial tree (from feeder artery to pre-capillary arterioles), which could indirectly be verified by increased vascular conductance in the exercising muscle.

Comparison of the brachial artery diameter between the two groups showed no significance. This is in agreement with results of a previous study in which brachial artery diameter did not change with age (Boutouyrie et al., 1992). However, it is worth noting that the present study showed a trend towards increasing brachial artery diameter with aging ($p = 0.07$). Such a trend was also shown in other studies (Safar et al.,1981; Kawasaki et al., 1987). Differences in the results of the studies may be due to the fact that aging appears to have different effects along the arterial tree, and the peripheral conduit arteries could represent the transition point. That is, for proximal arteries such as thoracic, abdominal aorta and common carotid, increased dilation predominates over increased thickness, while in more peripheral arteries, increased thickness predominates over dilation with aging(Learoyd and Taylor, 1966). However, this issue appears to warrant further studies.

When the forearm blood flow was calculated (MBV . cross-sectional area), the absolute values at rest, in both groups, were within the range observed in a previous study (Safar et al.,1981), however the mean blood flow (64.7 ± 10.6 ml . min^{-1}) found in the young group and 94.5±22.6 ml . min^{-1} in the old, are far smaller compared to 123 ± 20 ml. min^{-1} observed by Anderson and Mark (1989) in young participants. Since blood flow was calculated from MBV and vessel cross-sectional area, the same arguments used to explain the discrepancy in those variables between the two studies are appropriate to explain the difference in blood flow values.

The finding that resting forearm blood flow was greater in old compared to young is in agreement with a previous study (Safar et al., 1981) which also measured blood flow in the brachial artery and with another study (Foley et al., 1993) that measured blood flow in the anterior and posterior tibial arteries using Magnetic Resonance Angiography (MRA). However, Jasperse et al. (1994) observed no age-related changes in brachial artery blood flow. Once again, these conflicting results warrant further investigation.

The present study failed to show any significant difference in forearm blood flow changes from rest to exercise between young and old participants. The magnitude of changes in both groups were close to the 131 ml/min gain in young participants, reported by Hughson et al.(1995) using the same exercise protocol. These findings are also in line with the results observed by Jasperse et al. (1994) who did not find any age-related decline in submaximal and peak levels of FBF in response either to handgrip exercise or following blood flow occlusion plus exercise. Conversely, they observed greater FBF in the old compared to young , at the highest level (~ 60% of maximum) of sustained (8min) exercise. Based on these findings, they concluded that older subjects were not limited in the regulation of active muscle blood flow during handgrip

exercise.

The mean arterial perfusion pressure(MPP) increased slightly from rest to exercise in both groups(Table 3.3). The old group tended to present a higher MPP in response to the exercise (Figure 3.8), however, the difference was not statistically significant compared to the young group.

The site of the blood pressure measurement was approximately 20cm above the heart level. When the MPP resting values were corrected for this height factor, then the estimated mean arterial pressure (MAP) during rest was approximately 95 mmHg and this is close to the values reported by Safar et al. (1981) for young and old normotensives. The lack of difference between the groups is noteworthy in the present study, in spite of the extensive literature pointing to an increased blood pressure with aging either at rest or during exercise (Folkow and Svanborg, 1993; Lakata, 1993; Saltin, 1986). However, age-related increased blood pressure has been mostly reported in terms of systolic blood pressure. Decrease in blood pressure in the "very old" is also reported (Folkow and Svanborg, 1993). Also a selection factor (exclusion criteria: systolic and diastolic blood pressure greater than 140 and 90 mmHg, respectively) might have contributed to similar MAP between young and old groups. Such observations may explain the lack of any age-related changes in estimated MAP found in the present study in which the mean age in the old group was 72 years. In addition, similar MAP, found in the present study for young and old participants, is in agreement with the findings of Jasperse et al. (1994).

The small increase (5%) in cardiac output in the old group (Fig. 3.11), appears to be due solely to an increase in heart rate (Fig. 3.9) because the stroke volume did not change from rest to exercise(Fig.3.10). Since HR was not different from rest to exercise in the young group, CO

might not have changed in this group. In addition, an increase in CO in the old, might have contributed to the larger increase seen in blood pressure in old compared to the young group. CO changes cannot be ruled out as a contributor to the forearm blood flow changes; however, this extra amount of blood could go elsewhere. Interpretation of the implications of CO changes is very limited in this study because: 1)the vasoconstriction status of the splanchnia and other vascular beds was unknown and blood could go elsewhere; 2) the 5% change observed is less than the variability of measurements intrinsic to impedance cardiography (~10%), and 3) the normal physiological variability in cardiac output at rest is approximately 1 L/min (Eriksen et al., 1990), which is much greater than approximately 0.3 $L \cdot min^{-1}$ change observed in this study in older participants.

Conclusions

Most importantly, our data on blood flow kinetics have shown that there was not an age-related impairment in the regulation of blood flow kinetics at the onset of a moderate step change in dynamic handgrip exercise. It is noteworthy to mention that the present findings are limited: 1) to dynamic contractions of small muscle mass in which potential age-related limitations imposed by the central circulation do not appear to play any significant role. This may not be the case during large muscle dynamic exercise in which the blood flow kinetics may be limited by the kinetics of the pumping capacity of the heart; 2) to small muscle mass that appears to be recruited extensively throughout the lifespan. This may not be the case for muscles that can be largely affected by the age-related association with decreased physical activity levels and; 3) to a moderate level of exercise and the results may be different when higher exercise intensities challenge the system close to its maximal capacity.

Future Directions

1. Compare age-related blood flow kinetics during higher intensities of handgrip dynamic contractions.

2. Compare age-related blood flow kinetics during large-muscle dynamic exercise (e.g. blood flow in the femoral artery during exercise in the kicking ergometry).

3. Compare the effects of training on blood flow kinetics in older men and women.

CHAPTER IV

STUDY # 2

FOREARM BLOOD FLOW KINETICS IN RESPONSE TO HANDGRIP DYNAMIC CONTRACTIONS IN NORMALLY ACTIVE AND VERY ACTIVE OLDER PEOPLE.

Introduction

Martin et al. (1991) reported that highly trained older runners had higher leg vascular conductance and maximal calf blood flow compared to their sedentary counterparts. However, it is not yet clear whether habitually active elderly reach submaximal levels of blood flow and vascular conductance in response to exercise, faster than their less active counterparts is not yet clear.

There is a dearth of information regarding changes in the time course of the blood flow adaptation to the requirements imposed by exercise. Indirect evidence of blood flow kinetics based on phosphocreatine (PCr) and O_2 saturation rate of recovery revealed controversial results. In one of these studies (measuring PCr rate of recovery) older subjects were found to have significantly slower rates of recovery; that is, the time taken for PCr replenishment after exercise (McCully et al., 1991); while the other study (measuring O_2 saturation rate of recovery) found no significant age-related impairment (McCully et al., 1994). One explanation for this controversy was the different level of physical activity presented by the samples in different studies, in the sense that, only less habitually active older people presented a significant age-related impairment (McCully et al., 1994). Therefore, it seems fair to interpret that higher physically conditioned older people can show faster blood flow adaptation compared to their less active counterparts.

Although these indirect blood flow measurements (PCr and O_2 saturation rate of recovery) provide useful information, the results obtained through them seem to be a

combination of blood flow (O_2 delivery) and O_2 utilization. Therefore, further studies, particularly using techniques that provide more direct and non-invasive measurements with beat-by-beat resolution, are needed to improve the knowledge about the potential role played by the aging process and/or physical activity on blood flow kinetics. These requirements can be fulfilled by measurements of blood velocity and arterial diameter through ultrasound techniques during continuos exercise.

In study # 1, both young and old participants were normally active and no difference in blood flow kinetics was found between them. However, what is not known is whether chronically higher levels of physical activity affect blood flow kinetics in the elderly . Do their blood flow kinetics respond the same way as was seen in young people in response to training (Shoemaker et al., 1996)? Can older people demonstrate plasticity in the cardiovascular system that can lead to faster blood flow kinetics?

The hypothesis of this study was that the blood flow kinetics at the start of exercise would be enhanced in habitually active elderly compared with their less active counterparts.

METHOD

Detailed information on the methodology used in this study was presented in the previous study.

Subjects.

Five normally active and five very active healthy men participated in the study. They were all normotensives (resting systolic and diastolic pressure lower than 140 and 90 mmHg, respectively). None of them reported a medical history of cardiovascular disease nor was anyone on medication known to interfere with blood flow control. Informed consent was obtained from the participants and the study was approved by the Office of Human Research and Animal Care of the University of Waterloo.

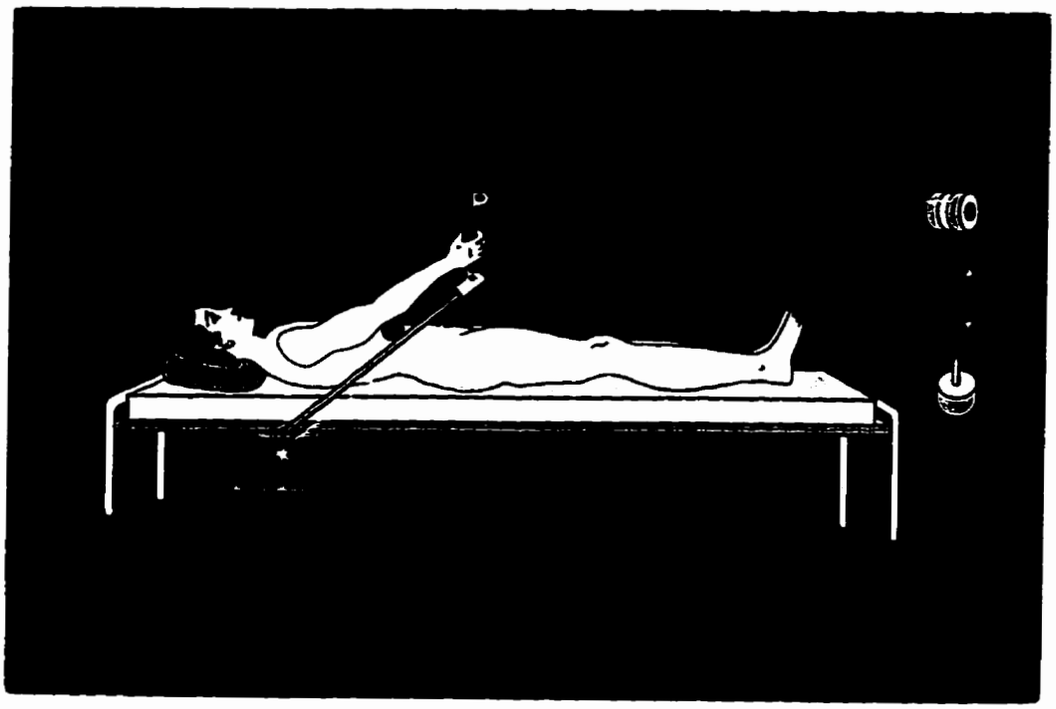
The so-called normally active group of participants (NA) comprised healthy older men who were living independently with involvement in common daily activities such as walking, gardening and snow shovelling, but none was engaged in a regular physical activity program. The very active group(VA) comprised healthy older men, who were playing hockey regularly (2 -3 times a week) in addition to the physical activities required in daily living. They were tested at the end of the hockey season. In addition, during the "off-season", they are regularly swimming, playing golf, bowling or riding their bikes. Therefore, they are very active all year-round.

Experimental Protocol.

Dynamic handgrip contractions were performed while the participants reclined

comfortably in the supine position. The exercising arm(which was the right one in all subjects) was supported on a platform that elevated it to an angle of 50° relative to the horizontal and extended to a handgrip device. The participant then lifted and lowered a weight of 4.4kg, through a vertical distance of 5cm, at a frequency of 20 contractions/min, in a pulley system (Figure 4.2) for five minutes. This sequence was repeated five times with a minimum ten minutes rest between trials to ensure that the subjects returned to baseline values.

Figure 4.1. Illustration of the experimental protocol.



Measurements.

Maximal Voluntary Isometric Handgrip Contraction (MVIC). The participant performed three maximal voluntary handgrip contractions with a rest period between them. The mean value was recorded as the participant's forearm MVIC.

Heart Rate. Heart rate was continuously monitored on a beat-by-beat basis with an electrocardiogram through 3 spot electrodes on the skin.

Blood Velocity. Measurements of MBV were taken similarly to that described by Tschakovsky et al. (1995). Doppler equipment (model 500 V, Multigon Industries, Mt. Vernon, NY) operating in pulsed wave mode was used. After determination of brachial artery position by palpation (~ 2cm proximal to the antecubital fossa) a 4-MHZ flat probe was manipulated over the brachial artery until optimal auditory and visual signals were obtained and then the probe was taped on the skin. The angle of insonation of the built-in transducer relative to the skin was 45°. It was assumed that the brachial artery ran parallel to the skin surface at the site of probe position. The gate was set at full width (12 mm) to facilitate insonation of the total width of the artery with approximately constant intensity. With this apparatus, it was possible to maintain a clear Doppler signal both at rest and during exercise.

Vessel Diameter. An ultrasound imaging (echo-Doppler) and Doppler System (model SSH-140-A, Toshiba Corporation, Japan) operated in Doppler (D) and brightness modulation (B) modes was used. After determination of brachial artery position by palpation proximal to the antecubital fossa, a 7.5 -MHZ hand-held probe, comprising an array of transmitting and receiving transducers, was manipulated over the brachial artery until optimal auditory and visual

signals were obtained. The vessel wall image and blood velocity profiles were video-taped for subsequent analysis. During the analysis, the velocity profiles generated by this system were only used to provide additional feedback about the optimal position of the probe.

Mean Arterial Perfusion Pressure (MPP): A photo plethysmograph finger blood pressure cuff (Ohmeda 2300, Finapres) was applied on the middle finger of the contralateral hand. The hand was rested at the same level as the ultrasound probe on the exercising arm.

Data Storage.

The electrocardiogram, Finapres, Doppler signals, including time, were recorded on a microcomputer data acquisition system. These signals were collected continuously for each 6-min trial. The Echo-Doppler images collected during 1 trial for each participant were stored on VCR tapes.

Data Analysis.

The MBV, HR, MPP data collected on a beat-by-beat basis from four repetitions for each subject were averaged at 1-s intervals to yield a single data set per subject. This method is similar to that described previously to examine breath-by-breath VO_2 (Phillips et al., 1995; Shoemaker et al., 1994) and MBV (Hughson et al.,1995; Shoemaker et al., 1994).

Mean arterial pressure was estimated by adding the MPP plus the difference in mmHg (2 mmHg per inch) due to the vertical distance between the site of measurement and the heart level. Forearm vascular conductance index (FVCI) was calculated by the quotient between MBV and MPP.

The MBV kinetic parameters for the response to the step increase in work rate were described from a two-component exponential model, already used in previous studies of MBV (Tschakovsky, 1993; Hughson et al., 1995). This model is the same used to describe $\dot{V}O_2$ kinetics (Cochrane et al, 1992; Hughson et al, 1993; Phillips, 1995).

Diameter (Dm) of the brachial artery was assessed by freezing the VCR image at 5 different points in time during the resting period (one for each ten second interval) and during the exercise period (one for each minute). For each point in time, three measurements were taken and the mean of these values was used to represent the diameter at that point in time, and the mean diameter values for rest and exercise were calculated for each participant.

Statistical Analysis

Intra-group differences between rest and exercise means of HR, Dm, MBV, MPP, and FVCI were compared through a paired T-test. Inter-group differences in these variables and in the MBV and FVCI kinetic parameters were compared through T-test for independent samples. The level of statistical significance was established at $p \leq 0.05$.

Results

Maximal voluntary isometric contraction

The mean values in Table 4.1 show that the old very active (VA) had stronger forearm muscles compared to the old normally active (NA) group. This difference in MVIC meant that the same absolute workload used in the study (4.4 kg.) required an extra 3.3 % MVIC to be moved by the participants in the NA group.

Mean Blood Velocity

The resting mean blood velocity was not different between the normally active and very active groups (9.14 ± 1.66 $cm.s^{-1}$ and 8.32 ± 1.0 $cm.s^{-1}$, respectively). With exercise MBV increased to 22.9 ± 4.6 in the NA and to 21.3 ± 3.3 in the VA, the difference between rest and exercise was significant within both groups ($p \leq 0.05$). However, there was no significant difference between the groups either at rest or during exercise.

Figure 4.2 illustrates the MBV data fitted by a two-component exponential model. The kinetic parameters obtained (refer to previous study for details), are shown on Table 4.2. Most importantly, the blood velocity mean response time (MBV-MRT) and total gains were not statistically different between the NA and the VA groups of participants. The MBV-MRT ranged from 18.82 to 36.64 cm . s^{-1} and from 17.79 to 25.35 cm. s^{-1} , in the NA and VA groups, respectively.

Table 4.1. Participants' characteristics in the normally active and very active groups of older people.

Group	Age (years)	Height (cm)	Weight (kg)	MVIC (kg)	Workload (% of MVIC)
NA	72.0 ± 6.4	172.8 ± 2.7	79.2 ± 5.1	34.0 ± 6.7	13.3± 2.0
VA	70.4 ± 5.6	173.8 ± 6.05	80.6 ± 10.3	42.0 ± 4.6 *	10.6 ± 1.1 *

NA = normally active; VA = very active; values are means ± SD; n = 5 in each group; * means that the differences were statistically significant between the two groups at $p \leq 0.05$; MVIC=maximal voluntary isometric contraction.

Figure 4.2. Curve-fitting procedures for mean blood velocity data (MBV). For illustration purposes only, data from the 5 participants in the normally active group were averaged over a one second window to produce one single data set (dotted line). Those data were than fitted by a two-component exponential model (continuous line). This procedure was repeated on each participant's data to obtain the kinetic parameters.

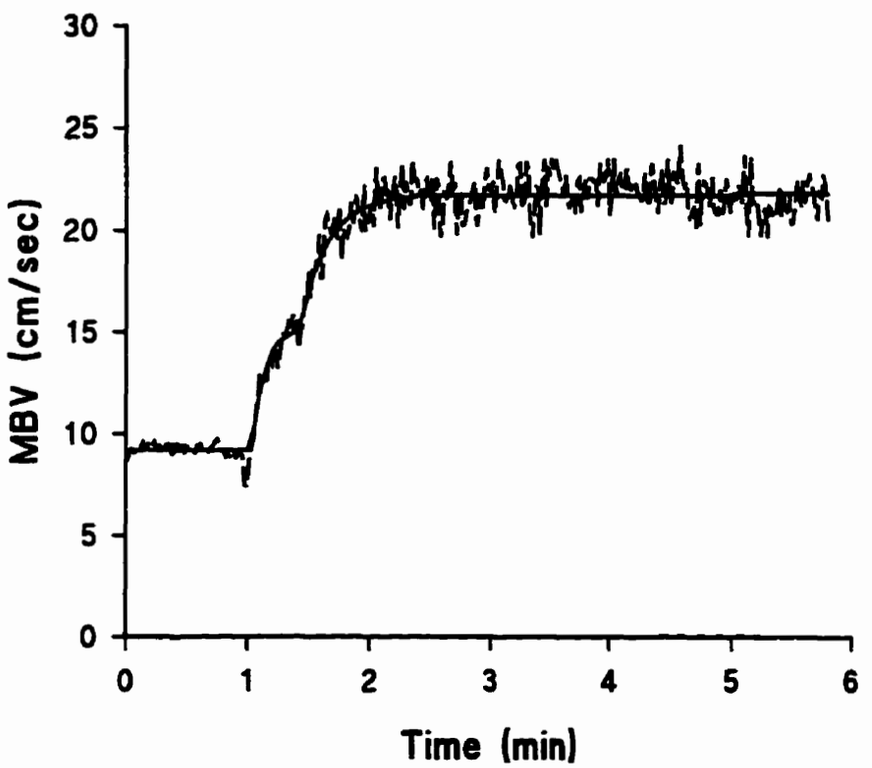


Table 4.2. Mean kinetic parameters of mean blood velocity for normally active and very active older people in response to a step change handgrip dynamic exercise.

Group	NA	VA
TD1(sec)	2.17±1.26	1.04 ± 0.33
g0 (cm/sec)	9.14±1.65	8.32 ± 1.02
tau 1(sec)	4.45±2.18	2.54 ± 1.12
g1 (cm/sec)	6.04±2.82	7.12 ± 1.51
TD2(sec)	23.49±5.00	18.28 ± 2.88
g2 (cm/sec)	7.75±1.61	5.88 ± 1.04
tau 2(sec)	17.07±10.34	20.59 ± 4.34
MRT (sec)	26.65±8.72	21.20 ± 2.96
tg (cm/sec)	13.80 ± 3.22	13.01 ± 2.38
mse (cm/sec)	1.72±1.09	2.35 ± 1.21

Values are means ± SD; n =5 for each group, respectively. td1 = time delay from the onset of exercise to the beginning of the response; tau1 is the time when 63% of the response in the first component of the exponential curve was achieved up to td2; td2 = time delay from the onset of the exercise to the beginning of the second component of t exponential curve; tau2 = time when 63 % of the response in the second component of the exponential curve was achieved; g0 = values at rest; g1= gain obtained during the first component of the exponential curve up to td2; g2 = gain obtained during the second component of the exponential curve; MRT = time taken to reach 63 % of the overall response; tg = total gain obtained; mse = standard error of mean of the curve fit data; * = the mean values between the two groups are statistically different.

Diameter of the brachial artery

Resting brachial artery diameters for NA (4.66 ± 0.42 mm) and VA (5.04 ± 0.94 mm) were not different ($p > 0.05$). Diameters during exercise for NA (4.72 ± 0.39) and VA (5.15 ± 1.03) were also not different from each other or their resting counterparts ($p > 0.05$). The mean diameter of the VA group was inflated by one participant whose brachial artery diameter was close to 7 mm.

Forearm blood flow

Calculated resting blood flows for NA (93.9 ± 20.7 $ml.min^{-1}$) and VA (100.7 ± 33.0) were not different ($p > 0.5$). Forearm blood flows during exercise for NA (238.8 ± 46.4) and VA (264.2 ± 73.8) were not different from each other, however they were different from their resting counterparts ($p \leq 0.5$).

Mean Arterial Perfusion Pressure

The mean arterial perfusion pressure difference was statistically significant when the rest values were compared to those at the last minute of exercise, in both groups (Table 4.3). The VA participants tended to present a smaller change in blood pressure during exercise , however, this difference did not reach statistical significance.

Forearm Vascular Conductance

An index of the forearm vascular conductance (FVCI) was calculated by dividing MBV by MPP on a beat-by-beat basis. The resting values of FVCI were 0.12 ± 0.03 and 0.11 ± 0.01 cm $(s . mmHg)^{-1}$ in the NA and VA groups, respectively. During exercise, the FVCI significantly increased to 0.25 ± 0.07 and to 0.27 ± 0.04 and $(s . mmHg)^{-1}$ in the NA and VA, respectively. Resting and exercise FVCI mean values were not different between the two groups ($p>0.05$).

The kinetic parameters obtained through a two-component exponential model showed that the values for FVCI-mean response time (FVCI-MRT), were not different in the NA (24.4 ± 8.24 seconds) compared to 17.79 ± 3.81 seconds in the VA group . However, it seems that the VA group tended to have a faster increase in FVCI compared to the NA group and this is illustrated in Figure 4.3.

Heart Rate

The heart rate values (Table 4.4), either during rest or at the end of the exercise period, were not statistically different between the two groups. The slight increase in heart rate due to the exercise was statistically significant ($p<0.05$) in both groups. The participants in the VA group tended to present lower heart rate at rest and during exercise.

Table 4.3. Mean Arterial Perfusion Pressure (MPP) changes in normally active and very active older people in response to moderate handgrip dynamic exercise.

	MPP		
Group	**Rest**	**Exercise**	**% increase**
NA	78.4 ± 10.8	88.5 ± 5.8 *	13
VA	79.6 ± 7.8	86.7 ± 7.4 *	9

NA= normally active group; VA=very active group; values are means ± SD in mmHg; n = 5 for each group. *= difference is statistically significant compared to resting values; Rest= blood pressure mean values during 1 minute rest; Exercise = blood pressure mean values at the end of exercise period (from 4th to 5th minute of exercise).

Figure 4.3. Changes in forearm vascular conductance index (Δ FVIC) in response to moderate dynamic handgrip exercise in normally and in very active older people. Data were averaged by each 4-s

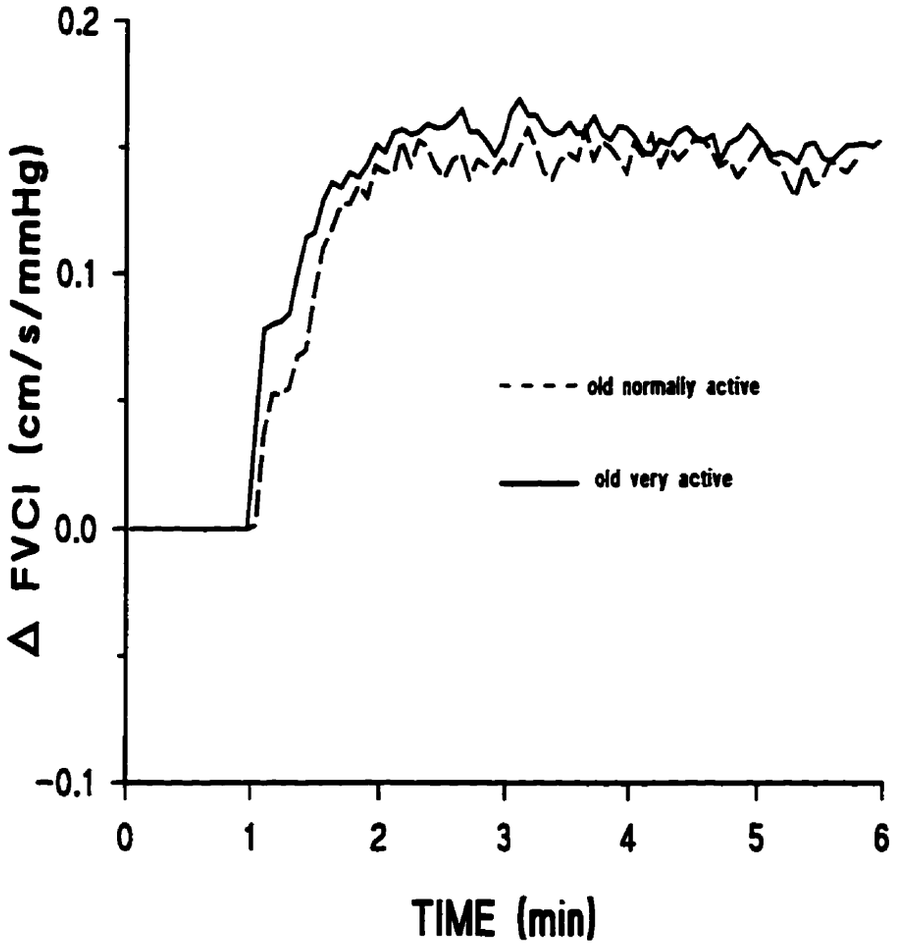


Table 4.4. Mean heart rate (HR) for normally active (NA) and very active (VA)groups of older people.

	Heart Rate	
Group	**Rest**	**Exercise**
NA	64.9 ± 10.6	69.3 ± 12.8 *
VA	56.7 ± 8.4	60.2 ± 9.0 *

Values are means ± SD; n = 5 for each group. *= difference is statistically significant compared to resting values; Rest= heart rate mean values during 1 minute rest while participants were lying instrumented prior to exercise; Exercise= heart rate mean values at the end of exercise period (from 4th to 5th minute of exercise).

Discussion

The present study showed that, in response to moderate dynamic handgrip exercise : 1) brachial artery MBV increased from rest to exercise in both groups, however, there was no significant difference between the two groups. The mean response time of MBV increase, in response to exercise, was not different in normally active compared to very active older people; 2) brachial artery diameter did not change with exercise; 3) Mean arterial pressure was not different between the two groups , at rest and during exercise; 4) FVCI increased in both groups, however, no significant differences between groups were found at rest, during exercise and in the mean response time to reach higher FVCI levels during exercise; 5) HR from rest to exercise was slightly increased in both groups, however, no significant inter-group difference was found.

Since this study found no difference in brachial artery diameter from rest to exercise, the blood flow kinetics are assumed to have been represented by MBV kinetics. Regarding the hypothesis of this study, the most important finding was that forearm blood flow and vascular conductance kinetics were not faster in very active compared to normally active older people. Therefore, this finding does not support the hypothesis that habitual vigorous activity speeds up the time course adaptation of blood flow kinetics or vascular conductance at the onset of small muscle moderate exercise. However, despite the fact that comparison of MBV-MRT and FVCI -MRT values did not reach statistical significance ($p > 0.05$) , it is worth noting that the MBV-MRT and FVIC - MRT mean values in the VA group tended to be 20% and 30%, respectively, faster than the NA group. In spite of lacking statistical significance, increased G1, faster tau 1, TD1, TD2 and smaller TG contributed to the trend for a faster MRT in the VA group. This trend is illustrated in Figure 4.3. This study has also shown that there is a large variability between

individuals in MBV- MRT values. The range of values in the groups overlapped each other. Therefore, the results of the present study are potentially limited by the size of the sample (five participants in each group), which might be too small for cross-sectional studies.

The above possible explanation is also reinforced when a statistical power calculation is done on the data. The calculated power was 0.245 which appears to be weak compared with the desired power of 0.800. To increase the power to the desired level, the sample size estimation is 21 participants in each independent sample.

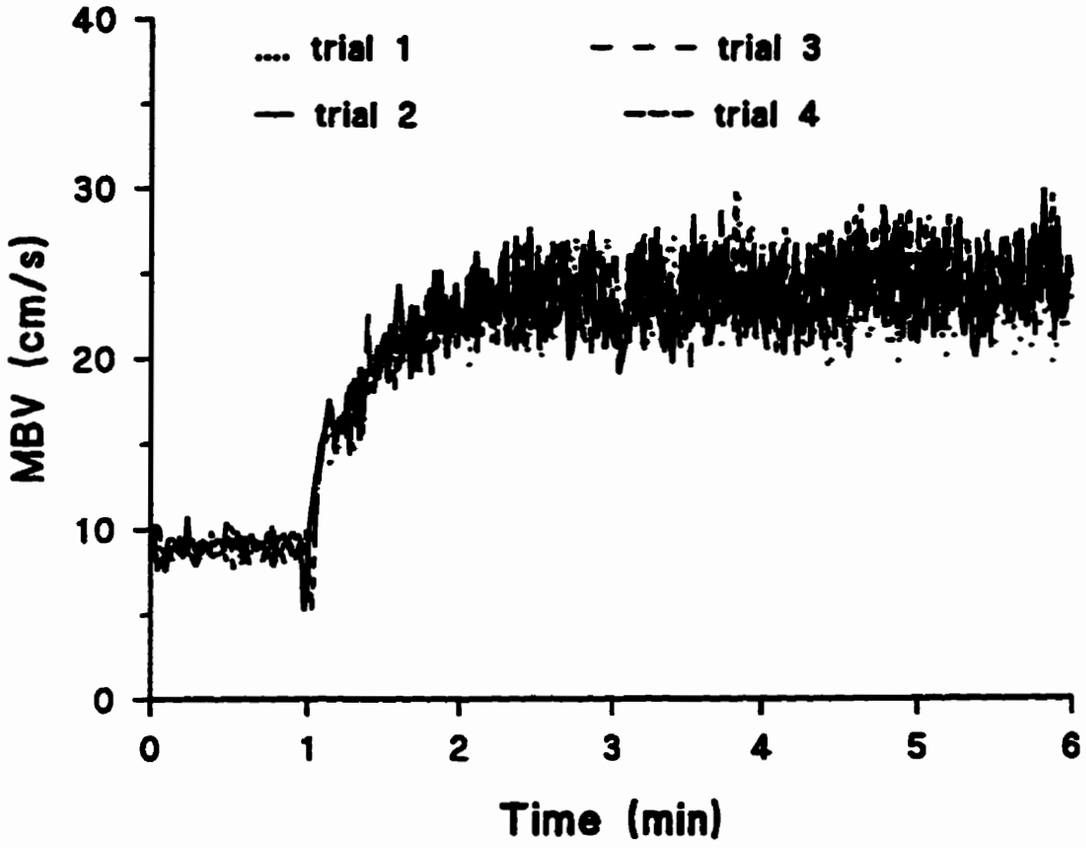
Another way to look at the blood flow kinetics results is by calculating the confidence interval (CI) of the parameter estimation (MBV-MRT). This can be done by applying the equation described by Lamarra et al., (1987) which follows:

$$n = (L \cdot S_o / K_n \cdot \Delta Y_{ss})^2$$

where L is a constant (47.5 for a 95% CI); S_o is the standard deviation of the MBV fluctuation; K_n is the confidence interval; and ΔY_{ss} is the difference between the MBV mean value during steady-state and at rest.

The calculated 95% CI for MBV-MRT was 3.16 seconds for one subject in the VA group (Figure 4.4) and 3.58 seconds for one subject in the NA group. Using these CI values for the MBV-MRT mean values in both groups, the MRT is expected to fluctuate between 24.36 and 18.04 seconds for the VA group and between 23.07 and 30.23 seconds in the NA group.

Figure 4.4. Mean blood velocity (MBV) beat-by-beat data, from one very active older man is plotted for each individual trial (total of four trials).



In an effort to explain the findings of the present study, we have to deal with complex and, as yet largely unclear group of mechanisms that control blood flow and their potential changes with physical conditioning e.g., augmented release of nitrous oxide with training (Sun et al., 1994) and enhanced vascular responsiveness to ischemic metabolites (Green et al., 1994) may have tended to act in favour of faster blood flow adaptation in the VA group.

If the habitual very active exercise pattern acted as an ongoing training stimulus for the old hockey players, it is reasonable to assume that they would have an elevated capillarization (Andersen and Henriksson, 1977). This morphological advantage might translate into a faster adaptation of blood flow in the VA group at the onset of an exercise challenge.

Increased rate of metabolite production due to lower oxidative capacity would tend to be in favour of a faster blood flow kinetics for the NA group. On the other hand, it is unlikely that the muscle pump could have a differential role between groups because both groups performed the same absolute workload, at the same contraction rate, in the same posture.

Changes in muscle sympathetic neural activity or vascular responsiveness to norepinephrine with training, which would favour faster blood flow adaptation in the NA group, remains a matter of debate (NG et al, 1994; Somers, 1988; Wiegman et al. 1981; Svedenhag et al. 1991). Furthermore, the mild workload performed dynamically by a small muscle mass (unilateral handgrip) may have prevented or minimized any exercise-induced effect on sympathetic tone.

Difference in the relative workload performed by each group might have, for some unknown reason, been involved in the lack of difference in blood flow kinetics between the groups. It can be seen from Table 1 that the two groups of subjects in this study were similar in

terms of age, height and weight. However, the VA were substantially more active than the NA group. While the NA did little more than activities of daily living, the VA were engaged in recreational hockey games lasting approximately 90 minutes at least twice per week. Moreover, in addition to total body involvement to the demands of hockey, there was substantial forearm activity in stick handling. Consequently, it is not surprising that the forearm voluntary isometric contraction values of the hockey players were substantially greater than the less active group.

In the development of a protocol to examine whether the history of habitual high level physical activity translates into faster blood flow kinetics at the onset of exercise, it was necessary to decide whether to use an absolute or relative exercise challenge. The decision was made to use an absolute load (4.4 kg) partly to reference previous data using that workload. In addition, it was felt that older adults are frequently confronted with fixed weight tasks which are not modified because of their age or level of physical fitness. This decision meant that the absolute workload (4.4 kg) of the VA group was relatively less demanding than the same workload for the NA group who had smaller strength capacity.

It has been demonstrated that in a muscle accustomed to daily activities, the $\dot{V}O_2$ kinetics (Chilibeck et al., 1996) and the rate of O_2 saturation (McCully et al., 1991) was not reduced with aging. Their findings might establish the basis for an additional explanation for the lack of difference in MBV and FVCI observed in the present study. That is, the normal daily activities, involving the forearm muscles, might have provided sufficient stimuli in the normally active group and compromised any significant difference.

In conclusion, the findings of the present study do not support the hypothesis that blood flow kinetics are faster in very active compared to normally active older men. However, there

was a tendency for faster blood flow kinetics and forearm vascular conductance in the very active group of participants and statistical significance may have been compromised by the small number of subjects and high inter-individual variability. Therefore, it is suggested that further investigations including larger samples and/or longitudinal studies need to be carried out to further clarify the relationship between blood flow kinetics and level of physical activity in older highly active people. It is also noteworthy that the findings of the present study are limited to dynamic contractions of a small muscle mass in which potential changes in central circulation with training may not play any significant role. This may not be the case during large muscle dynamic exercise in which the muscle blood flow kinetics may be strongly influenced by the kinetics of the pumping capacity of the heart. Finally, the exercise intensity in this study was not more than moderate. It is possible that a more extreme exercise challenge might reveal differential blood flow adaptations if, in fact, they do exist in very active older males.

CHAPTER V

STUDY # 3

A 30-DAY FOREARM TRAINING PROTOCOL IMPROVES FOREARM BLOOD FLOW KINETICS.

Introduction

Young people have been shown to increase their maximal work-related forearm blood flow and vascular conductance in response to chronic handgrip training in a very short period of time (30 min per day, 4 days per week and during 4 weeks). This increase was shown to occur independently of any significant effect on uninvolved vascular beds (that is, it is specific and unilateral to the trained limb) or $\dot{V}O_2$ max. (systemic aerobic capacity) (Sinoway et al.,1987). This increase in peripheral vasodilatory capacity with a relatively short period of training was also shown for older people regarding calf blood flow and vascular conductance. Martin et al (1990, 1991) concluded that with physical conditioning of sedentary older people, changes in the resistance vasculature occurred fairly rapidly and increased to a level equivalent to that of the highly trained older people.

On the other hand, a mild exercise regimen (20 to 40 toe raise repetitions a day for 7 weeks) did not change the PCr rate of recovery (McCully et al., 1991), which may reflect no changes in blood flow kinetics. Marsh et al. (1993) observed no change in blood flow at rest and immediately following wrist flexion exercise, following an endurance training period. However, they observed a delay of the onset of intracellular acidosis threshold. Shoemaker (1996) observed faster femoral artery mean blood velocity (MBV) and leg vascular conductance (LVC) in young men following 2 h endurance training for 10 days. However, training did not change the MBV and LVC gain, from rest to exercise steady-state. No studies appeared to be done reporting blood flow kinetics in response to an exercise regimen in older people using beat-by-beat measurements and this was thought to warrant further investigation.

In the present study, the hypothesis was that the adaptation to endurance training in older people includes a faster increase in blood flow kinetics at the onset of exercise.

METHOD

Detailed information on the methodology used in this study was presented in the previous study (Forearm blood flow kinetics in response to handgrip dynamic contractions in young and old people).

Participants

Four men and one woman (67.8 ± 2.6 year-old), all of whom were normally active, participated in the study. They were all normotensive (resting systolic and diastolic pressure lower than 140 and 90 mmHg, respectively). Using a medical screening form, none of them reported a medical history of cardiovascular disease and no one was on medication known to interfere with blood flow control. Informed consent was obtained from the participants and the study was approved by the Office of Human Research and Animal Care of the University of Waterloo.

Testing Protocol (laboratory)

Dynamic handgrip contractions were performed while the participants reclined comfortably in the supine position. The exercising arm was supported on a platform that elevated it to an angle of 50° relative to the horizontal and extended to a handgrip device. The participant lifted and lowered a weight of 4.4kg, through a vertical distance of 5cm, at a frequency of 20 contractions/min, using a pulley system for 5 minutes. This sequence was repeated five times with a minimum 10 min. rest between trials to ensure the participants returned to baseline values.

Exercise Training (at home)

Training was conducted daily for 30 days in the participants' homes at a time of their choice. The exercise consisted of repeated handgrip contractions of the dominant limb using a handgrip exerciser (Power Hand Grip, Weider, Montreal, Quebec). The workload was set to 5.4 kg and had to be moved over a distance of 4.5 cm at a rate of 20 contractions per minute . This protocol was repeated for 20 min daily (400 contractions). This workload was similar to that used by Sinoway et al. (1987) at the beginning of their training protocol. In order to mimic the test protocol, the participants were asked to actively control their movement back to the rest position which results in a working distance of 9cm (4.5 cm concentric and 4.5 cm eccentric). The resultant workload was then 1.6W. Neither the intensity nor duration of the exercise was changed during the exercise period.

Measurements.

All measurements were taken at day 0, day 10 and day 30 of the training period, with room temperature around 20° C. The contralateral arm was only measured at rest.

Maximal Voluntary Isometric Handgrip Contraction (MVIC). The participant performed three maximal voluntary handgrip contractions with a rest period between them. The mean value was recorded as the participant's forearm MVIC.

Heart Rate. Heart rate was continuously monitored on a beat-by-beat basis with an electrocardiogram through 3 spot electrodes on the skin.

Blood Velocity. Measurements of MBV were taken similarly to the method described by Tschakovsky et al. (1995). The Doppler equipment (model 500 V, Multigon Industries, Mt.

Vernon, NY) operated in pulsed wave mode was used. After determination of brachial artery position by palpation (~ 2cm proximal to the antecubital fossa) a 4-MHZ flat probe was manipulated over the brachial artery until optimal auditory and visual signals were obtained and then the probe was taped on the skin. The angle of insonation of the built-in transducer relative to the skin was 45°. It was assumed that the brachial artery ran parallel to skin surface at the site of probe position. The gate was set at full width (12 mm) to facilitate insonation of the total width of the artery with approximately constant intensity. With this apparatus, it was possible to maintain a clear Doppler signal both at rest and during exercise.

Vessel Diameter. An ultrasound imaging (echo-Doppler) and Doppler System (model SSH-140-A, Toshiba Corporation, Japan) operated in Doppler (D) and brightness modulation (B) modes was used. After determination of brachial artery position by palpation proximal to the antecubital fossa, a 7.5 -MHZ hand-held probe, comprising an array of transmitting and receiving transducers, was manipulated over the brachial artery until optimal auditory and visual signals were obtained. The vessel wall image and blood velocity profiles were assessed only during the first trial. During the analysis, the velocity profiles generated by this system were only used to provide additional feedback about the optimal position of the probe.

Mean Arterial Perfusion Pressure (MPP): A photoplethysmograph finger blood pressure cuff (Ohmeda 2300, Finapres) was applied on the middle finger of the contralateral hand. The hand was rested at the same level as the ultrasound probe on the exercising arm.

Data Storage.

The electrocardiogram, finapres, Doppler signals, including time, were recorded on a microcomputer data acquisition system. These signals were collected continuously for each 6-min trial. The Echo-Doppler images collected during the first trial for each participant were stored on VCR tapes.

Data Analysis.

The MBV, $HR_{,}$, MPP data collected on a beat-by-beat basis from four repetitions for each subject were ensemble averaged at 1s-intervals to yield a single data set per subject. This method is similar to that described previously to examine breath-by-breath $\dot{V}O_2$ (Phillips et al., 1995; Shoemaker et al., 1994)) and MBV (Hughson et al.,1995; Shoemaker, 1994).

Forearm vascular conductance index (FVC) was calculated by the quotient between MBV and MPP.

The MBV kinetic parameters for the response to the step increase in work rate were described from a two-component exponential model, already used in previous studies of MBV (Tschakovsky, 1993; Hughson et al., 1995). This model is the same as used to describe $\dot{V}O_2$ kinetics (Cochrane et al, 1992; Hughson et al, 1993; Phillips, 1995). One subject's data were best fitted with a one-component exponential model. The one-component was chosen because it provided the best fit, that is, the least residual sum of squares. In addition, the shape of the curve of this particular subject's data suggested a one-component exponential model. Usually, the on-transient MBV data depicts a pattern consistent with a two-component exponential model; however, a one or even three-component model is the most appropriate (Shoemaker, 1996).

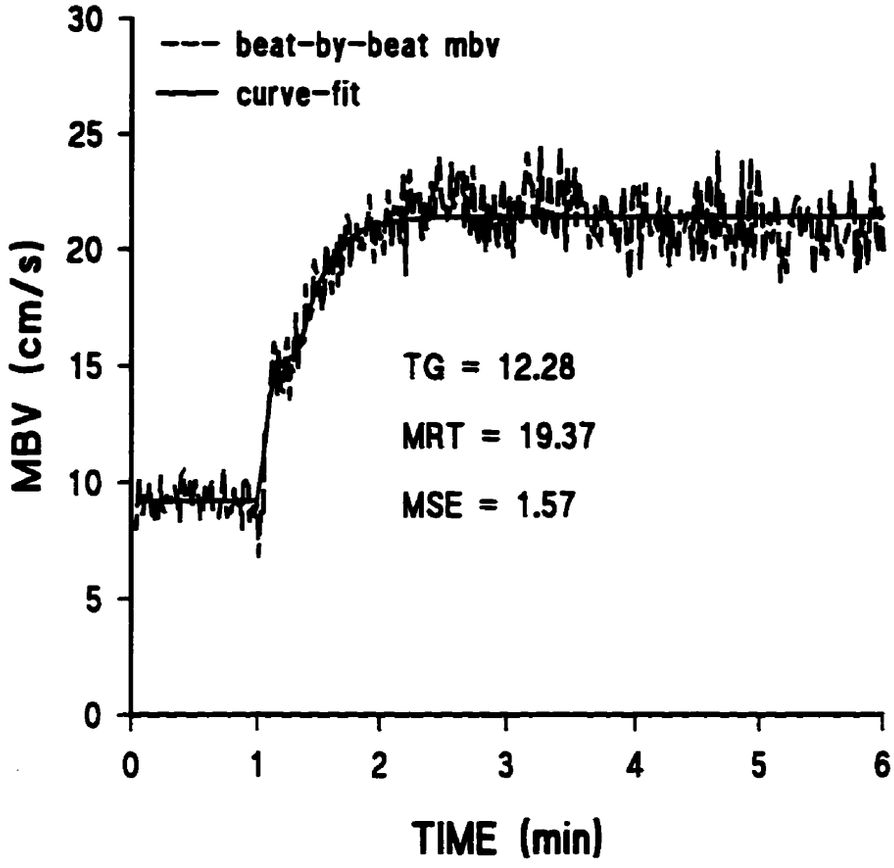
Therefore, with the exception of the above participant, the remaining participants' data were fitted with the two-component exponential model. Figure 5.1 illustrates the MBV data fitted to a two-component exponential model in one of the participants' data..

Diameter (Dm) of the brachial artery was assessed by freezing the VCR image at 5 different points in time during the resting period (one for each 10 seconds) and during the exercise period (one for each minute). For each point in time, three measurements were taken and the mean of these values was used to represent the diameter at that point in time, and the mean diameter values for rest and exercise were calculated for each participant.

Statistical Analysis

Rest and exercise mean values, in the trained arm, at each day of measurement (day 0, day 10 and day 30) were compared through a paired T-test. Rest, exercise, total gain and kinetic mean values across the three days of measurement were compared through ANOVA repeated measures. The Bonferroni's method was used to isolate the day or days that differed from the others. The level of statistical significance was established at $p \leq 0.05$.

Figure 5.1. Mean blood velocity (MBV) data from one subject at the 30th day of training fitted with a two-exponential model.



Legend:

TG= total gain in centimetres per second; MRT= mean response time in seconds; and MSE= mean squared error in centimetres per second.

Results

The diameter of the brachial artery, in the trained arm, did not change from rest to exercise at day 0 (4.85 ± 0.37 and 4.82 ± 0.49 mm, respectively); at day 10 (4.67 ± 0.36 and 4.85 ± mm, respectively) and at day 30 (4.71 ± 0.40 and 4.92 ± 0.44 mm, respectively).

It can be noted in Table 5.1 that the mean blood velocity during exercise (MBVEX) and consequently forearm blood flow (FBFEX) were approximately 2.5 times larger during exercise steady-state compared to the resting values.

All resting values of mean blood velocity, mean diameter of brachial artery (DM) and calculated forearm blood flow, either in the untrained or in the trained arm, were unaffected by time alone or training. Similarly, the exercise steady-state values of these measurements were also unaffected by the exercise protocol in the trained arm (Table 5.1).

The mean blood velocity kinetic parameters, except MBV-MRT values which were better included in a separate table (Table 5.3), are showed in Table 5.4.

The mean blood velocity kinetics in terms of the mean response time (MBV-MRT) were significantly enhanced ($p<0.05$) for the trained arm following 30 days of training (Table 5.3). The improved kinetics were obvious after 10 days of training but not statistically significant until the 30th day. An illustration of the change in mean blood velocity is shown in figure 5.2.

In response to a moderate handgrip challenge, mean arterial pressure was slightly but significantly increased ($p<0.05$) during the exercise by approximately 10-12 mmHg (Table 5.4). However, neither the mean arterial perfusion pressure responses nor the total gain in mean arterial perfusion pressure from rest to exercise were changed with exercise training ($p>0.05$).

Table 5.1. Blood velocity, brachial artery diameter and blood flow at rest and during moderate handgrip exercise in trained and untrained arm in older people.

	UNTRAINED ARM			TRAINED ARM						
DAY	**MBVR**	**DM**	**FBFR**	**MBVR**	**MBVEX**	**TGMBV**	**DM**	**FBFR**	**FBFEX**	**TGFBF**
	($cm.s^{-1}$)	**(mm)**	**($ml.min^{-1}$)**	**($cm.s^{-1}$)**	**($cm.s^{-1}$)**	**($cm.s^{-1}$)**	**(mm)**	**($ml.min^{-1}$)**	**($ml.min^{-1}$)**	**($ml.min^{-1}$)**
day 0	9.11 ± 1.05	4.67 ± 0.31	98.9 ± 24.1	8.85 ± 1.40	23.1 * ± 3.1	14.2 ± 2.7	4.84 ± 0.42	98.4 ± 25.8	254.6 * ± 42.1	156.2 ± 23.1
day 10	9.22 ± 1.14	4.49 ± 0.30	89.1 ± 17.4	8.65 ± 1.20	22.9 * ± 3.3	14.3 ± 3.2	4.76 ± 0.32	92.5 ± 17.0	244.3 * ± 33.4	151.9 ± 30.0
day 30	8.80 ± 0.88	4.52 ± 0.31	87.5 ± 15.7	8.39 ± 1.20	21.9 * 2.3	13.5 ± 2.0	4.82 ± 0.41	91.3 ± 14.8	239.2 * ± 37.3	147.9 ± 29.8

n=5 for each day. Values are mean ± SD. Day 0=measurement taken at pre-training status; day 10=measurements taken at the 10th day of training and; day 30= measurements taken at the 30th day of training. MBVR = mean blood velocity at rest; DM= mean diameter of brachial artery; FBFR=forearm blood flow at rest; MBVEX= mean blood velocity during exercise steady state; TGMBV=total gain in mean blood velocity from rest to exercise; FBFEX= forearm blood flow during exercise steady - state; TGFBF= total gain in forearm blood flow from rest to exercise. * = differences are statistically significant compared to resting values ($p \leq 0.05$).

Table 5.2. Mean blood velocity (MBV) kinetic parameters.

MBV kinetic Parameters	Participant 1			Participants 2 to 5 (values are mean and SD for the 4)		
	Day 0	Day 10	Day 30	Day 0	Day 10	Day 30
td1 (sec)	0.92	0.16	0.53	1.25 ± 0.92	1.82 ± 1.38	1.32 ± 0.8
tau 1 (sec)	40.75	36.18	34.35	3.31 ± 3.10	2.10 ± 1.16	2.33 ± 1.01
td 2 (sec)	-	-	-	19.10 ± 3.38	22.60 ± 6.70	21.5 ± 6.19
tau 2 (sec)	-	-	-	22.90 ± 11.39	18.80 ± 8.33	18.2 ± 9.23
g0 (cm/sec)	9.51	8.77	9.04	8.94 ± 1.45	8.62 ± 1.42	8.22 ± 1.29
g1 (cm/sec)	18.85	19.74	16.85	5.47 ± 1.41	6.29 ± 1.50	6.53 ± 0.44
g2 (cm/sec)	-	-	-	7.44 ± 1.32	6.62 ± 0.80	6.11 ± 0.75
tg (cm/sec)	18.85	19.74	16.85	12.90 ± 0.62	12.96 ± 1.08	12.60 ± 0.79
mse (cm/sec)	2.53	2.46	2.60	2.12 ± 1.10	1.87 ± 1.04	1.79 ± 0.60

td1 = time delay from the onset of exercise to the beginning of the response; tau1 is the time when 63% of the response in the first component of the exponential curve was achieved up to td2; td2 = time delay from the onset of the exercise to the beginning of the second component of the exponential curve; tau2 = time when 63 % of the response in the second component of the exponential curve was achieved; g0 = values at rest; g1= gain obtained during the first component of the exponential curve up to td2; g2 = gain obtained during the second component of the exponential curve; tg = total gain obtained; mse = standard error of mean of the curve fit data; * = the mean values between the two groups are statistically different.

Table 5.3. Mean response time individual values of mean blood velocity (MBV-MRT)at day 0, day 10 and day 30 of training in older people.

	DAY 0	DAY 10	DAY 30
P 1	41.7	36.2	34.9
P 2	29.3	25.2	19.4
P 3	16.0	16.9	15.1
P 4	35.6	26.7	29.8
P 5	23.1	23.2	20.1
MEAN ± SD	**29.1 ± 10.1**	**25.6 ± 7.0**	**23.8 ± 8.2 ***

All values are in seconds. P= participant; Day 0=measurement taken at pre-training status; day 10=measurements taken at the 10th day of training and; day 30= measurements taken at the 30th day of training. *=statistically significant at $p \leq 0.05$ compared to day 0.

Figure 5.2. The change in mean blood velocity (Δ MBV) data from one of the participants at day 0 and day 30 (the data were averaged at each 3 seconds) to include one complete contraction-relaxation cycle (1 second contraction by two seconds relaxation).

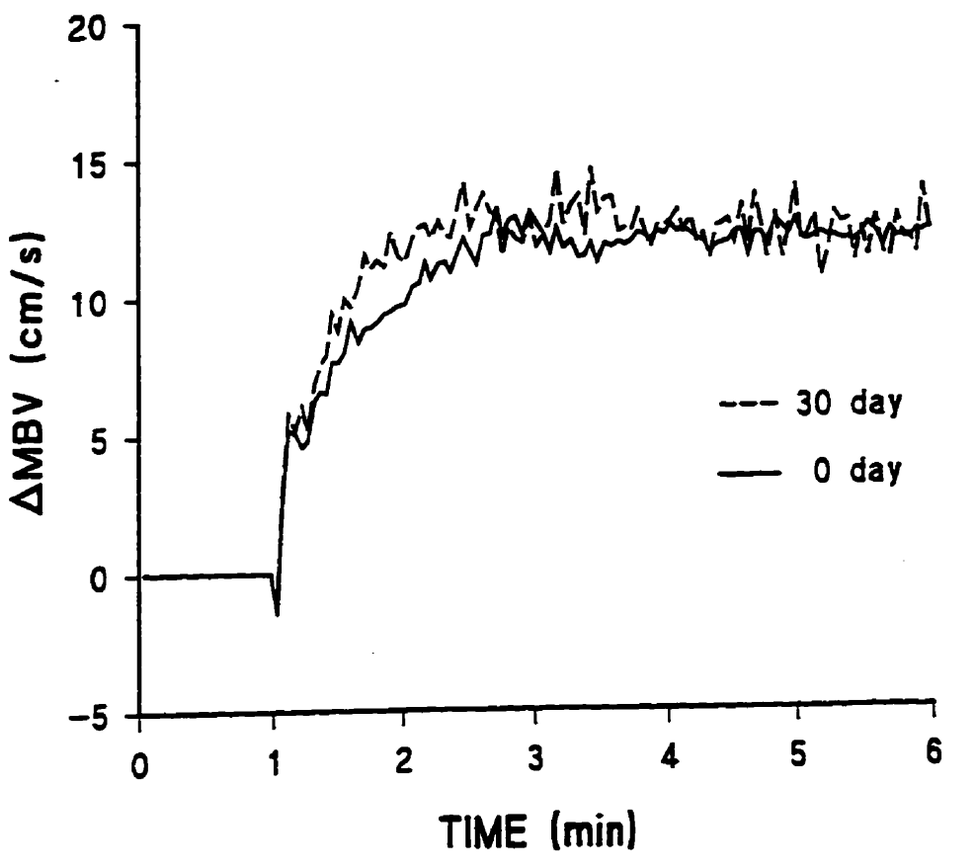


Table 5.4. Blood pressure at rest and during moderate handgrip exercise before and during training older people.

DAY	MPPR (mmHg)	MPPEX (mmHg)	TGMPP (mmHg)
day 0	76.9 ± 4.1	88.5 ± 3.9 *	11.6 ± 2.7
day 10	78.3 ± 5.8	90.5 ± 5.5 *	12.1 ± 2.8
day 30	76.5 ± 4.2	87.4 ± 3.1*	10.9 ± 3.1

n=5 for each day. Values are mean ± SD. Day 0=measurement taken at pre-training status; day 10=measurements taken at the 10th day of training and; day 30= measurements taken at the 30th day of training. MPPR = mean arterial perfusion pressure at rest; MPPEX= mean arterial perfusion pressure during exercise steady state; TGMPP=total gain in mean arterial perfusion pressure from rest to exercise. * = differences are statistically significant compared to resting values ($p \leq 0.05$).

The forearm vascular conductance (FVC) was calculated from the division of mean blood velocity by mean arterial perfusion pressure. Resting values for both the untrained and trained arms were not different during the period of the training protocol. Also, the FVC of the trained arm was unaffected by the length of training (10 days or 30 days). FVC of the trained arm was significantly larger during exercise compared to resting values on each day of measurement (Table 5.5).

Although resting and exercising FVC were not significantly affected by training (noted above), the mean response time of forearm vascular conductance (MRT-FVC) for the trained arm was significantly faster ($p<0.05$) after 30 days of training (Table 5.6) . The differences between day 0 and day 10 or day 10 and day 30 were not statistically significant.

Similar to the blood pressure response, heart rate increased slightly from rest to exercise ($p<0.05$), however, the magnitude of change was no different as a result of the training process (day 0 = 65.9 ± 7.1 to 71.1 ± 8.42 b.p.m.; day 10= 66.7 ± 6.8 to 72.1 ± 6.25 b.p.m; day 30 = 64.7 ± 9.42 and 69.4 b.p.m.).

The maximal voluntary isometric contraction (MVIC) of the trained arm was slightly increased between day 0 (35.8 ± 6.5 kg) and day 30 (38.4 ± 7.1 kg); day 10 (36.1 ± 6.6 kg) and day 30, however it was not different between day 10 and day 30.

Table 5. 5. Forearm vascular conductance ($cm.s^{-1}$.$mmHg^{-1}$) at rest and during moderate handgrip exercise in trained and untrained arm of older people.

	UNTRAINED ARM	TRAINED ARM		
	REST	REST	EXERCISE	TOTAL GAIN
Day 0	0.12 ± 0.02	0.12 ± 0.02	0.26 ± 0.03*	0.14 ± 0.02
Day 10	0.12 ± 0.03	0.11 ± 0.02	0.25 ± 0.03 *	0.14 ± 0.03
Day 30	0.11 ± 0.02	0.10 ± 0.02	0.25 ± 0.02*	0.15 ± 0.02

n=5 for each day. Values are mean ± SD. Day 0=measurement taken at pre-training status; day 10=measurements taken at the 10th day of training and; day 30= measurements taken at the 30th day of training. * = differences are statistically significant compared to resting values ($p \leq 0.05$).

Table 5.6. Mean response time individual values of forearm vascular conductance (FVC-MRT) at day 0, day 10 and day 30 of training in older people.

	DAY 0	DAY 10	DAY 30
P 1	31.6	27.9	26.8
P 2	21.2	20.3	14.6
P 3	13.9	16.0	13.5
P 4	28.6	22.2	22.8
P 5	22.0	22.2	19.7
MEAN ± SD	**23.5 ± 6.9**	**21.8 ± 4.3**	**19.5 ± 5.6 ***

All values are in seconds. P= participant; Day 0=measurement taken at pre-training status; day 10=measurements taken at the 10th day of training and; day 30= measurements taken at the 30th day of training.

*= statistical significance at $p \leq 0.05$ compared to day 0.

DISCUSSION

The most significant result of this study was the faster increase from rest to exercise values of forearm blood velocity kinetics and vascular conductance in response to endurance training with a moderate workload for a relatively short period of time. Since the brachial artery diameter did not change from rest to exercise, the MBV kinetics are assumed to represent the forearm blood flow kinetics. These findings are of practical significance for older individuals who may be more prone to participate in light activities rather than in a training regimen requiring intense efforts. A practical consequence is that faster blood flow kinetics can result in a more efficient energy production through aerobic metabolism and consequent lower levels of metabolites (Pi, H^+, La). For example, faster blood flow kinetics could result in a lower rate of perceived exertion for the same absolute submaximal level of physical activity and the individual should feel more comfortable while participating in physical activities.

Faster forearm blood flow kinetics with endurance training found in this study seem to provide an additional explanation to the findings of Marsh et al. (1993) who found a delay in the onset of intracellular acidosis and increased endurance performance in response to forearm endurance training in older people. A faster blood flow kinetics would produce a lower Pi/PCr ratio and $[H^+]$ at any submaximal exercise intensity.

The findings of the present study are not in agreement with those reported by McCully et al. (1991), who reported no changes in PCr kinetics following an endurance training regimen. A possible explanation for this controversy may be related to the fewer number of repetitions used in

their training regimen (20 to 40 toe raise repetitions a day) compared to 400 repetitions per day in the present study. One limitation in the present study (related to the training protocol) was not having tested the maximal endurance capacity during the handgrip exercise. The mean value of 15% of maximum MVIC, used in the exercise protocol, may not provide a good idea of the exercise intensity. A rough idea of what it might have meant in terms of percentage of endurance capacity is the comparison of the workload used in the present study (1.6 w) with the values reported by Jasperse et al.(1994) for older people, during intermittent and progressive bouts of handgrip exercise for 8 minutes . They showed that at the workload of 3.1J (2.1 w), the rate of perceived exertion was practically maximal and approximately 30% of the sample had already stopped the exercise due to muscle fatigue.

The results of the present study are in line with those reported by Shoemaker et al.(1996) who observed faster femoral artery mean blood velocity (MBV) and leg vascular conductance (LVC) in young men following short-term endurance training (10 days). However, the present study only showed approximately 20 % faster MBV compared to approximately 40 % in Shoemaker et al's study where the intensity of exercise was higher. Methodological differences between these two studies prohibit further comparison of the results.

The forearm model used in this study appeared to have minimized the influence of central circulation and stressed peripheral adaptations. From rest to exercise, the heart rate and MPP only increased approximately 7% and 11%, respectively. The training protocol did not change any of the two mentioned parameters of central circulation. Furthermore, the simultaneous improvement in MBV and FVC kinetics at the onset of the exercise means that the change in MBV kinetics was not parallelled by a similar increase in blood pressure. This suggests that the training protocol might

have improved vasodilation downstream from the brachial artery at the onset of exercise. Sinoway et al. (1987) has already shown that maximal forearm blood flow and minimal resistance can be improved in response to 30-day of forearm training without change in mean arterial pressure, systemic aerobic capacity or uninvolved vascular beds. Green et al. (1994) have also shown increased forearm peak vasodilatory capacity in response to endurance training (30-min sessions, 4 days per week, during 4 weeks). They suggested that this increase could be due to structural modification of the vasculature by a mechanism localized to the vascular bed involved in the conditioning process.

Several mechanisms intrinsic to the trained arm may be involved in facilitating faster blood flow kinetics. However, there is considerable debate about the mechanisms that control blood flow and how they can be modified with training. Since the same arm position (same hydrostatic pressure), absolute workload and contraction rate were used during each day of measurement, it is unlikely the muscle pump may have played any significant role in the changes observed. Changes in muscle sympathetic activity or vascular responsiveness to norepinephrine with training remains a matter of debate (NG et al, 1994, Somers, 1988; Wiegman et al., 1981; Svedenhag et al. 1991). Furthermore, the moderate workload performed dynamically by the small muscle mass (unilateral handgrip) may have prevented or minimized any exercise-induced effect on sympathetic tone. Previous research has shown that the smaller the muscle mass involved, the less likely that exercise would affect sympathetic tone (Clausen et al., 1973; McCloskey et al.,1975). Increased responsiveness to metabolites (Green et al. ,1994) released from active skeletal muscle such as Pi, La and H^+, is another proposed mechanism to increase blood flow with short-term training. Increased release of nitric oxide (NO) has been observed in canine femoral arteries exposed to

chronic flow (Miller and Burnett, 1992).Therefore, there is a possibility that repeated increases in flow generated by exercise training induced an increase in the rate of nitric oxide (NO) release which could have led to faster vasodilation of resistance vessels. In rodents, endurance training as short as 2 weeks, appeared to have increased the rate of nitric oxide production leading to greater vasodilation (Sun et al., 1994). Another important vasodilator that may also be involved in the changes in blood flow kinetics are prostaglandins. Koller and Kaley (1990) have demonstrated flow-induced prostaglandin release from skeletal muscle artery but, Shoemaker (1996) has shown that prostaglandins probably play no important role in adaptation of blood flow at the onset of exercise.

Increased forearm vasodilatory capacity observed with a short period of endurance training has been suggested to be due to structural changes in the muscle vasculature (Sinoway et al., 1987; Green et al., 1994). We did not observe any difference in the brachial artery diameter with training, however this does not rule out potential structural modifications occurring downstream in the resistance vessels. Increased capillarization would also contribute to a faster MBV kinetics seen in the conduit artery, however , increased capillarization within 30 days of endurance training has not been reported.

In summary, we cannot be sure which specific mechanism was responsible for the faster MBV observed in the present study. However, changes in central circulation, muscle pump and sympathetic tone are the most unlikely to have contributed. More likely, local functional adaptations might have occurred which led to faster vasodilation of resistance vessels downstream to the brachial artery.

The slight increase in MVIC found was unexpected, because the exercise protocol was not designed to increase muscle strength. Different motivational states of the subjects, in the sense that

they might have expected a higher level of strength after the training period might also have played a role in the observed changes. Psychological aspects have been shown to modify the expression of human strength (McArdle et al, 1991). Marsh et al. (1993) and Sinoway et al. (1987) did not find changes in maximal strength after an endurance training period, however Green et al. (1994) reported increased grip strength following a forearm endurance training for 4 weeks.

The lack of change in exercise steady- state blood velocity, blood flow, blood pressure and vascular conductance with training is in line with the results reported by Sinoway et al. (1987), who found no changes in blood flow and minimal resistance at any submaximal workloads.

Potential limitations. The major concern about the changes in blood flow kinetics found in this study is associated with the learning process that might have occurred. The training protocol involved the same task as in the test protocol and it is possible that the participants have mastered its execution. Since the absolute change observed was small (5.3 sec), the achievement of a better relaxation during each duty-cycle would increase the rate of blood flow at the onset of the exercise. Nevertheless, having chosen the dominant arm to be trained, may have prevented or minimized changes due to the learning process.

A second concern is related to how accurately the MBV kinetics represents blood flow kinetics at the onset of exercise. In the present study, the vessel diameter was not measured either on a beat-by-beat basis as MBV was or simultaneously with MBV, which would represent an extremely ideal condition.

Another potential limitation refers to the fact that the duration and intensity of the exercise were kept constant throughout the training period. This procedure may have prevented any further changes in MBV and FVC kinetics between days 10 and 30.

Finally, the results and conclusion are based on a small number of subjects and without a control group during the exercise measurements. Therefore statements regarding the universality of the present observations must be viewed cautiously. The reason for this limitation was the difficulty in finding volunteers willing to report to the lab at least four times with each lab visit lasting between 3 to 4 hours, and meet the requirements to participate in the study.

In conclusion, this study documents that the rate of changes in blood flow and vascular conductance at the onset of the exercise can be improved by endurance training in older people. We suggest that the main mechanism (s) responsible for these changes is peripheral and lead to faster vasodilation of the resistance vessels downstream from the conduit artery.

APPENDIX I

Blood velocity and blood pressure responses to premature heart beats before, during and after moderate small muscle mass exercise: A case study

Introduction

During the phase of participants' recruitment for a study designed to analyse blood flow responses to moderate small muscle mass exercise in older people, one of the volunteers showed elevated resting heart rate (around 100 beats-per-minute) and some premature heart beats. The premature heart beats appeared before (rest), during exercise and after exercise (recovery).

Since one of the requirements to participate in the study was to be free of cardiovascular disease and, in order to be sure that the exercise protocol involved in the study would not present any increased risk to the participant's health, it was decided to refer the volunteer to his family physician.

The physician reported that the volunteer could tolerate the exercise protocol and participate in the study. However, he also reported that: 1) the volunteer had a positive exercise test which showed ischaemic changes in leads V4 - V6; and 2) the heart rate varied due to underlying lymphoma. Because such variation in heart rate could have presented a confounding factor in the results of the study, it was decided not to include this volunteer in the study. However, the blood velocity and blood pressure responses to those premature beats were judged to be worthy of analysis and the results could motivate future study.

Therefore, the aim of the present report is to describe the blood velocity and blood pressure responses to those premature heart beats in one individual.

Method

Participant : Male, 78 years-old, 173 cm tall, 72 kg, non- smoker and normally active.

Exercise Protocol: The participant performed handgrip contractions while in supine position (reclined comfortably on his back). The exercising arm was supported on a platform and extended to a handgrip device at 50° above the heart level. The participant then moved a weight of 4.4kg, through a distance of 5cm, at a frequency of 20 contractions/min, in a pulley system. The load to be moved corresponded to approximately 15% of the participant maximum voluntary contraction.

The participant was given 30 min. rest before starting data collection as follows:

rest(1min) Recovery (3min)

Measurements :

Heart Rate: Heart rate was continuously monitored by an electrocardiogram through 3 spot electrodes on the skin.

Doppler Blood Velocity: Brachial artery mean blood velocity (MBV) was determined by using pulsed Doppler ultrasound velocimetry. Blood velocity spectra was be obtained by a Doppler

(model 500 V, Multigon Industries) operating in pulsed mode. A 4 MHZ ultrasound probe was taped to the skin directly over the brachial artery at the elbow. The angle of the transducer crystal relative to the skin was assumed to be 45° , and the ultrasound gate was maintained at full width to facilitate insonation of the total width of the artery with approximately constant intensity. With this apparatus, it was possible to maintain a clear Doppler signal both at rest and during exercise (1). The Doppler shift frequency spectra was processed by a quadrature audio demodulator (2) that provided instantaneous MBV in real time allowing collection of MBV in analog-to-digital units. The quadrature audio demodulator also generated the appropriate Doppler shift frequency signals to produce a two-point calibration. Beat-by-beat MBV was calculated by integrating the total area under the instantaneous MBV profile, with the marked QRS complex of the electrocardiogram tracing signalling the end of one heartbeat and the beginning of the next (Shoemaker et al., 1994).

The main source of error in MBV determination is the angle of insonation. To reduce the magnitude of this error, the body position during the handgrip trials was fixed. In addition, a flat probe, which maintains the angle of the sound transducer with the skin was used. Further limitations associated with Doppler measurements of blood velocity include random error attributable to the operator, improper alignment of the ultrasound beam with the artery, and Doppler signal processing and frequency estimation (Gill, 1985). Random error was reduced by averaging four repeated trials into one data set. The latter two sources of error was minimized by using both auditory and visual feedback of the Doppler signal.

Blood Pressure Using Finapres Finger Cuff : Mean Arterial Perfusion Pressure (MPP) was collected beat-by-beat using a Finapres finger cuff blood pressure monitor (Finapres, Ohmeda

Inc.). The finapres has been shown to provide values similar to those obtained simultaneously by intra-arterial measurements during performance tests inducing rapid changes in blood pressure (Parati et al., 1989). The small finger cuff was placed around the middle finger of the contra lateral hand, with the arm and hand resting above the heart level (that is, at the same level where the blood velocity was measured in the exercising arm).

Data Storage

The electrocardiogram, finapres and Doppler signals were stored on a microcomputer data acquisition system. These signals were collected continuously for each 9 min. trial

Data Analysis:

All beat-by-beat data for the participant's four trials were ensemble averaged to produce a single data set for that participant.

The beat-by-beat heart rate data for trials 1,3 and 4 were analysed without any average procedure to be compared with the averaged four trials data.

Four small sets of data consisting of 10 heart beats which included premature beats were extracted from trials 1,3 and 4 to illustrate the blood pressure and blood velocity responses.

Mean Blood Velocity was calculated from the area under the curve by a computer program.

Mean Arterial Blood Pressure was estimated by the equation MPP = 1/3 * pulse pressure + diastolic pressure.

Results and Discussion

Averaged values for the four trials - Heart rate increased from 95 ± 1.6 bpm (mean ± SD) during rest to 103 ± 3.4 bpm during exercise and decreased to 99 ± 2.7 bpm during 3 minutes of recovery. The 8 % increase in heart rate observed from rest to exercise is close to the increase found for other participants in a previous study (Gobbi et al., 1995). However, the absolute heart rate values (95 during rest and 103 during exercise) are higher than those values (67 for rest and 71 for exercise) found in that study.

The mean blood velocity significantly increased from 10 ± 0.6 to 37 ± 7.0 cm/sec during exercise and went down to 31 ± 9.0 cm/sec during recovery. The resting value of blood velocity is close to 9 cm/sec found in the previous study. However, the MBV is higher during exercise than the value found previously (21 cm/sec) (Gobbi et al., 1995).

The mean arterial perfusion pressure (MPP) significantly increased from 78 ± 1 during rest to 94 ± 9 during exercise and decreased to 91 ± 4 mmHg during recovery. The resting value is close to the 80 mmHg observed in previous study , however the exercise value is somewhat higher than the 87 mmHg observed in the same study (Gobbi et al., 1995). It is worth noting that the MPP can be thought to be representative of the central mean arterial pressure (MAP), and the difference in terms of the latter being higher was due to the effect of negative hydrostatic pressure when the measurement point is above the heart level.

Figure A.1 shows the plot obtained for heart rate (HR), MBV and MPP when the values were averaged for all the four trials. It is interesting to observe the relatively small variability of the data, except for heart rate and blood pressure during the recovery phase. It seems that when data are analysed in this way, it can provide a useful tool to control for the high variability (due to the contraction relaxation process) found in MBV during exercise.

Non-averaged heart rate values for trials 1,3 and 4 - Figure A.2 shows the plots of heart rate beat -by -beat values for each of the 3 out of 4 trials comprised in the exercise protocol. Rest values are shown up to 60 seconds, exercise from 60 to 360 seconds and recovery from 360 to 540 seconds. This figure enables one to appreciate the extent and the location of the premature heart beats. It can be seen that such premature beats occur in each of the three phases of the exercise protocol (rest, exercise and recovery) and normally is represented by a sudden increase followed by a decrease in heart rate compared to regular values. It also can be observed that the premature heart beats occur preferentially during the recovery phase. This observation warrants further study. Is this post-exercise preponderance associated with an immediate post-exercise increased hyperaemia (increased blood velocity) in conjunction with a concomitant decrease in blood pressure? (see Figure A.1)

Extracted portions of data containing premature heart beats

Figure A.3 shows 10 heart beats extracted at the very beginning of the data collection. It can be observed that the premature heart beat occurred on QRS complex numbered as 5 which causes an increase in HR from 90 (QRS 4) to 122 with a simultaneous decrease in brachial artery MBV from 14 to 3 cm/sec and in MPP from 91 to 77 mmHg. This HR increase was immediately followed by a decrease from 122 to 71 bpm (QRS 6) with a simultaneous increase in MBV from 3 to 10 cm/sec and an increase in blood pressure from 77 to 86 mmHg. This decrease in HR was then followed by a new increase (QRS 7), and a decrease in both MBV and BP, after which the values tended to stabilize.

Figure A.1. Averaged values of heart rate (HR), mean blood velocity (MBV) and mean arterial perfusion pressure (MPP) for four trials for each second

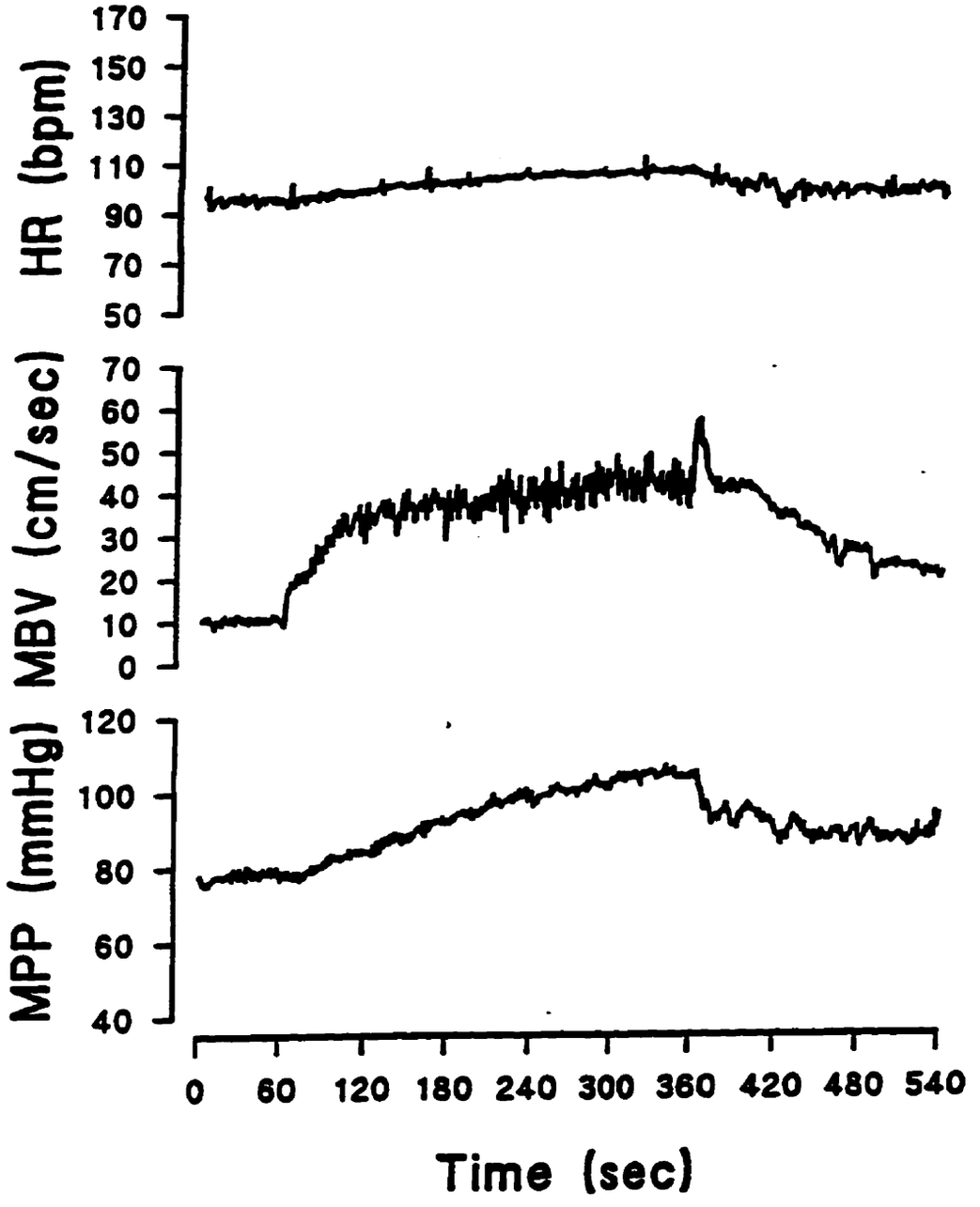


Figure A.2. Heart rate (HR) beat-by-beat values for each trial

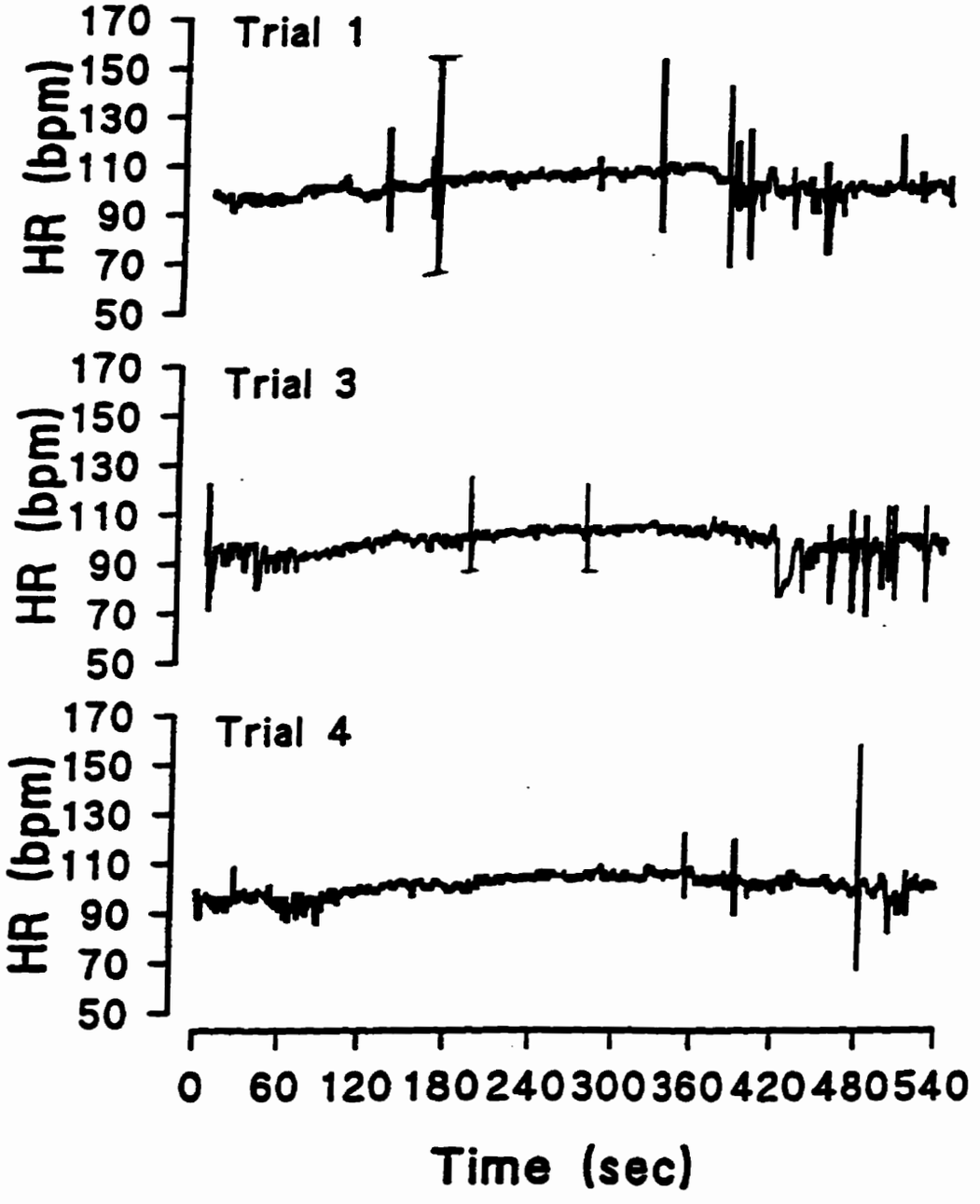
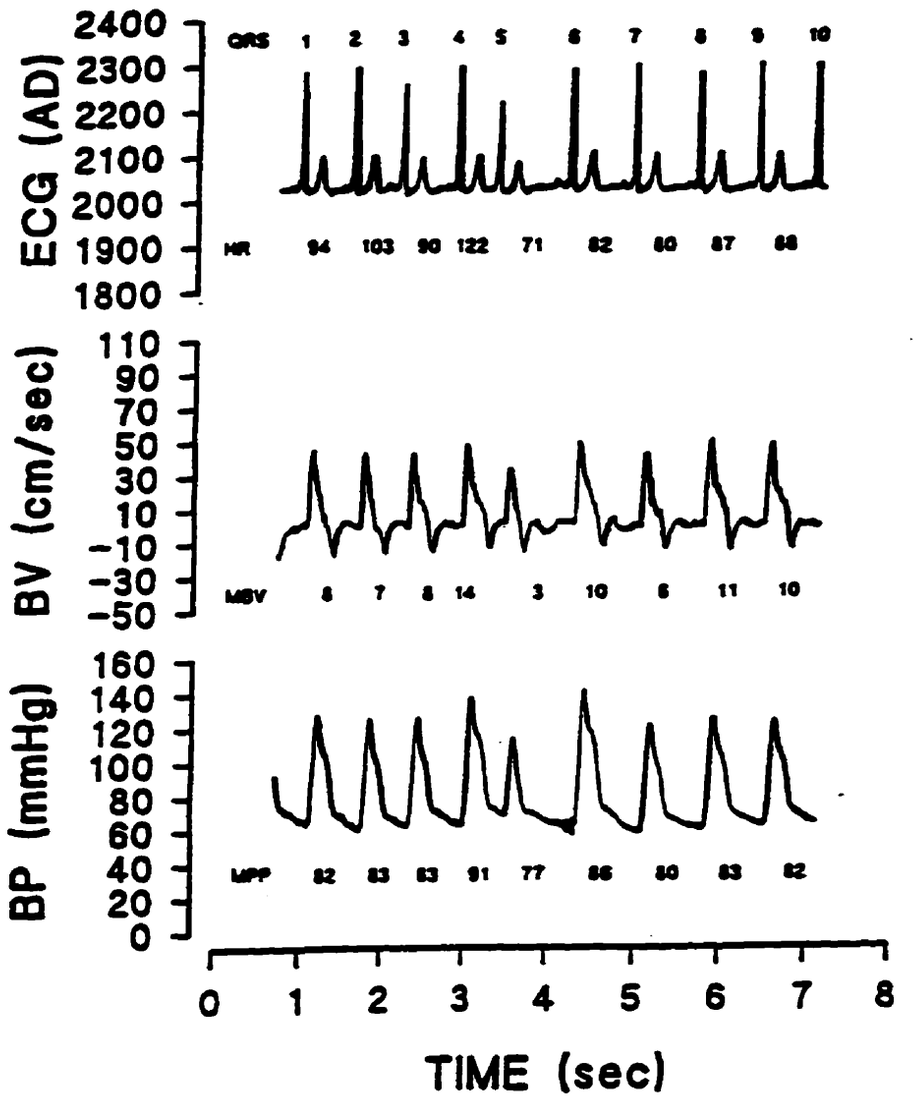


Figure A.3. Blood velocity (BV) and blood pressure(BP) response to heart rate (HR) variation during rest in trial 3.



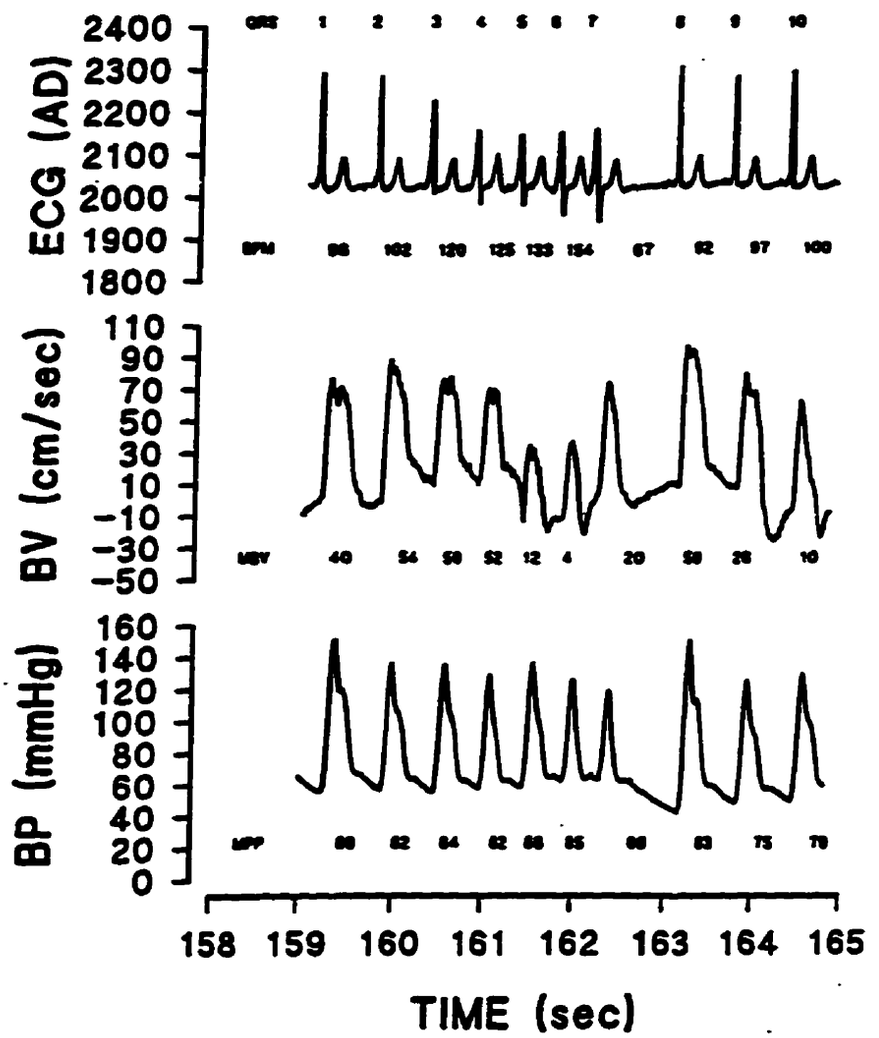
Legend: QRS= the electrical waves of depolarization (Q, R and S waves); MBV=mean blood velocity; MPP= mean arterial perfusion pressure; ECG (AD)= electro cardiographic values in analog-to-digital units.

Figure A.4 also shows 10 heart beats extracted during exercise when the MBV had already reached a plateau. The analysis of the responses during this period is somewhat more complex because the effects of contraction relaxation phases of the exercise are added to the effects caused by the premature beats, especially for the MBV response. Furthermore, it is not possible to know with great accuracy when the contraction and relaxation started and finished. Considering that forearm muscle contraction decreases brachial artery blood velocity and relaxation causes the opposite effect, it can be estimated that one relaxation phase started somewhere near QRS 1 and lasted 2 seconds reaching a point between the QRS 4 and 5. A contraction phase then started near QRS 5 and lasted 1 second reaching a point near QRS 7. The following relaxation phase seems to start near QRS 7 and lasted almost to QRS 9 when a new contraction started.

The first premature beat occurred at QRS 4 and was followed by four other premature beats while the HR progressively increased from 102 to 154bpm. The trend was to decrease MBV and MPP. It is important to keep in mind that brachial artery MBV was further decreased by the exercise contraction phase between QRS 5 and 7. It is also noteworthy that, contrary to what could be expected, the arterial blood pressure increased with further increase in heart rate due to the premature heart beat at QRS 5. Because QRS 5 coincides with the beginning of the contraction phase it is speculated that the muscle contraction may have triggered a small increase in TPR which in turn increased arterial blood pressure.

It appears that the most significant effects of these series of premature heart beats during dynamic exercise were: 1) an amplified decrease in blood velocity during contraction . Compare velocities at QRS 5 and 6 (12 and 4 cm/sec respectively) with those at QRS 9 and 10 (26 and 10 cm/sec respectively); 2) the increase in blood velocity is less during the relaxation phase following premature beats (compare the mean of 34 cm/sec for QRS 7 to 9 with 51 cm/sec for QRS 1 to 4). Therefore, in both contraction and relaxation phases of the exercise, blood underperfusion to exercising muscles may occur due to premature beats.

Figure A.4. Blood velocity (BV) and blood pressure (BP) responses to heart rate (HR) variation during forearm exercise in trial 1.

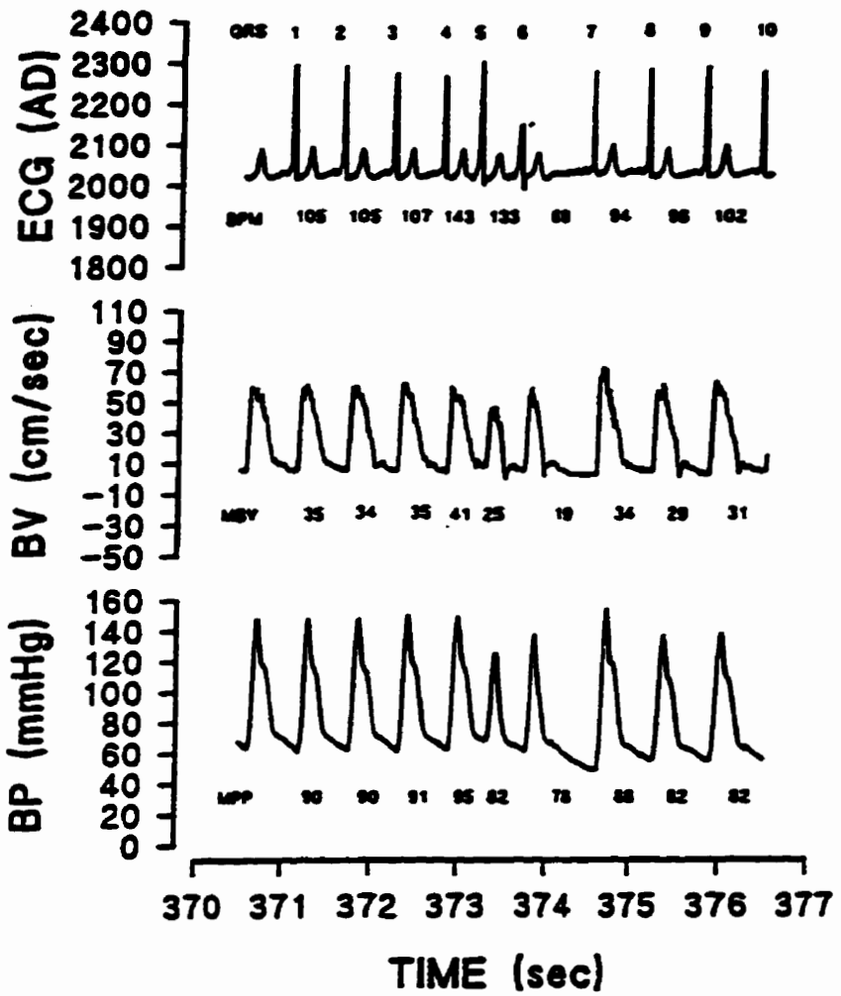


Legend: QRS= the electrical waves of depolarization (Q, R and S waves); MBV=mean blood velocity; MPP= mean arterial perfusion pressure; ECG (AD)= electro cardiographic values in analog-to-digital units.

Figure A.5 shows two subsequent premature beats QRS 5 and 6 causing the HR to be increased from 107 to 143 and 133 respectively. These premature heart beats occurred in the beginning of the recovery period during trial 1. In response to the first premature heart beat, the MBV decreased from 41 to 25 and the MPP from 95 to 82 mmHg. The second premature heart beat causes a further drop in MBV from 25 to 19 cm/sec and in MPP from 82 to 78 mmHg. Again, it seems that when these premature heart beats occur in sequence, the blood flow is lower compared to the other beats. Consistent with what happened during rest and exercise, these premature heart beats are followed by a substantial decrease in HR, and an increase in MBV and MPP towards the stable values.

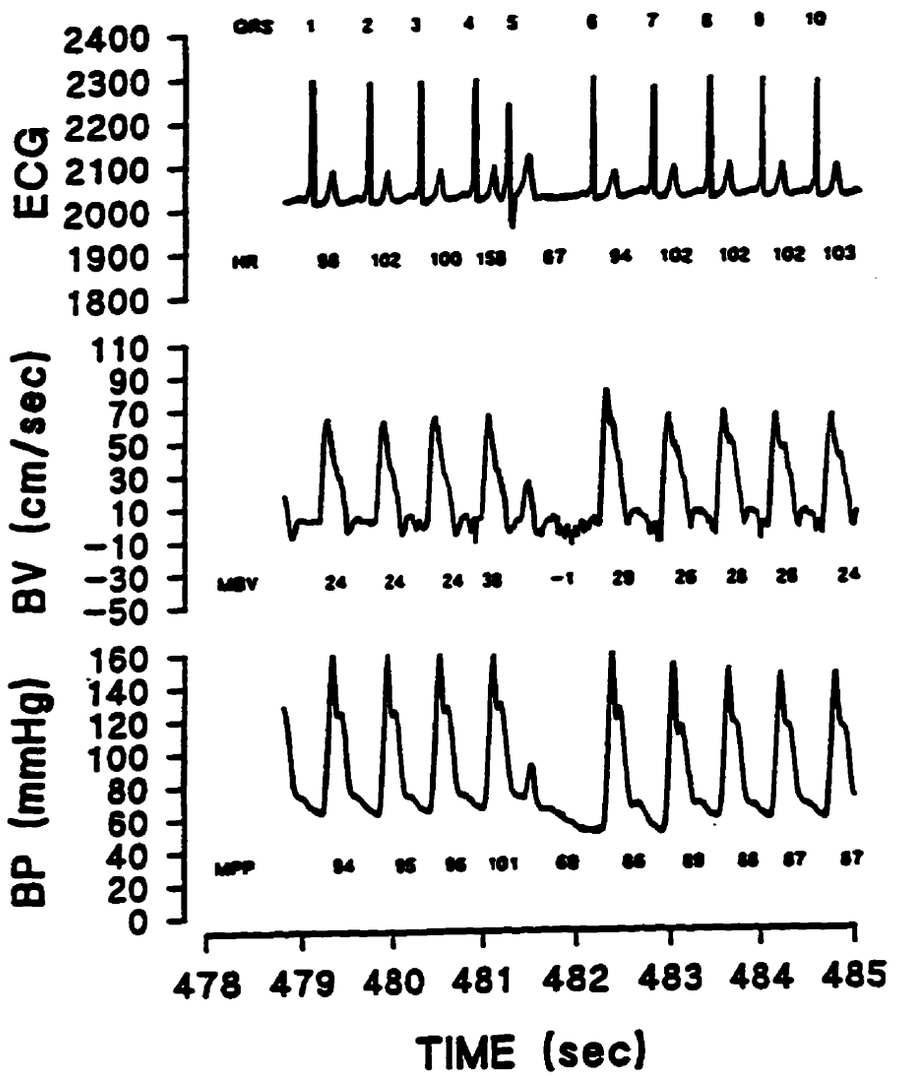
Figure A.6 shows 10 heart beats which were extracted from the data collected during late recovery in trial 4. The premature beat occurred at QRS 5. The heart rate increased from 100 to 158, the MBV decreased from 38 to -1 cm/sec and MPP from 101 to 69 mmHg. Immediately after , the HR fell to 67, the MBV increased to 29 and the MPP also increased 86. Following this bradycardia, the HR, MBV and MPP tended towards more consistent values. It is worth noting that, even in situations when the tissue requires a relatively large amount of blood, as in recovery from exercise, the mean blood velocity can be negative for an entire beat, due to one premature beat.

Figure A.5. Blood velocity (BV) and blood pressure (BP) responses to heart rate (HR) variation during early recovery in trial 1.



Legend: QRS= the electrical waves of depolarization (Q, R and S waves); MBV=mean blood velocity; MPP= mean arterial perfusion pressure; ECG (AD)= electro cardiographic values in analog-to-digital units.

Figure A.6. Blood velocity (BV) and blood pressure (BP) response to heart rate (HR) variation during late recovery in trial 4.



Legend: QRS= the electrical waves of depolarization (Q, R and S waves); MBV=mean blood velocity; MPP= mean arterial perfusion pressure; ECG (AD)= electro cardiographic values in analog-to-digital units.

Conclusions

1. Premature heart beats appear to occur preferentially during the recovery phase. The relatively high blood flow still required during the recovery phase, in conjunction with the sudden decrease in blood pressure at the end of exercise may be involved in this preponderance.

2. When one or more premature heart beats occur, both the mean arterial perfusion pressure and mean blood velocity decrease and this represents tissue underperfusion by blood. There may be two possible explanations: 1) that blood availability to the heart is not enough for the premature beat to maintain or increase the stroke volume and/or 2) premature heart beats are not sufficient to maintain the ejection fraction.

3. The contraction phase of dynamic exercise during premature heart beats appears to exert opposite effects on MBV and on MPP. That is, it amplifies the decrease in blood flow to exercising muscle and counteracts the decrease in mean arterial blood pressure. In addition, the blood flow during the relaxation phase appears to be lower following a series of premature heart beats.

4. Even during the presence of variation in HR, MBV and MPP due to premature heart beats, such as in the present case, the averaging procedure for four trials of data seems to be a useful tool for further data analysis. However, the inclusion of a participant with such a condition can impose a confounding factor on a beat- by- beat analysis.

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