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Muscle contraction duration and fibre recruitment influence blood flow and VO₂ independent of contractile work during steady-state exercise in humans

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Abstract

We tested the hypothesis that, among conditions of matched contractile work, shorter contraction durations and greater muscle fibre recruitment result in augmented skeletal muscle blood flow and oxygen consumption ($\dot{V}O_2$) during steady-state exercise in humans. To do so, we measured forearm blood flow (FBF; Doppler ultrasound) during 4 minutes of rhythmic handgrip exercise in 24 healthy young adults and calculated forearm $\dot{V}O_2$ via blood samples obtained from a catheter placed in retrograde fashion into a deep vein draining the forearm muscle. In Protocol 1 (n = 11), subjects performed rhythmic isometric handgrip exercise at mild and moderate intensities under conditions in which tension time index (TTI; isometric analog of work) was held constant but contraction duration was manipulated. In this protocol, shorter contraction durations led to greater FBF (184 ± 25 vs. 164 ± 25 ml·min⁻¹) and $\dot{V}O_2$ (23 ± 3 vs. 17 ± 2 ml·min⁻¹; both *P*<0.05) among mild workloads, whereas this was not the case for moderate intensity exercise. In Protocol 2 (n = 13), subjects performed rhythmic dynamic handgrip exercise at mild and moderate intensities under conditions of matched total work, but muscle fibre recruitment was manipulated. In this protocol, greater muscle fibre recruitment led to significantly greater FBF (152 ± 15 vs. 127 ±13 ml·min⁻¹) and $\dot{V}O_2$ (20 ± 2 vs. 17 ± 2 ml·min⁻¹; both *P*<0.05) at mild workloads and there was a trend for similar responses at the moderate intensity but this was not statistically significant. In both protocols, the ratio of the change in FBF to change in $\dot{V}O_2$ was similar across all exercise intensities and manipulations, and the strongest correlation among all variables was between $\dot{V}O_2$ and blood flow. Our collective data indicate that, among matched workloads, shorter contraction duration and greater muscle fibre recruitment augment FBF and $\dot{V}O_2$ during mild intensity forearm exercise, and that muscle blood flow is more closely related to metabolic cost ($\dot{V}O_2$) rather than contractile work *per se* during steady-state exercise in humans.

Keywords

vasodilatation; metabolic cost; oxygen delivery

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INTRODUCTION

Skeletal muscle blood flow increases to active muscles during exercise, the magnitude of which is typically graded with the level of contractile work. Although many substances have been proposed to be involved in exercise hyperemia such as K^+, adenosine, adenosine triphosphate (ATP), nitric oxide, prostaglandins, and lactate, as well as mechanical factors such as shear stress and compression/distortion of resistance vessels (Clifford & Hellsten, 2004), it has proved difficult to elucidate the precise mechanisms involved in the control of local vascular tone. Traditionally, contractile work has been considered the main determinant of skeletal muscle metabolic cost (i.e. oxygen consumption; $\dot{V}O_2$) which, according to the metabolic autoregulatory theory, would subsequently determine skeletal muscle blood flow to the active tissue (Anrep & von Saalfeld, 1935; Bockman et al., 1980; Bockman, 1983; Mohrman & Regal, 1988). However, recent data derived from both animal and human studies have challenged this concept (Bergstrom & Hultman, 1988; Hogan et al., 1998; Ferguson et al., 2001; Hamann et al., 2004; Hamann et al., 2005). Although not fully understood, it appears that conditions of prolonged heavy intensity exercise (Poole et al., 1991), as well as manipulation of contraction duration (Bergstrom & Hultman, 1988; Hogan et al., 1998; Hamann et al., 2005) and potentially muscle fibre recruitment (VanTeeffelen & Segal, 2000; Hamann et al., 2004), are capable of dissociating contractile work, metabolic cost, and muscle blood flow during exercise. Understanding the relations among these variables during steady-state exercise may provide insight into the determinants of and/or mechanisms underlying blood flow control to skeletal muscle.

For a given level of total work performed, metabolic cost ($\dot{V}O_2$) and contractile work have been observed to be dissociated when contractions are of shorter duration but also occur more frequently (Bergstrom & Hultman, 1988; Hogan et al., 1998). Further, within matched total time-tension indexes (TTI; the isometric analog of work), repeated stimulation of canine gastrocnemius-plantaris muscle for 0.25 seconds resulted not only in a significantly elevated $\dot{V}O_2$ compared to a longer 1 second stimulation with similar contraction-relaxation ratios, but also a greater muscle blood flow response (Hamann et al., 2005). In these studies, the elevated metabolic cost associated with shorter contraction durations was attributed to greater ATP utilization required for cross bridge activation and relaxation (Chasiotis et al., 1987; Bergstrom & Hultman, 1988). An important observation in this study was that the ratio of the change in muscle blood flow per unit change in $\dot{V}O_2$ was not different between exercise conditions, leading the investigators to conclude that the metabolic cost of contractions (not contractile work per se) is the primary determinant of blood flow during steady-state exercise (Hamann et al., 2004).

To date, the only study designed to address this issue and determine both muscle blood flow and $\dot{V}O_2$ in humans was performed by Ferguson and colleagues (Ferguson et al., 2001) during knee extensor exercise, and the data derived from this study indicate that muscle contractions performed at 100 rpm elicited a greater blood flow and $\dot{V}O_2$ response compared with contractions performed at 60 rpm, which is generally consistent with the previous studies in canines. However, in this study, the total work performed was matched by reducing the external load in an attempt to adjust for greater internal work (mechanical cost needed to overcome inertial and gravitational forces opposing limb movement) associated with higher intensity contractions, and close inspection of this data indicates significant variability between subjects (i.e. range of total work performed was overestimated by 25% and underestimated ~20% in some subjects) (Ferguson et al., 2000). Conversely, Hoelting and colleagues observed lower leg blood flow as the frequency of the contractions increased (40 vs. 60 and 80 contractions per minute) (Hoelting et al., 2001), and this was attributed to a reduction in the time spent in the relaxation phase of contractions when arterial inflow is unimpeded. In this study, $\dot{V}O_2$ during the various contraction frequencies was not assessed.

Thus, the effect of contraction frequency on muscle blood flow in humans remains equivocal. Further, in the studies determining both blood flow and $\dot{V}O_2$, only one workload was used in all of the aforementioned studies in dogs and humans where blood flow increased approximately 3-fold, and therefore it remains unknown whether these responses occur at higher workloads that elicit a greater haemodynamic response.

Another recent study demonstrated that muscle contractile work and blood flow can potentially be dissociated by altering muscle fibre recruitment for a given level of work. In 2004, Hamann *et al.* demonstrated that within matched work conditions (similar TTI), the blood flow response to a single forearm isometric contraction followed the degree of muscle fibre recruitment, suggesting that muscle fibre recruitment can independently influence skeletal muscle blood flow. However, given the transient and rapid hyperemia in response to a single contraction (Corcondilas *et al.*, 1964; Naik *et al.*, 1999; Kirby *et al.*, 2007; Carlson *et al.*, 2008), blood gases were not measured and thus it remains unknown whether the observed increase in muscle blood flow with greater muscle fibre recruitment was associated with an increase in metabolic cost of contractions ($\dot{V}O_2$). Manipulation of contractile work and muscle fibre recruitment during repeated (continuous), steady-state contractions is required to address this question.

Accordingly, the purpose of the present study was to determine the independent influence of (1) *muscle contraction duration* and (2) *muscle fibre recruitment* on skeletal muscle blood flow and $\dot{V}O_2$ during repeated handgrip exercise in humans utilizing two exercise protocols and two workloads that do not require an estimation of internal work to match total work performed. We hypothesized that, among equivalent workloads, (1) shorter contraction durations and (2) greater muscle fibre recruitment would elevate skeletal muscle blood flow during steady-state exercise. Further, we hypothesized that blood flow under all exercise conditions would be more closely associated with changes in metabolic cost ($\dot{V}O_2$), not contractile work *per se*.

METHODS

Subjects

With Institutional Review Board approval and following written informed consent, 11 individuals participated in protocol 1 and 13 participated in protocol 2 (Table 1) with 5 subjects having performed both protocols (protocols described below). Female subjects were studied during the early follicular phase of their menstrual cycle to minimize any potential cardiovascular effects of sex-specific hormones. All participants were non-smokers, non-obese, normotensive, and not taking any medications. Studies were performed after a 4-hour fast with the subjects in the supine position. The experimental arm of the subject was slightly elevated above heart level to minimize any potential influence of the muscle pump on forearm haemodynamics. All studies were performed according to the Declaration of Helsinki.

Venous Catheterization and Blood Gas Measurements

An 18 gauge catheter (3.8 cm) was inserted in retrograde fashion into an antecubital vein of the experimental arm for deep venous blood samples. Heparinized saline was continuously infused through this catheter at a rate of approximately 3 ml·min^{-1} for the duration of the study to keep it patent(Crecelius *et al.*, 2011a). A single venous blood sample (~5 ml) was collected at rest and at the end of each exercise bout into a heparinized syringe following a 5 ml waste sample that was disposed of. The blood sample was immediately analyzed with a clinical blood gas analyzer (Siemens Rapid Point 405 Automatic Blood Gas System, Los

Angeles, CA, USA) for partial pressures of venous oxygen and carbon dioxide (PO_2 and PCO_2), venous oxygen content (ctO_2), pH, and oxygen saturation (SO_2).

Forearm Blood Flow, Vascular Conductance and Oxygen Consumption

A 12 MHz linear-array ultrasound probe (Vivid 7, General Electric, Milwaukee, WI, USA) was used to determine brachial artery mean blood velocity (MBV) and brachial artery diameter. The probe was securely fixed to the skin over the brachial artery proximal to the venous catheter insertion site as previously described (Crecelius *et al.*, 2011a; Crecelius *et al.*, 2011b). For blood velocity measurements, the probe insonation angle was maintained at <60 degrees and the frequency used was 5 MHz. The Doppler shift frequency spectrum was analyzed via a Multigon 500M TCD (Multigon Industries, Mt Vernon NY, USA) spectral analyzer from which mean velocity was determined as a weighted mean of the spectrum of Doppler shift frequencies. Brachial artery diameter measurements were made in duplex mode at end-diastole at rest and between contractions (in triplicate) during steady-state conditions. Forearm blood flow (FBF) was calculated as:

FBF = MBV × π (brachial artery diameter/2)^2 × 60, where the FBF is in ml·min^{-1}, the MBV is in cm·s^{-1}, the brachial diameter is in cm, and 60 was used to convert from ml·s^{-1} to ml·min^{-1}. A fan was directed toward the experimental arm to minimize the potential contribution of skin blood flow to forearm haemodynamics.

As an index of vascular tone, forearm vascular conductance (FVC) was calculated as: (FBF/ Mean Arterial Pressure)*100 and expressed as (ml·min^{-1}·100mmHg^{-1}) (Crecelius *et al.*, 2011b).

Forearm $\dot{V}O_2$ was calculated in the experimental arm as: FBF × (arterial – venous O_2 content). Arterial oxygen content was assumed to be 20 ml·dL^{-1} (Holmgren & Linderholm, 1958; Roach *et al.*, 1999). Importantly, several studies have shown that arterial oxygen content does not change during mild-to-moderate forearm (handgrip) exercise in humans (Casey *et al.*, 2010; Crecelius *et al.*, 2011b).

Heart Rate, Cardiac Output and Mean Arterial Pressure

Heart rate (HR) was monitored with a 3-lead ECG. Cardiac Output (CO) was determined with a Finometer (Finapres Medical Systems BV, Amsterdam, The Netherlands) which calculates CO from HR and estimated Stroke Volume (SV) utilizing the modelflow method (Sugawara *et al.*, 2003). Mean arterial pressure (MAP) was measured by placing a finger pressure cuff around the middle phalanx of the middle finger on the non-experimental arm (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands). Resting arterial blood pressure was measured over the brachial artery following 30 minutes of supine rest, and just prior to each exercise trial (Cardiocap 5, Datex Ohmeda, Louisville,CO), and resting Finometer MAP was corrected for differences between the two readings (Kirby *et al.*, 2005).

Handgrip Exercise

Maximum voluntary contraction (MVC) was determined for each subject as the average of at least three maximal handgrips on a dynamometer (Stoelting, Chicago, IL, USA) that were within 3 percent of each other. All handgrip exercise (protocols 1 and 2; described below) was performed using both audio and visual cues to ensure correct timing of contraction and relaxation (Dinenno & Joyner, 2003, 2004). In addition, one research team member monitored exercise performance to ensure target force and duration were attained and that contraction execution was consistent.

Experimental Protocols

Figure 1 is a timeline for the specific trials. In each protocol, following 2 minutes of resting baseline measurements, 4 minutes of handgrip exercise was performed. Workloads were counterbalanced to eliminate any potential effect of order on the primary outcome variables. A minimum of 15 minutes of rest occurred between exercise bouts.

Protocol 1 (n =11) was designed to test the effect of *contraction duration* on FBF and $\dot{V}O_2$. All contractions were held for either 1 second or 4 seconds. In order to match work, the rest duration was varied such that a 1 second contraction was followed by a 2 second relaxation (1:2) and a 4 second contraction was followed by an 8 second relaxation (4:8). With this design, the duty cycles were equal and conditions of shorter contraction duration were also those in which contractions were performed more frequently.

Handgrip exercise was performed utilizing rhythmic isometric handgrip exercise on a dynamometer (Stoelting, Chicago, IL, USA). Within this protocol, two exercise intensities were examined, mild (20% MVC) and moderate (40% MVC). Total "work" performed among mild or moderate workload conditions was equal at end exercise by matching the time tension index (TTI), the isometric analog of work (Hamann *et al.*, 2005). The workload was matched by calculating the total tension generated over 4 minutes. For instance, with an MVC of 60 kg, the mild workload (20% 1:2 and 20% 4:8) time-tension index was calculated as follows: 20% MVC × 60 kg × 20 seconds of tension per minute (twenty 1 second contractions) × 4 minutes = 960 kg over 4 minutes and 20% × 60 kg × 20 seconds of tension per minute (five 4 second contractions) × 4 minutes of exercise = 960 kg over 4 minutes.

Protocol 2 (n = 13) was designed to determine the independent role of *muscle fibre recruitment* on FBF and $\dot{V}O_2$. The degree of muscle fibre recruitment was varied within subjects by altering the % MVC (10 % vs. 20%) (Taylor *et al.*, 1988; Adams *et al.*, 1992; Hamann *et al.*, 2004). In this protocol contraction duration was not varied and all contractions were performed over a 1 second period. In an effort to match total work, fewer contractions were performed with 20% MVC compared to 10% MVC. Fewer contractions and longer relaxation times led to varying duty cycles being performed to match total work. For example, within the mild (10% MVC) or moderate (20% MVC) contraction intensity conditions, the duty cycle was adjusted such that a 1 second contraction was followed by a 1, 3, or 7 second relaxation (30, 15, or 7 contractions per minute).

Weights corresponding to 10% and 20% MVC were attached to a pulley system and lifted 3.5 cm over the pulley to varying contraction: relaxation cycles. To calculate each workload, the load lifted (kg) was multiplied by the distance (0.035 m) traveled and the force of gravity (9.81 m·(sec^2)$^{-1}$) for each contraction and multiplied by the total number of contractions performed over 4 minutes. The two mild workloads were 10% MVC (1:3 ratio) and 20% MVC (1:7 ratio). The two moderate workloads were 10% MVC (1:1 ratio) and 20% MVC (1:3 ratio). The goal of both protocols was to use a mild workload that would elevate forearm blood flow ~3-fold as shown in previous studies (Dinenno & Joyner, 2004; Schrage *et al.*, 2004), and to further increase blood flow (~6-8 fold) during moderate intensity exercise (Dinenno & Joyner, 2003).

Data acquisition and analysis

Data (HR, CO, MAP, and mean blood velocity) were collected and stored on a computer at 250 Hz and were analyzed off-line with signal-processing software (WinDaq, DATAQ Instruments, Akron, OH, USA). The last 30 seconds of baseline were used to calculate average FBF, HR, CO and MAP for all experimental trials. For steady-state exercise, the last 30-36 seconds were used to ensure that the haemodynamic responses represented complete contraction: relaxation cycles for each trial. In addition, to determine the relation between

FBF and $\dot{V}O_2$ in response to exercise, we calculated the ratio of the change in FBF to the change in $\dot{V}O_2$ in response to exercise ($\Delta FBF/\Delta\dot{V}O_2$).

Statistics

Data are presented as mean ± S.E.M. For each experimental protocol, specific hypothesis testing comparing the two experimental conditions within each exercise workload was performed using two-tailed paired t-tests. The relations between contractile work, FBF, and $\dot{V}O_2$ were examined using simple linear regression analysis. Significance was set *a priori* at $P < 0.05$.

RESULTS

Protocol 1: Role of Contraction Duration

Forearm Haemodynamics and Oxygen Consumption—At rest, there were no differences in FBF and $\dot{V}O_2$ (Table 2). At rest and during exercise, MAP, CO, and HR were not different within mild and moderate workloads (Table 3). Among matched mild workloads, shorter contraction duration (20% 1:2 vs. 20% 4:8) exhibited greater FBF (184 ± 25 vs. 164 ± 25 ml·min^{-1}) and $\dot{V}O_2$ (23 ± 3 vs. 17 ± 2 ml·min^{-1}; $P < 0.05$; Figure 2). Conversely, among matched moderate TTI workloads, there were no differences in the exercise FBF or $\dot{V}O_2$ responses with shorter contraction duration (40% 4:8 vs. 40% 1:5; Figure 2).

The ratio of $\Delta FBF/\Delta\dot{V}O_2$ from rest to exercise was not different within workloads as the duration of the muscle contraction or degree of muscle fibre recruitment was varied, demonstrating that changes in FBF and $\dot{V}O_2$ paralleled one another (Figure 4A). Additionally, we determined the relations between contractile work performed and $\dot{V}O_2$, contractile work and FBF, as well as that between $\dot{V}O_2$ and FBF. There were significant relations between contractile work (TTI) and $\dot{V}O_2$ ($r^2 = 0.52$; $P < 0.001$), contractile work and FBF ($r^2 = 0.54$; $P < 0.005$) as well as $\dot{V}O_2$ and FBF ($r^2 = 0.88$; $P < 0.001$), with the strongest correlation between $\dot{V}O_2$ and FBF.

Venous Blood Gases—Table 3 presents resting and exercising venous blood gas data. There were no differences in resting blood gases within workloads. Among the mild workloads, 20% 4:8 had lower PCO_2 and higher PO_2 and %SO_2 compared to the other mild workload (20% 1:2). Venous oxygen content and pH were not different within mild workloads during exercise and there were no differences among exercising blood gas variables within the moderate workloads.

Protocol 2: Role of Muscle Fibre Recruitment

Forearm Haemodynamics and Oxygen Consumption—There were no differences in resting FBF and $\dot{V}O_2$ (Table 2). MAP, CO, and HR were not different within mild or moderate workloads at rest and exercise (Table 4). Among matched mild workloads, as the degree of muscle fibre recruitment increased from 10% to 20% MVC, there was a significant increase in FBF (152 ± 15 vs. 127 ± 14 ml·min^{-1}) and $\dot{V}O_2$ (20 ± 2 vs. 17 ± 2 ml·min^{-1}; both $P < 0.05$; Figure 3). In contrast, although FBF and $\dot{V}O_2$ tended to be elevated with greater muscle fibre recruitment among the moderate workloads, these did not achieve statistical significance ($P = 0.13$ and $P = 0.29$, respectively; Figure 3).

The ratio of $\Delta FBF/\Delta VO_2$ in response to exercise was not different within workloads as the degree of muscle fibre recruitment was varied (Figure 4B), demonstrating that changes in FBF and $\dot{V}O_2$ always paralleled one another. As in Protocol 1, there were significant relations between contractile work and $\dot{V}O_2$ ($r^2 = 0.72$, $P < 0.001$), and contractile work and

FBF ($r^2 = 0.78$, $P<0.001$), however the strongest correlation existed between $\dot{V}O_2$ and FBF ($r^2 = 0.90$, $P<0.001$).

Venous Blood Gases—Table 4 presents resting and exercising venous blood gas data. There were no differences in resting blood gas variables within workloads. Among the mild workloads there was a significantly lower PCO_2 and greater PO_2 and $\%SO_2$ in the 20% 1:7 vs. 10% 1:3 condition. Venous oxygen content and pH were not different within mild workloads during exercise and there were no differences among exercising blood gas variables within the moderate workloads.

DISCUSSION

The purpose of this study was to determine the independent influence of (1) contraction duration and (2) muscle fibre recruitment on skeletal muscle blood flow and $\dot{V}O_2$ during rhythmic handgrip exercise utilizing two exercise protocols in which total contractile work was matched in humans. The primary new findings are as follows. First, for a given amount of absolute work performed at *mild* exercise intensities, shorter contraction durations (Protocol 1) and greater muscle fibre recruitment (Protocol 2) were associated with greater levels of muscle blood flow and $\dot{V}O_2$. In contrast, altering contraction duration or muscle fibre recruitment at higher intensity exercise (moderate workloads) did not significantly influence blood flow or $\dot{V}O_2$, although there was a trend for muscle fibre recruitment to have an independent influence on these variables. Second, the ratio of the change in forearm blood flow to the change in $\dot{V}O_2$ was similar across all experimental manipulations in both exercise protocols. Third, in both experimental protocols, the strongest correlation among all variables was found between $\dot{V}O_2$ and FBF. Collectively, these findings indicate an ability to dissociate total work from the haemodynamic and metabolic cost of muscle contractions, via alterations in contraction duration or muscle fibre recruitment, but importantly demonstrate that muscle blood flow and $\dot{V}O_2$ changed in parallel during steady-state exercise in humans.

Protocol 1: role of contraction duration

Several investigations have demonstrated that shorter contraction durations are associated with a greater metabolic cost ($\dot{V}O_2$) compared with longer duration contractions for a given amount of total work performed (Chasiotis *et al.*, 1987; Bergstrom & Hultman, 1988; Hogan *et al.*, 1998; Ferguson *et al.*, 2001; Hamann *et al.*, 2005). Previous studies in humans using electrical stimulation of the leg musculature (Chasiotis *et al.*, 1987; Bergstrom & Hultman, 1988) observed greater ATP utilization in the condition with more frequent contractions and it has been estimated that the energy cost of the activation and relaxation phases of a contraction comprise ~40% of total energy requirements (Bergstrom & Hultman, 1988). Therefore, in the present study, although ATP utilization was not determined, the greater metabolic cost associated with shorter/more frequent contractions could be due to a larger energy requirement.

More recently, Hamann and colleagues demonstrated in the canine hindlimb that the augmented $\dot{V}O_2$ observed with shorter contraction durations was associated with a greater muscle blood flow response (Hamann *et al.*, 2005). Importantly, this study also demonstrated that the Δ muscle blood flow/$\Delta \dot{V}O_2$ was similar across exercise conditions, indicating that the muscle blood flow responses followed the elevated metabolic cost associated with shorter duration contractions. To the best of our knowledge, only one previous study has attempted to address this in humans. Ferguson et al. varied the number of contractions performed during single knee extensor exercise (60 rpm vs. 100 rpm) while matching the total work performed on a leg ergometer in humans (Ferguson *et al.*, 2001).

Greater FBF and $\dot{V}O_2$ were observed within the 100 rpm condition; however, in order to match total power output, the external load was estimated and adjusted in an effort to account for internal work associated with inertia, which led to large variability in the actual total work performed (Ferguson *et al.*, 2000).

In the present study, we utilized mild-to-moderate intensity isometric handgrip exercise (in the supine position), an exercise modality which eliminated the need to account for internal power or inertia to address this question at two different exercise intensities. Thus, TTI (isometric analog of work) was matched by having subjects contract at the same intensity while varying the duration of the contraction. In the mild workload condition of the present study, shorter contraction durations elicited greater muscle blood flow and $\dot{V}O_2$. Further, the Δ muscle blood flow/$\Delta\dot{V}O_2$ ratio was similar across all experimental conditions (mild and moderate workloads; Figure 4A), and this observation is consistent with those in the canine hindlimb (Hamann *et al.*, 2004). These collective observations support the notion that greater metabolic demand associated with shorter contraction durations provides local feedback which facilitates elevations in skeletal muscle blood flow. This hypothesis regarding local metabolic autoregulation of blood flow is supported, in part, by significant differences among blood gas variables between mild exercise conditions (20% 1:2 and 20% 4:8). Specifically, in addition to greater blood flow and $\dot{V}O_2$, the shorter contraction duration condition (20% 1:2) resulted in a significantly higher PCO_2 and lower $SO_2\%$, PO_2, and venous CtO_2 (Table 3), all of which would imply a greater metabolic perturbation providing local feedback to facilitate muscle blood flow.

Protocol 2: role of muscle fiber recruitment

Recent data (Hamann *et al.*, 2004) indicate that among matched work conditions (equal time tension index [TTI]), the blood flow response to a single, brief forearm muscle contraction increased as the number of muscle fibres recruited increased. Furthermore, VanTeeffelen and colleagues demonstrated that among matched tension conditions, arteriolar vasodilatation increased with motor unit recruitment during rhythmic contraction of the hamster retractor muscle (VanTeeffelen & Segal, 2000). Thus, these investigators were able to dissociate the blood flow/vasodilatory response to muscle contractions from the absolute work performed. However, in these studies, it was not possible to determine whether this augmented hyperemic response was associated with an elevated metabolic demand due to greater muscle fibre recruitment. Further, single contractions do not result in an accumulation of metabolites and thus the increase in blood flow represents more of a local feedforward versus a feedback mechanism (Kirby *et al.*, 2007). In the present study, we manipulated muscle fibre recruitment by altering the absolute load lifted by each subject (Taylor *et al.*, 1988; Hamann *et al.*, 2004) but kept total work constant by adjusting the number of contractions during four minutes of dynamic steady-state exercise. Within the mild workload condition, we observed uncoupling of contractile work from muscle blood flow and $\dot{V}O_2$ during rhythmic (continuous) contractions, such that greater blood flow and $\dot{V}O_2$ were observed when muscle fibre recruitment was increased. In the moderate workload condition, similar trends were observed yet they did not achieve statistical significance. As in protocol 1, the Δ muscle blood flow/$\Delta\dot{V}O_2$ ratio was similar across all experimental conditions (mild and moderate workloads; Figure 4B). Thus, again, we were able to dissociate contractile work and the metabolic cost of contractions, but we were unable to uncouple muscle blood flow and $\dot{V}O_2$.

In Protocol 2, it is unclear how increasing muscle fibre recruitment may enhance the metabolic demand for a given amount of contractile work, however, the stimulation of a greater number of muscle fibres may require more ATP for the initiation and relaxation of cross-bridge formation across a larger number of muscle fibres (Krustrup *et al.*, 2008). Further, given that each subject lifted a heavier load within exercise intensities to increase

muscle fibre recruitment, it is likely that there was also greater recruitment of fast twitch muscle fibres. Consistent with the metabolic autoregulatory theory of local blood flow control, recent work indicates that activation of fast twitch muscle fibres is associated with greater metabolic demand and blood flow responses compared with slow twitch fibres (Krustrup *et al.*, 2008; Hellsten *et al.*, 2009). Although this mechanism may be attributing to the elevation in metabolic demand and subsequent blood flow response, it is also plausible that activation of a greater number of muscle fibres may lead to higher potassium (K+) efflux from the muscle and/or greater mechanical compression/distortion of resistance vessels, both of which would result in greater local feedforward mechanisms of vasodilatation (Mohrman & Sparks, 1974; Lott *et al.*, 2001; Armstrong *et al.*, 2007; Kirby *et al.*, 2005, 2007).

Experimental Considerations

There are two primary experimental considerations with the current set of experiments. First, why shorter contraction duration or greater muscle fibre recruitment would only result in significant elevations in blood flow and $\dot{V}O_2$ at mild workloads is unclear. Although there was a trend for muscle fibre recruitment to have an independent influence on blood flow and $\dot{V}O_2$ at the moderate workload in Protocol 2, any independent influence of contraction duration or fibre recruitment was clearly more robust a mild intensity exercise. Perhaps this indicates that the relative contribution of the associated mechanisms involved in increasing blood flow and $\dot{V}O_2$ are greater at lower exercise intensities and become reduced as exercise intensity increases. Future studies would be required to address this issue.

Second, the results from the present study must be interpreted within the experimental conditions employed. Subjects in both protocols performed mild-to-moderate rhythmic handgrip exercise in which systemic haemodynamics and perfusion pressure were relatively constant. In this context, recent data indicate that acute manipulations of perfusion pressure (via movement of the exercising forearm above and below heart level) during constant load exercise lead to alterations in muscle blood flow that are not fully restored to control levels (Walker *et al.*, 2007). Although blood gases and thus $\dot{V}O_2$ were not measured in this study, these data indicate that perfusion pressure may have an independent influence on exercise hyperemia under certain experimental conditions.

Experimental Limitations

In an effort to match total work performed during repeated continuous contractions the number of contractions performed, the duration with which they were held, and the time between contractions was varied. In Protocol 1, the contraction duration and force output were varied to match a predetermined amount of "work" (TTI). Although a trained researcher monitored exercise performance, TTI was not actually measured and therefore individual variability in the performance of the task could have led to us over or under estimate work performed. In Protocol 2, we did not directly quantify muscle fibre recruitment, however previous studies clearly indicate that altering muscle contraction intensity (i.e. increasing %MVC) leads to a greater EMG signal indicating augmented muscle fibre recruitment (Taylor *et al.*, 1988). Even if EMG measures were obtained in the present study, we still would not know exactly how many additional muscle fibres were recruited by increasing the workload from 10 to 20% MVC. Additionally in Protocol 2, a greater amount of time spent in the relaxation phase between contractions could potentially augment the blood flow response as muscle contraction can impede arterial inflow. It is unlikely that augmented relaxation time can explain our blood flow responses because in Protocol 1, 20% 4:8 resulted in a lower blood flow response than 20% 1:2, even though the total time in the relaxation phase was greater during steady-state exercise when blood flow was quantified (24 vs. 20 seconds). Further, we did not observe a significant elevation in

blood flow in Protocol 2 during moderate intensity exercise in which the relaxation time for the 20% 1:3 trial (greater muscle fibre recruitment) was longer than the 10% 1:1 trial (24 vs. 15 seconds). These data are consistent with that of Shoemaker and colleagues (Shoemaker *et al.*, 1998) who demonstrated no difference in the total blood flow response when the relaxation time varied between 1 and 2 seconds when contractile work was matched.

Conclusions

The findings from the present study indicate that both shorter skeletal muscle contraction duration and greater muscle fibre recruitment under conditions of matched total contractile work can independently influence muscle blood flow and $\dot{V}O_2$ during steady-state handgrip exercise in humans. The apparent independent influence of these variables is more robust during mild exercise intensities. Further, the changes in blood flow and $\dot{V}O_2$ during exercise always paralleled one another regardless of exercise intensity and whether contraction duration or muscle fibre recruitment was manipulated. The collective observations indicate that muscle blood flow responses under the experimental conditions employed are more closely related to metabolic cost ($\dot{V}O_2$) than contractile work *per se*.

Acknowledgments

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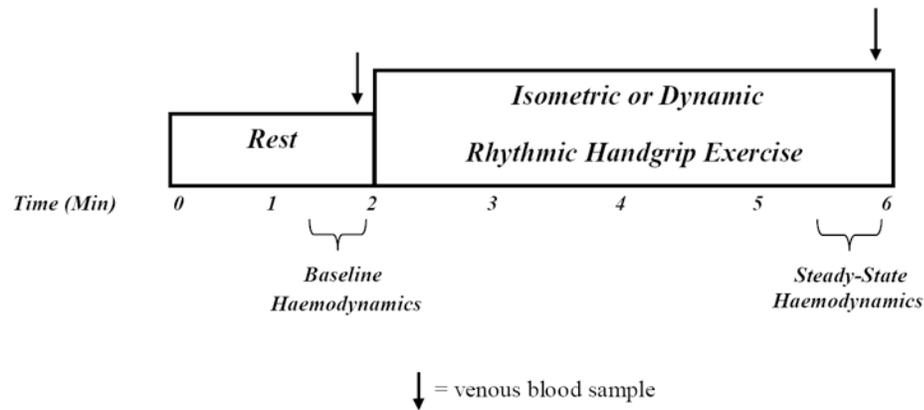
Figure 1. General Experimental Trial
Each trial consisted of a 2-minute baseline period. After this time period, subjects performed 4 minutes of either rhythmic isometric handgrip exercise (Protocol 1) or rhythmic dynamic handgrip exercise (Protocol 2). A venous blood sample for determination of $\dot{V}O_2$ was collected from the experimental arm at rest and during the final minute of exercise. The final 30 seconds of rest and 30-36 seconds of exercise were used to ensure that the average steady-state forearm blood flow and $\dot{V}O_2$ represented complete contraction: relaxation cycles for all condition.
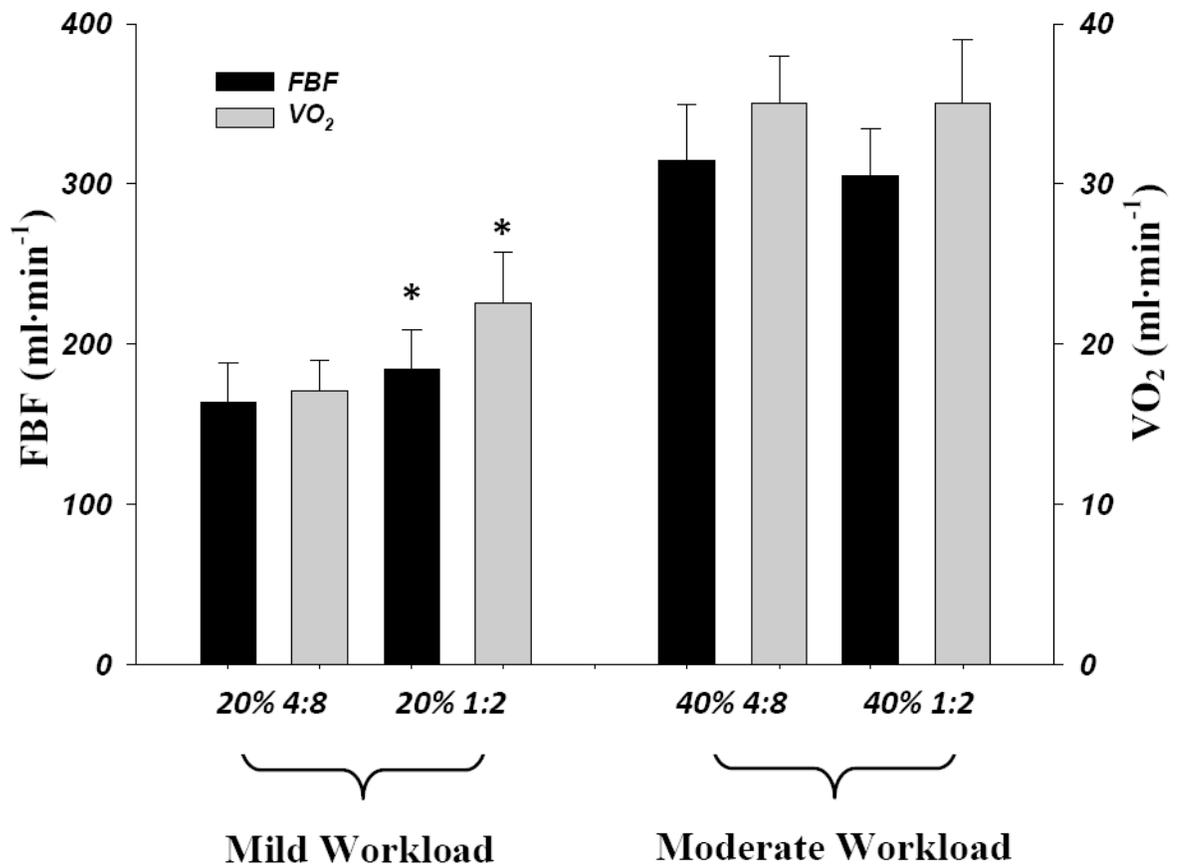
Figure 2. Forearm blood flow and $\dot{V}O_2$ during exercise in Protocol 1
Among the mild workloads, shorter contraction duration resulted in greater FBF and $\dot{V}O_2$.
However, there was no significant increase in FBF or $\dot{V}O_2$ among the moderate workloads
as the contraction duration was shortened.
*$P < 0.05$ vs. 20% 4:8 condition
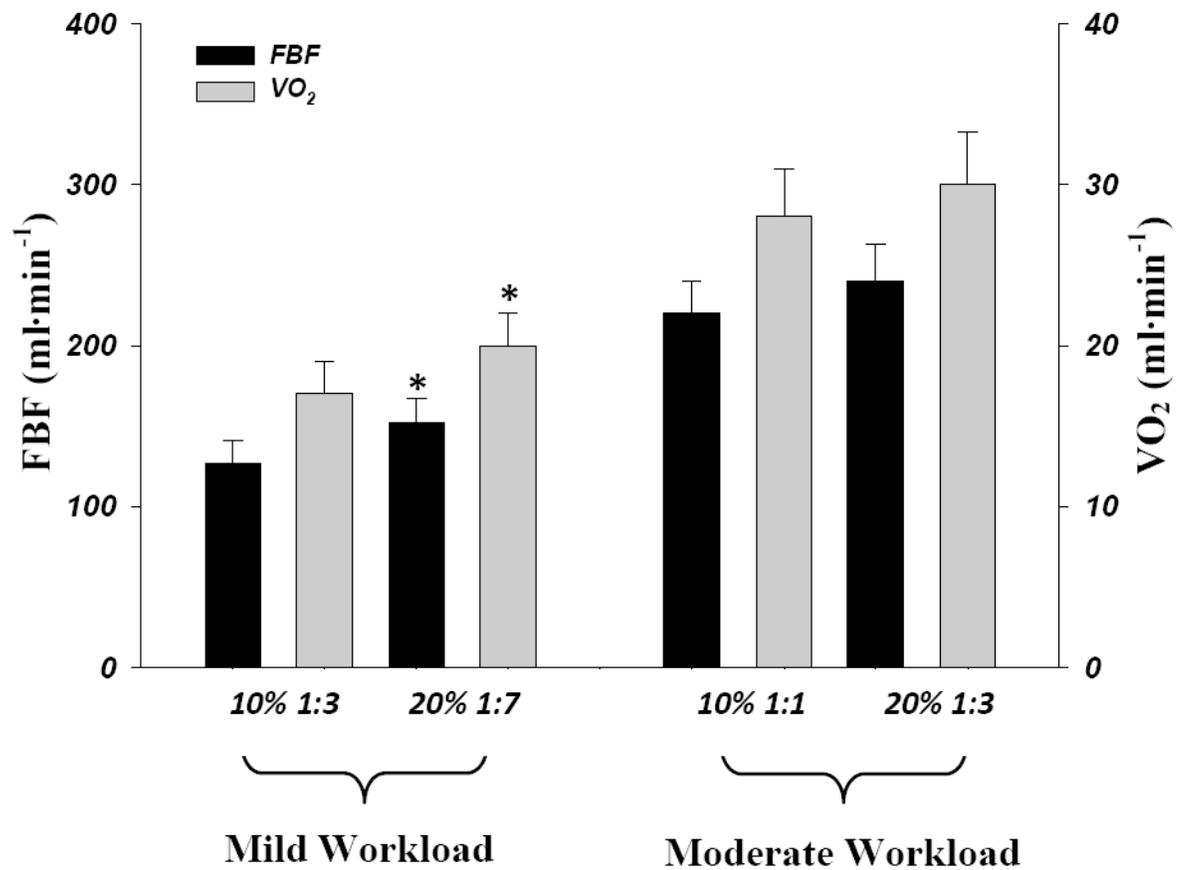
Figure 3. Forearm blood flow and V̇O₂ during exercise in Protocol 2
In the mild workloads, as the degree of muscle fibre recruitment increased from 10% MVC to 20% MVC, there was a significant increase in FBF and V̇O₂. However, there was no significant difference in FBF or V̇O₂ response among the moderate workload as the degree of muscle fibre recruitment increased.
* $P < 0.05$ vs. 10% 1:3 condition
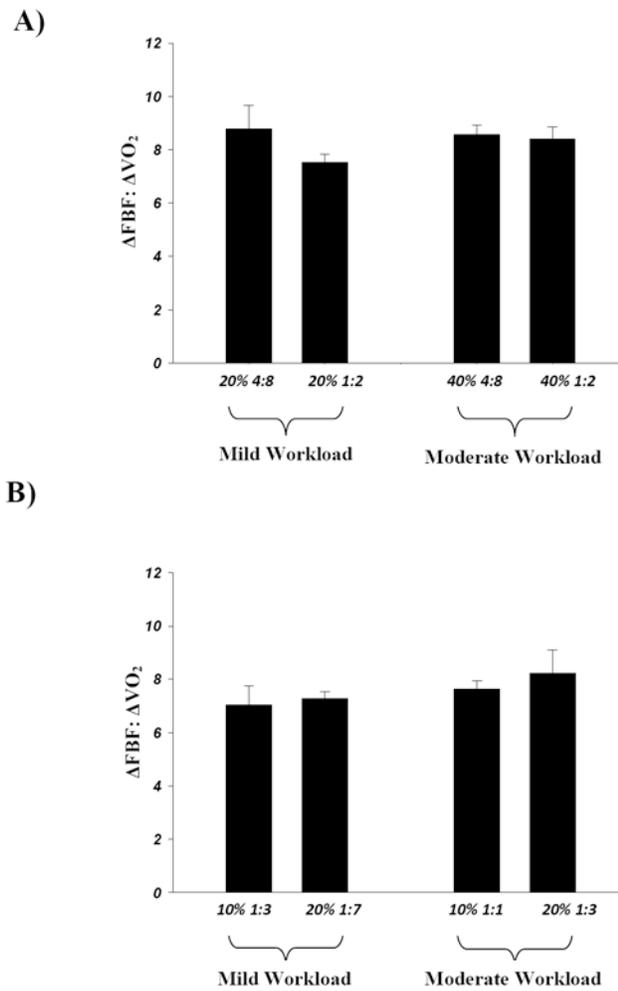
Figure 4. Ratio of change in forearm blood flow: change in $\dot{V}O_2$ in response to exercise in Protocol 1 (A) and Protocol 2 (B)
As the contraction duration (A) or degree of muscle fibre recruitment (B) varied, the ratio of change in forearm blood flow (FBF): $\dot{V}O_2$ from rest to exercise did not vary between workloads suggesting that changes in FBF and $\dot{V}O_2$ always paralleled one another.

Table 1

Subject Characteristics

	Protocol 1	**Protocol 2**
Male/Female	9/2	11/2
Age (Years)	20 ± 1	20 ± 1
Height (cm)	178.5 ± 0.1	176.7 ± 0.1
Weight (Kg)	74.7 ± 3.4	75.7 ± 2.5
BMI (kg·m2)	24 ± 1	24 ± 1
MVC (Kg)	42 ± 4	47 ± 4
Forearm FFM (g)	915 ± 91	966 ± 78

All values are Mean ± S.E.M.;

BMI = body mass index; MVC = maximum voluntary contraction; FFM = fat-free mass

Table 2

Resting forearm blood flow and VO$_2$ for protocols 1 and 2.

	FBF (ml·min^{-1})	VO$_2$ (ml·min^{-1})
Protocol 1- Contraction Duration (n = 11)		
20% 4:8 (mild)	37 ± 5	2.6 ± 0.2
20% 1:2 (mild)	35 ± 5	2.7 ± 0.3
40% 4:8 (moderate)	37 ± 5	2.8 ± 0.3
40% 1:2 (moderate)	36 ± 5	3.0 ± 0.3
Protocol 2- Muscle Fibre Recruitment (n = 13)		
10% 1:3 (mild)	33 ± 1	2.9 ± 0.3
20% 1:7 (mild)	32 ± 4	3.0 ± 0.3
10% 1:1 (moderate)	31 ± 4	2.8 ± 0.3
20% 1:3 (moderate)	33 ± 5	3.0 ± 0.3

All values are Mean ± S.E.M

Table 3

Protocol 1 Rest and Exercise Cardiovascular and Blood Gas Data

Protocol 1: Contraction Duration Rest and Exercise Cardiovascular and Blood Gas Variables

Condition	Time	Heart Rate (beats·min⁻¹)	Cardiac Output (liters·min⁻¹)	Mean Arterial Pressure (mmHg)	Forearm Vascular Conductance (ml·min⁻¹·100mmHg⁻¹)	pH	PCO₂ (mmHg)	SO₂ %	PO₂ (mmHg)	CtO₂ (ml·dL⁻¹)
Mild 20% 4:8	Rest	60 ± 4	4.9 ± 0.5	91 ± 2	40 ± 4	7.35 ± 0.00	46.4 ± 1.2	59.1 ± 4.5	33.5 ± 1.8	13 ± 1
	Exercise	$66 \pm 3^*$	$5.5 \pm 0.4^*$	95 ± 2	$170 \pm 23^*$	7.34 ± 0.01	48.3 ± 1.1	$40.7 \pm 2.2^*$	$25.3 \pm 0.9^*$	$8 \pm 1^*$
Mild 20% 1:2	Rest	59 ± 3	5.2 ± 0.4	91 ± 1	38 ± 5	7.35 ± 0.00	46.5 ± 1.1	54.2 ± 5.2	31.5 ± 2.2	11 ± 1
	Exercise	$64 \pm 4^*$	5.6 ± 0.4	92 ± 1	$198 \pm 25^{*\dagger}$	$7.32 \pm 0.01^*$	$52.6 \pm 1.6^{*\dagger}$	$36.4 \pm 2.3^{*\dagger}$	$23.5 \pm 1.0^{*\dagger}$	$8 \pm 1^*$
Moderate 40% 4:8	Rest	63 ± 4	5.3 ± 0.4	91 ± 1	41 ± 5	7.35 ± 0.01	47.2 ± 1.1	57.2 ± 4.1	31.9 ± 1.7	12 ± 1
	Exercise	67 ± 4	$5.9 \pm 0.5^*$	$97 \pm 3^*$	$318 \pm 29^*$	$7.30 \pm 0.01^*$	$52.9 \pm 1.5^*$	$41.9 \pm 1.5^*$	$27.3 \pm 1.0^*$	$9 \pm 1^*$
Moderate 40% 1:2	Rest	62 ± 3	5.2 ± 0.6	92 ± 2	38 ± 5	7.34 ± 0.01	49.5 ± 2.0	51.6 ± 4.8	29.7 ± 1.9	13 ± 1
	Exercise	$69 \pm 4^*$	$5.6 \pm 0.6^*$	$97 \pm 2^*$	$315 \pm 30^*$	$7.30 \pm 0.01^*$	$54.5 \pm 2.0^*$	$39.3 \pm 1.8^*$	$26.5 \pm 0.9^*$	$9 \pm 1^*$

All values are Mean ± S.E.M

*
P<0.05 vs. Rest,

†P<0.05 vs. 20% 4:8 Exercise Condition.

Table 4

Protocol 2 Rest and Exercise Cardiovascular and Blood Gas Data

Protocol 2: Muscle Fibre Recruitment Rest and Exercise Cardiovascular and Blood Gas Variables

Condition	Time	Heart Rate (beats·min⁻¹)	Cardiac Output (liters· min⁻¹)	Mean Arterial Pressure (mmHg)	Forearm Vascular Conductance (ml·min⁻¹. 100mmHg⁻¹)	pH	PCO₂ (mmHg)	SO₂ %	PO₂ (mmHg)	CtO₂ (ml·dL⁻¹)
Mild 10% 1:3	Rest	55 ± 3	5.2 ± 0.3	91 ± 2	36 ± 5	7.36 ± 0.01	49.6 ± 1.6	50.3 ± 0.3	27.8 ± 1.8	10 ± 1
	Exercise	$60 \pm 3^*$	5.5 ± 0.3	93 ± 2	$137 \pm 15^*$	$7.34 \pm 0.01^*$	52.8 ± 1.1	$31.5 \pm 2.7^*$	$19.6 \pm 0.6^*$	$7 \pm 1^*$
Mild 20% 1:7	Rest	56 ± 3	5.3 ± 0.3	92 ± 2	35 ± 5	7.36 ± 0.01	48.2 ± 0.8	47.5 ± 2.7	27.2 ± 1.3	10 ± 1
	Exercise	$60 \pm 3^*$	5.5 ± 0.4	93 ± 2	$163 \pm 16^{*\dagger}$	7.34 ± 0.01	$49.5 \pm 1.1^\dagger$	$33.8 \pm 1.6^*$	$21.2 \pm 0.6^{*\dagger}$	$7 \pm 1^*$
Moderate 10% 1:1	Rest	56 ± 3	5.1 ± 0.3	92 ± 1	35 ± 5	7.36 ± 0.01	49.2 ± 1.4	51.8 ± 3.1	28.7 ± 1.4	11 ± 1
	Exercise	$61 \pm 4^*$	$5.6 \pm 0.3^*$	94 ± 2	$235 \pm 20^*$	$7.30 \pm 0.01^*$	$58.8 \pm 1.0^*$	$35.7 \pm 1.6^*$	$23.2 \pm 0.6^*$	$7 \pm 1^*$
Moderate 20% 1:3	Rest	56 ± 3	5.2 ± 0.3	91 ± 2	36 ± 5	7.36 ± 0.01	48.2 ± 0.7	49.1 ± 3.5	27.9 ± 1.5	10 ± 1
	Exercise	$62 \pm 4^*$	$5.5 \pm 0.3^*$	93 ± 2	$258 \pm 24^*$	$7.31 \pm 0.01^*$	$56.5 \pm 1.2^*$	$35.8 \pm 2.6^*$	$22.3 \pm 0.6^*$	$8 \pm 1^*$

All values are Mean ± S.E.M

*
P<0.05 vs. Rest,

†
P<0.05 vs. 10% 1:3 Exercise Condition.