Temporal profile of rat skeletal muscle capillary haemodynamics during recovery from contractions

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In skeletal muscle capillaries, red blood cell (RBC) flux (F_{RBC}), velocity (V_{RBC}) and haematocrit (Hct_{CAP}) are key determinants of microvascular O_2 exchange. However, the mechanisms leading to the changes in F_{RBC}, V_{RBC} and Hct_{CAP} during muscle contractions and recovery thereafter are not fully understood. To address this issue we used intravital microscopy to investigate the temporal profile of the rat spinotrapezius muscle ($n = 5$) capillary haemodynamics during recovery from 3 min of twitch muscle contractions (1 Hz, 4–6 V). Specifically, we hypothesized that (1) during early recovery F_{RBC} and V_{RBC} would decrease rapidly and F_{RBC} would display a biphasic response (consistent with a muscle pump effect on capillary haemodynamics), and (2) there would be a dynamic relationship between changes (Δ) in V_{RBC} and Hct_{CAP}. The values at rest (R) and end-recovery (ER) were significantly lower ($P < 0.05$) than at end-contraction (EC) for F_{RBC} (in cells s^{-1}, R $= 30.1 \pm 7.8$, EC $= 46.2 \pm 7.3$ and ER $= 26.0 \pm 6.1$), V_{RBC} (in $\mu m\ s^{-1}$, R $= 368 \pm 83$, EC $= 497 \pm 62$ and ER $= 334 \pm 59$) and Hct_{CAP} (R $= 0.193 \pm 0.016$, EC $= 0.214 \pm 0.023$ and ER $= 0.185 \pm 0.019$). The first data point where a significant decrease in F_{RBC}, Hct_{CAP} and V_{RBC} occurred was at 5, 5 and 20 s post-contraction, respectively. The decrease in F_{RBC} approximated a monoexponential response (half-time of ~26 s). The relationship between ΔV_{RBC} and ΔHct_{CAP} was not significant ($P > 0.05$). Based on the early decrease in F_{RBC} (within 5 s), overall dynamic profile of F_{RBC} and the ~20 s 'delay' to the decrease in V_{RBC} we conclude that the muscle pump does not appear to contribute substantially to the steady-state capillary haemodynamics in the contracting rat spinotrapezius muscle. Moreover, our findings suggest that alterations in V_{RBC} do not obligate proportional changes in Hct_{CAP} within individual capillaries following muscle contractions.

(Received 4 January 2006; accepted after revision 26 March 2006; first published online 31 March 2006)
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Several mechanisms have been proposed to explain the skeletal muscle hyperaemia during exercise including the muscle pump, nitric oxide and endothelium-derived hyperpolarizing factor (for review see Clifford & Hellsten, 2004; Tschakovsky & Sheriff, 2004). Currently, the role of the muscle pump in the exercise hyperaemia remains controversial (Sheriff, 2005; Clifford *et al.* 2005).

The key microcirculatory variables that influence O_2 exchange are red blood cell flux (F_{RBC}), velocity (V_{RBC}) and capillary haematocrit (Hct_{CAP}). In the microcirculation, the biphasic increase in F_{RBC} following the onset of contractions is associated with a rapid increase in V_{RBC} (within 2 s) to values similar to, or greater than, the steady state (Kindig *et al.* 2002). These results suggested a prominent role of the muscle pump or some very rapid vasodilatory process on skeletal muscle capillary haemodynamics (i.e. F_{RBC}, V_{RBC} and Hct_{CAP}). However, the mechanisms controlling F_{RBC}, V_{RBC} and Hct_{CAP} during recovery from exercise remain elusive. Qualitatively similar to the onset of exercise, the recovery dynamics of estimated capillary blood flow (equivalent to F_{RBC}) displayed an initial fast decrease (half-time, $t_{0.5}$, ~6.5 s) followed by a slower response ($t_{0.5}$ ~21 s) (Ferreira *et al.* 2005*a*). However, these estimated responses relied on only one variable (~F_{RBC}) and a set of assumptions that limit their interpretation (Ferreira *et al.* 2005*a*,*b*). In this context, insights into the potential effect of the muscle pump on skeletal muscle capillary haemodynamics (steady state and recovery) could be gained by determining, concurrently, the time course of F_{RBC}, V_{RBC} and Hct_{CAP} after cessation of contractions.

During the steady state of muscle contractions there is an ~25–30% increase in Hct_{CAP} (Klitzman & Duling, 1979; Kindig *et al.* 2002) that is thought to stem from arteriolar vasodilatation (Desjardins & Duling, 1990) and increases in V_{RBC} (Duling *et al.* 1982; Desjardins & Duling, 1987,

 DOI: 10.1113/jphysiol.2006.104802

1990). These suggestions are based on observations made during steady-state contractions (Klitzman & Duling, 1979) or during pharmacologically induced vasodilatation (Desjardins & Duling, 1990). If the increase in Hct_{CAP} with contractions is a consequence of an elevated V_{RBC}, we would expect a positive (linear or non-linear) relationship between ΔV_{RBC} and ΔHct_{CAP}. Moreover, this relationship would be maintained during the steady state and recovery from contractions when physiologically induced changes in both Hct_{CAP} and V_{RBC} occur.

In the present study we examined, for the first time, the temporal profile of skeletal muscle capillary haemodynamics (F_{RBC}, V_{RBC} and Hct_{CAP}) following the cessation of muscle contractions to elucidate the potential role of the muscle pump on capillary hyperaemia and the effects of changes in V_{RBC} on Hct_{CAP} during muscle contractions. Assuming that there is a time delay to the decrease in arteriolar diameter following the cessation of muscle contractions (Gorczynski & Duling, 1978; Bearden *et al.* 2004) and that the muscle pump imparts mechanical energy affecting V_{RBC} and F_{RBC} with a rapid on–off time course, we hypothesize that the muscle pump effect on capillary hyperaemia would be manifested by a rapid decrease in V_{RBC} and a biphasic response of F_{RBC} (fast decrease followed by slower dynamics), which is analogous (but directionally opposite) to the onset of exercise (see Fig. 1 and Kindig *et al.* 2002). Regarding the factor(s) determining the increase in Hct_{CAP} with contractions, we hypothesized that following the cessation of muscle contractions there would be a dynamic relationship between changes in V_{RBC} and Hct_{CAP}, suggesting a mechanistic association between these variables as previously proposed (Duling *et al.* 1982).

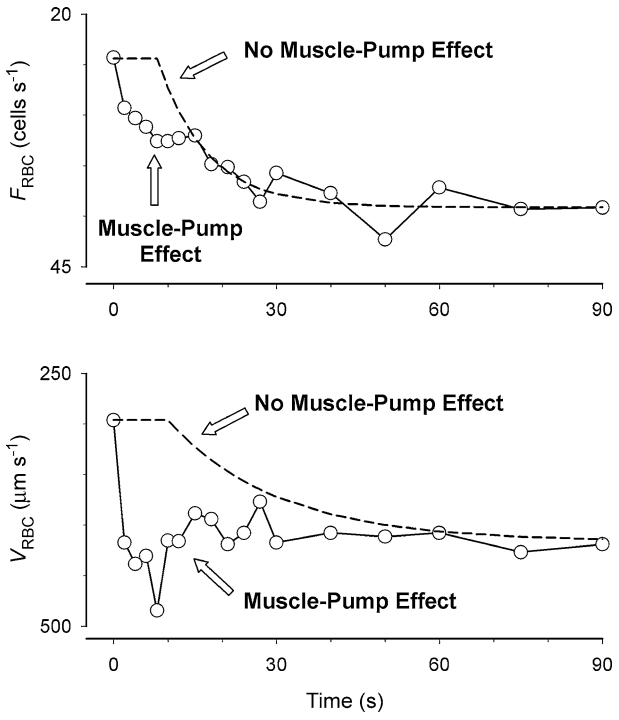

Figure 1
Schematic hypothesized representation of capillary red blood cell flux (F_{RBC}) and velocity (V_{RBC}) during recovery from muscle contractions in the presence (circles) or absence (dashed lines) of a muscle pump effect on capillary hyperaemia. Open circles: 'mirror image' of data from Kindig *et al.* (2002) for capillary haemodynamics following the onset of exercise. Note that the *y*-axis was modified to be visually equivalent to the profiles expected during recovery from contractions. Dashed lines: theoretical representation assuming a delay before the onset of changes in arteriolar diameter (Gorczynski & Duling, 1978; Bearden *et al.* 2004).

Methods

Animals

Experiments were performed in seven female Sprague-Dawley rats (279 ± 6 g). The focal plane could not be maintained over the entire 3 min of recovery from muscle contractions in two animals therefore capillary haemodynamics data were collected in five animals. Upon completion of the experimental procedures the animals were killed with an overdose of pentobarbital sodium. The study was approved by the Institutional Animal Care and Use Committee at Kansas State University.

Muscle preparation

The procedures used in the current study were described in detail by Kindig *et al.* (2002). Briefly, animals were anaesthetized (pentobarbital sodium 40 mg kg^{-1} I.P. to effect) and their right carotid artery cannulated for measurement of blood pressure and heart rate (Digi-Medical BPA model 200, Louisville, KY, USA). The rat was placed on a circulation-heated (38°C) Lucite platform and the left spinotrapezius muscle was exteriorized, sutured at five equidistant positions to a thin wire horseshoe and superfused continuously with a Krebs-Henseleit bicarbonate-buffered solution equilibrated with 5% CO_2–95% N_2. The remaining exposed tissue was kept moist and covered with Saran wrap (Dow, Indianapolis, IN, USA). Importantly, this surgical procedure preserves the spinotrapezius microvascular blood flow compared to the intact muscle (Bailey *et al.* 2000). A microvascular field typically containing 6–10 capillaries was selected and muscle sarcomere length set at ~2.7 μm, as confirmed by on-screen measurements. Thereafter, twitch muscle contractions were induced (1 Hz, 4–6 V, 2 ms pulse duration) for 3 min and the same microvascular field was followed for 3 min after cessation of muscle contractions. This stimulation protocol has been shown to result in an increase in blood flow of ~2–3 times the resting value (Behnke *et al.* 2001) and has the advantage

of not inducing fatigue, as determined by maintenance of initial tension development for up to 10 min (D. C. Poole, T. I. Musch & S. A. Hahn, unpublished observations).

Image aquisition and analysis

Images were obtained by bright-field microscopy (Eclipse E600-FN, Nikon; ×40 objective; numerical aperture, 0.8) and recorded at 30 frames per second on super-VHS cassettes (BR-S822 U, JVC, Elmwood Park, NJ, USA) for off-line analysis. The super-VHS tapes were played back and the recorded microvascular fields (270 μm × 210 μm) viewed on a high-resolution monitor (Trinitron PVM-1954Q, Sony, Ichinoniya, Japan). Final magnification was ×1184, as verified by calibration with a stage micrometer.

For each microvascular field we identified capillaries in which haemodynamic measurements could be made during the time periods of interest (see below) over the 3 min of recovery and randomly selected five capillaries per muscle (on one muscle the focal plane limited measurements to four capillaries). Based on this criterion capillaries not supporting RBC flow at rest were not excluded from the study *a priori*; however, reflecting the fact that the vast majority of capillaries are flowing at rest all capillaries examined supported RBC flow prior to the onset of muscle contractions and continued to do so during and following cessation of contractions (see Results). At the end of muscle contractions capillary diameter (Dia_{CAP}) was measured at three sites along the capillary length for those capillaries in which haemodynamics were assessed. Red blood cell velocity (V_{RBC}) and capillary haematocrit (Hct_{CAP}) were assessed over several frames within a 1 s time window (30 frames). Measurements were made twice within 30 s prior to the onset of contractions (rest, R), within ∼16 frames (Kindig *et al.* 2002) after the microvascular field resumed its focus subsequent to the last contraction (end-contraction, EC) and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120 and 180 s (end-recovery, ER). Based on preliminary measurements we determined that this sampling strategy would be sufficient to address the primary hypotheses of our study (L. F. Ferreira, D. J. Padilla & D. C. Poole, unpublished observations). V_{RBC} was determined by following the RBC path length over a period of time (determined from total number of frames). The number of RBCs within a capillary was determined by tracing individual RBCs over an average capillary length of approximately 120 μm. V_{RBC} and number of RBCs were usually measured within the same frames; however, on some occasions this was not possible and measurements of V_{RBC} and RBC spacing were then performed on the closest possible frames. Capillary haematocrit was then calculated as $Hct_{CAP} = (N_{RBC} \times Vol_{RBC})/[\pi \times (Dia_{CAP}/2)^2 \times$ capillary length], where N_{RBC} is number of RBCs and Vol_{RBC} is RBC volume. Red blood cell flux was calculated as $F_{RBC} = [Hct_{CAP} \times \pi \times (Dia_{CAP}/2)^2 \times V_{RBC}]/Vol_{RBC}$. For these calculations we assumed that capillaries were circular in cross-section and $Vol_{RBC} = 61\ \mu m^3$ (Altman & Dittmer, 1974). The methods used in this study to calculate Hct_{CAP} and F_{RBC} have been validated theoretically (Cokelet, 1974) and empirically (Sarelius & Duling, 1982). To determine the percentage of capillaries supporting RBC flow we observed the microvascular field at R (during 15 s prior to the onset of contractions), EC and ER. Capillaries not containing RBCs or with stagnant RBCs during the 15 s of rest prior to contractions, 15 frames after the last contraction (see above) and within a 1 s window (30 frames) at EC were considered as not supporting RBC flow. The number of capillaries supporting RBC flow was multiplied by 100 and divided by the total number of capillaries within the microvascular field (i.e. percentage of capillaries supporting RBC flow).

Data analysis

The normalcy of the data was tested by the Kolmogorov-Smirnov test. The comparison of means was performed with one-way repeated-measures ANOVA and *post hoc* analyses were conducted with the Holm-Sidak test (SigmaStat 3.0, Systat Software, Richmond, CA, USA). Changes in V_{RBC} and Hct_{CAP} induced by muscle contractions were determined in each capillary by subtracting the resting values from post-contraction data (1–180 s). The relationship between variables was examined with linear regression (SigmaPlot 7.01, Systat Software). For all tests significance was accepted when $P \leq 0.05$. The coefficient of variation for V_{RBC} was determined for R, EC and ER as CV = (S.D. × 100)/mean. All data are presented as mean ± S.E.M.

Results

Systemic cardiovascular variables did not change significantly from EC to ER (mean arterial pressure, 104 ± 8 *versus* 105 ± 9 mmHg; heart rate, 284 ± 19 *versus* 282 ± 20 beats min^{-1}, respectively; $P > 0.05$ for both). Mean capillary diameter was 5.7 ± 0.1 μm.

We observed that the vast majority of capillaries supported RBC flow at R, EC and ER (Fig. 2). V_{RBC}, F_{RBC} and Hct_{CAP} increased significantly with contractions ($P \leq 0.05$) and recovered to resting values at 180 s post-contraction (Fig. 3). The increase in F_{RBC} (∼50%) with contractions was greater than that of V_{RBC} (∼35%), which accounts mathematically for the increase in Hct_{CAP} (Fig. 3). The Kolmogorov-Smirnov test showed a normal distribution of V_{RBC} at R, ER and EC. The temporal profile of V_{RBC}, F_{RBC} and Hct_{CAP} during

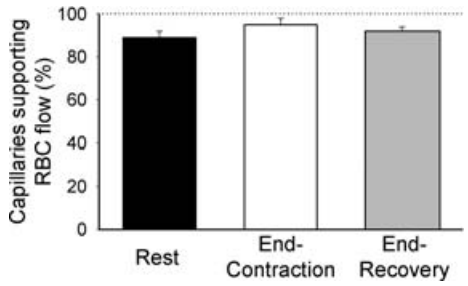

Figure 2
Percentage of capillaries supporting red blood cell (RBC) flow at rest, end-contraction and end-recovery. Note that most capillaries contain moving RBCs at rest, during muscle contractions and after 3 min of recovery. Thus, 'capillary recruitment' does not appear to be an important mechanism by which the muscle pump increases skeletal muscle blood flow during contractions.

recovery from contractions for a representative muscle (5 capillaries) is shown in Fig. 4. It is compelling that there is little (F_{RBC}) or no (V_{RBC}) decrease in these variables for 20 s following cessation of contractions. Figure 5 depicts the mean data for F_{RBC}, V_{RBC} and Hct_{CAP} of five microvascular fields (24 capillaries) measured over 3 min of recovery from contractions. F_{RBC} decreased significantly at 5 s compared to EC and subsequently displayed a progressive decrease. V_{RBC} did not change significantly up to 15–20 s and decreased significantly thereafter reaching a steady state ~90 s post-contraction. The values for Hct_{CAP} were significantly lower at 5 s compared to EC (i.e. 1 s, Fig. 5, bottom panel) and showed no further decrease until 60 s into recovery; however, the difference between 50 and 60 s was of borderline significance ($P = 0.056$).

There was little dynamic association between ΔHct_{CAP} and ΔV_{RBC} ($P > 0.05$, Fig. 6), which suggests that changes in V_{RBC} had no simple or proportional effect on the alterations of Hct_{CAP} that occurred following muscle contractions. Notably, there was a marked heterogeneity of changes in Hct_{CAP} from R to EC where ΔHct_{CAP} varied from −21% to 49% of the pre-contraction value. Similarly, there was a heterogeneous distribution of V_{RBC} and the degree of heterogeneity, assessed by the coefficient of variation, was not significantly different ($P > 0.05$) when comparing R (40.0 ± 2.0%), EC (43.4 ± 5.2%) and ER (49.6 ± 6.7%).

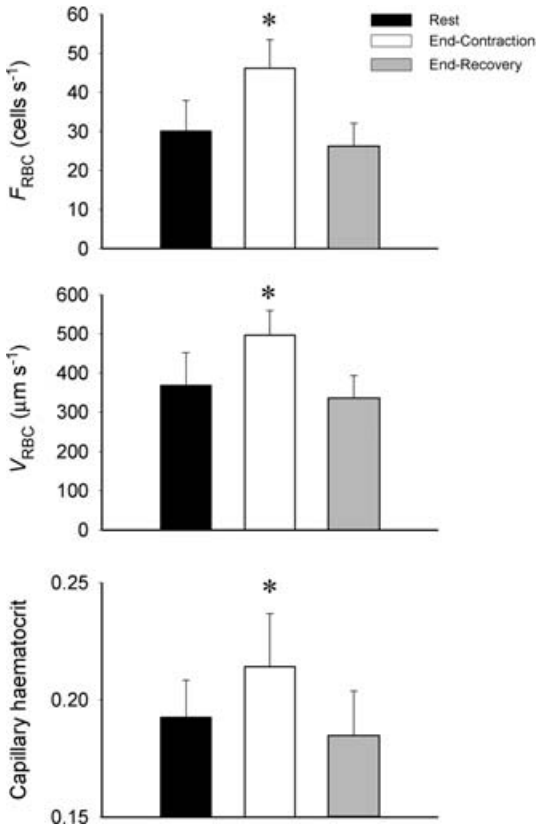

Figure 3
Capillary red blood cell velocity (V_{RBC}), flux (F_{RBC}) and haematocrit in 5 microvascular fields (24 capillaries). *Significantly ($P < 0.05$) different from Rest and End-Recovery.

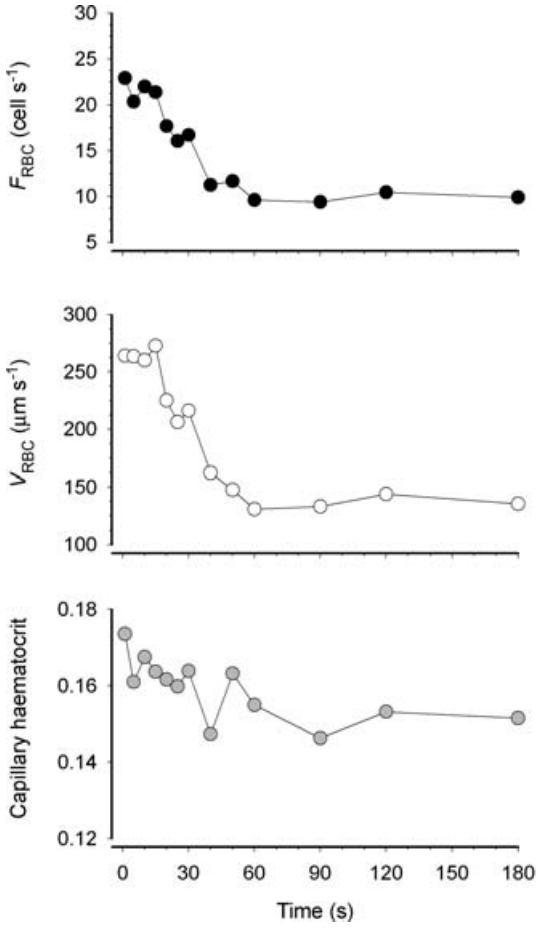

Figure 4
Temporal profile of red blood cell velocity (V_{RBC}), flux (F_{RBC}) and capillary haematocrit (Hct_{CAP}) for a representative animal (mean of 5 capillaries).

To further examine the association between microcirculatory variables we determined the relationship between means of V_{RBC} and F_{RBC}. This analysis demonstrated a significant ($P \leq 0.05$) relationship with similar slopes and intercepts when analysing the steady state (EC and ER, Fig. 7, top panel) and dynamic recovery from EC to ER (Fig. 7, bottom panel).

Discussion

Two principal novel findings arise from the present study. First, we observed that F_{RBC} decreased by ~25% of the overall response within 5 s of the last muscle contraction with a temporal profile that approximated a monoexponential response whilst V_{RBC} showed a 'delay' of ~20 s before a significant decrease was observed. Thus, our concurrent analysis of F_{RBC} and V_{RBC} argues against a substantial muscle pump effect on capillary haemodynamics at the steady state and early recovery of muscle contractions (see Figs 1 and 8). Secondly, dynamic changes in Hct_{CAP} within individual capillaries during contractions were dissociated from changes in V_{RBC} measured at the steady state and during recovery from exercise.

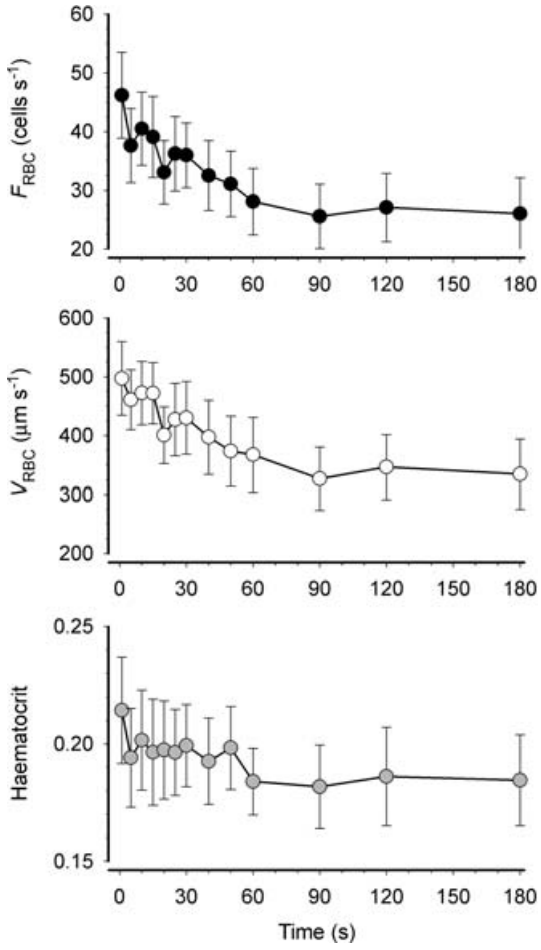

Figure 5
Temporal profile of red blood cell flux (F_{RBC}), velocity (V_{RBC}) and capillary haematocrit (Hct_{CAP}) during recovery from contractions for 5 muscles (total of 24 capillaries). F_{RBC} was decreased at 5 s compared to end-contraction ($P < 0.05$ *versus* 1 s) and the half-time of recovery of mean F_{RBC} was ~26 s. V_{RBC} remained relatively constant up to 15 s after cessation of muscle contractions ($P > 0.05$ *versus* 1 s) and decreased thereafter achieving a steady state at ~90 s (half-time ~30 s). Hct_{CAP} at 5 s was significantly lower than end-contraction (1 s); however, Hct_{CAP} did not appear to decrease further until 50–60 s after the last contraction.

Muscle contractions and (lack of) 'capillary recruitment'

The opinion is widely held that many skeletal muscle capillaries do not support RBC flux at rest and are recruited (i.e. support RBC flux) during contractions. If true, one could hypothesize that the muscle pump facilitates the increase in 'capillary recruitment' and this phenomenon would help explain the increased muscle O_2 diffusing capacity seen during exercise (see 'Capillary gas exchange'). However, there is compelling experimental evidence in intact conscious animals (Hudlicka *et al.* 1982; Kayar & Banchero, 1985) and a variety of individual muscles examined by intravital microscopy (Poole *et al.* 1997; Kindig & Poole, 1998, 2001; Kindig *et al.* 1999, 2002) that the overwhelming majority of capillaries do support RBC flux (i.e. flow) at rest. Consistent with these studies, we observed that ~90% of capillaries supported RBC flow at rest with no significant change during the steady state of contractions and at the end of recovery (Fig. 2) refuting the idea that the muscle pump could facilitate flow in stagnant capillaries (i.e. capillary recruitment). Moreover, the absence of 'capillary recruitment' raises

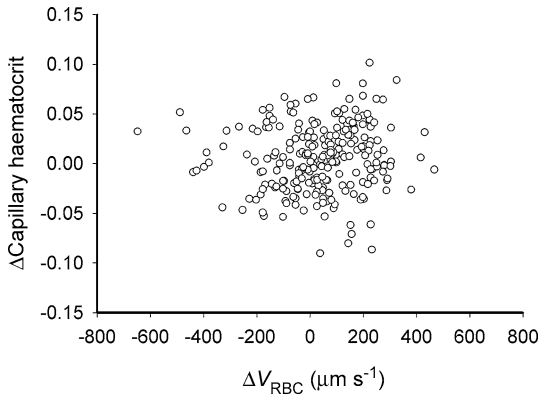

Figure 6
Relationship between changes (Δ) in capillary haematocrit (Hct_{CAP}) and red blood cell velocity (V_{RBC}) during recovery from muscle contraction over each measured interval (from 1 to 180 s; $n = 4$) for each capillary studied. Some capillaries displayed a sustained (i.e. until 180 s post-contraction) decrease in V_{RBC} and Hct_{CAP} below pre-contraction values while others showed only a temporary decrease compared to the pre-contraction state.

the probability that the bulk of the increased capillary O_2 diffusing capacity in exercising muscle comes from a combination of: (i) a better utilization of capillary surface area along the length of individual capillaries, in part due to exercise-induced elevation of tube haematocrit (and thus RBC-to-capillary surface contact) and also increasing the capillary length over which O_2 is exchanged, and (ii) intramyocyte effects that act to increase intracellular diffusivity as myoglobin becomes progressively more O_2 desaturated (Honig *et al.* 1997).

Temporal profile of recovery of capillary haemodynamics

The overall time course of F_{RBC} measured directly in skeletal muscle capillaries (Fig. 5) is in close agreement with two previous investigations of the dynamics of recovery of muscle microvascular blood flow after cessation of exercise (Lash, 1994; Ferreira *et al.* 2005*a*). The recovery of F_{RBC} approximated an exponential profile with a $t_{0.5}$ of ∼26 s (Fig. 5). Based on the relationship between $t_{0.5}$ of recovery of blood flow and contraction frequency (Fig. 4 in Lash, 1994), we estimate that the $t_{0.5}$ of recovery of blood flow to resting levels in feed arteries of spinotrapezius muscle after 1 Hz contractions (as used here) was ∼28 s (Lash, 1994). For exercising humans the dynamics of vastus lateralis muscle microvascular blood flow (estimated) during recovery from cycling exercise at 1 Hz (60 r.p.m.) had a $t_{0.5}$ of ∼25 s (Ferreira *et al.* 2005*a*). Thus, the recovery of rat spinotrapezius muscle hyperaemia measured in capillaries appears to have a similar time course to that measured in 'larger' vessels (Lash, 1994) and human muscles (Ferreira *et al.* 2005*a*). The recovery dynamics of F_{RBC} were 140% slower (i.e. $t_{0.5}$ off/on ∼2.4; Fig. 8) than the kinetics of F_{RBC} following the onset of muscle contractions ($t_{0.5}$ ∼11 s; Kindig *et al.* 2002). These data confirm the on–off asymmetry of O_2 delivery kinetics (Barstow *et al.* 1990; Yoshida & Whipp, 1994; McDonough *et al.* 2001), which implies that different mechanisms may be involved in the blood flow response following the onset and recovery from exercise.

The mechanisms controlling skeletal muscle hyperaemia during contractions and subsequent recovery have yet to be fully discriminated (Clifford & Hellsten, 2004; Tschakovsky & Sheriff, 2004). While there appears to be solid evidence for the participation of nitric oxide (Hirai *et al.* 1994; Shoemaker *et al.* 1997), prostaglandins

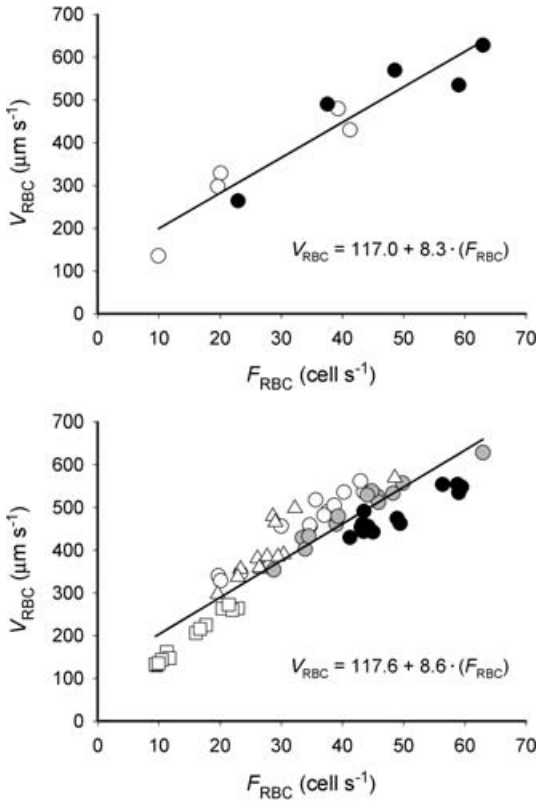

Figure 7
Mean capillary red blood cell velocity (V_{RBC}) and flux (F_{RBC}) during recovery from muscle contractions (from 1 to 180 s). Upper panel shows the steady-state relationship (●, end-contraction; ○, end-recovery) for each animal. Lower panel depicts the dynamic association between V_{RBC} and F_{RBC} from end-contraction to end-recovery. Symbols represent each individual muscle studied. For both panels the continuous line denotes the linear regression.

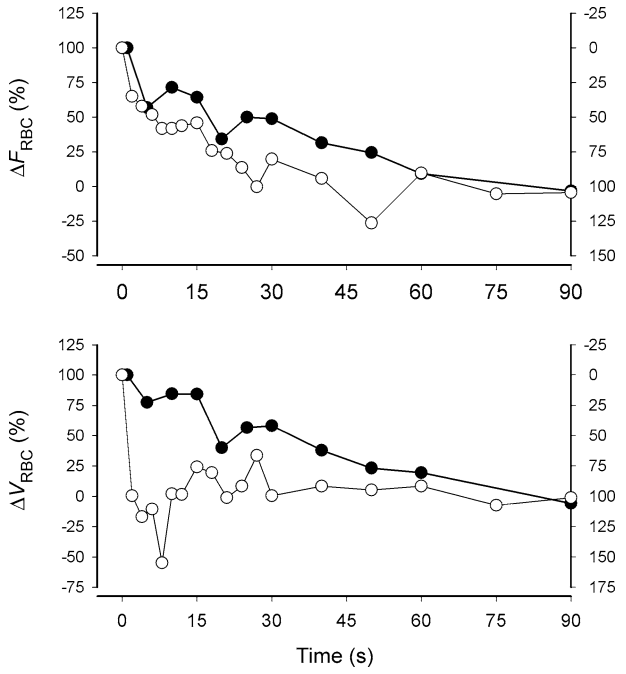

Figure 8
Changes (Δ) in F_{RBC} and V_{RBC} after the onset (○; right *y*-axis) and end of muscle contractions (●; left *y*-axis). The data are normalized for the amplitude of the response at 90 s (percentage change) and the right *y*-axes are modified to produce a 'mirror image' of the onset of contractions (Kindig *et al.* 2002) as for Fig. 1.

(Shoemaker *et al.* 1996) and adenosine (Kille & Klabunde, 1984) in this process, the flow-enhancing effect of the muscle pump is more controversial (Laughlin, 1987; Lutjemeier *et al.* 2005; Valic *et al.* 2005; Sheriff, 2005; Clifford *et al.* 2005). The recovery from exercise has been used to tease out the potential effect of the muscle pump on the steady state of hyperaemia (Van Leeuwen *et al.* 1992; Lutjemeier *et al.* 2005; Ferreira *et al.* 2005*a*). In our study, the similar overall dynamics of F_{RBC} and V_{RBC} during recovery from contractions might support the hypothesis that the muscle pump contributes to the steady state of capillary haemodynamics. However, assuming that there is a time delay (~10 s) to the onset of recovery of arteriolar diameter towards its pre-contraction state (Gorczynski & Duling, 1978; Bearden *et al.* 2004) we reasoned that, analogous to the onset of muscle contractions (Fig. 1 and Kindig *et al.* 2002), a rapid decrease in V_{RBC} and F_{RBC} plus a biphasic profile of F_{RBC} (i.e. dynamics of V_{RBC} faster than that of F_{RBC}) would be considered evidence for the muscle pump effect on blood flow during the steady state of exercise. Thus, the ~15 s latency to the decrease in V_{RBC} and the close to monoexponential profile of F_{RBC} with only a small decrease at 5 s (Fig. 5) argue against a major role for the muscle pump effect on the spinotrapezius muscle capillary hyperaemia during the steady state of contractions. At first glance these results appear to disagree with data suggesting the presence of the muscle pump during exercise in humans (Van Leeuwen *et al.* 1992; Lutjemeier *et al.* 2005), running dogs (Sheriff *et al.* 1993) and rats (Sheriff, 2003), and in the spinotrapezius muscle (Kindig *et al.* 2002). However, it is important to consider differences in exercise protocols and integrate results from previous studies to help explain our findings.

The effect of the muscle pump has been demonstrated during the steady state of upright exercise (Shiotani *et al.* 2002; Lutjemeier *et al.* 2005), while it is assumed that during supine exercise (or with limbs above the level of the heart; Tschakovsky *et al.* 1996) and electrically stimulated contractions the muscle pump effect is either less pronounced or non-existent (Laughlin, 1987; Shiotani *et al.* 2002; Tschakovsky & Sheriff, 2004). The spinotrapezius muscle preparation used in our study approximates more closely isotonic small muscle mass exercise of light-to-moderate intensity (i.e. no change in MAP; Lutjemeier *et al.* 2005) in the supine position with the limbs at or above the level of the heart (Shoemaker *et al.* 1996). Under these conditions, as for upright exercise or limbs below the level of the heart, the kinetics of blood flow following the onset of contractions still displayed an early rapid increase (e.g. Shoemaker *et al.* 1996; Kindig *et al.* 2002) that is thought to be determined predominantly, but not exclusively (Hamann *et al.* 2003; Tschakovsky *et al.* 2004; VanTeeffelen & Segal, 2005), by the muscle pump (Sheriff *et al.* 1993; Sheriff, 2003). Consistent with this notion, the very rapid increase in V_{RBC} (1–2 s) and F_{RBC} (within one contraction), with a 15–18 s delay to the increase in Hct_{CAP} following the onset of contractions provides strong evidence for the presence of a muscle pump effect in the spinotrapezius muscle (for a detailed discussion see Kindig *et al.* 2002). However, it is relevant to consider that the muscle pump effect is not seen when contractions are performed with the muscle in a vasodilated state (Dobson & Gladden, 2003; Hamann *et al.* 2003). Therefore, reconciling the skeletal muscle capillary haemodynamics following the onset (Kindig *et al.* 2002) and recovery from exercise (present study), we propose that the blood flow-enhancing effect of the muscle pump is present early during muscle contractions and progressively disappears as contractions are repeated and vasodilatation proceeds (Gorczynski & Duling, 1978; Dobson & Gladden, 2003; Hamann *et al.* 2003) such that the muscle pump does not contribute to sustaining the steady state of capillary hyperaemia in the spinotrapezius muscle (present data). In this setting, the muscle pump contributes to the rapid kinetics of F_{RBC} (and V_{RBC}) following the onset of exercise while the absence of the muscle pump after cessation of contractions permits a slow recovery of F_{RBC} such that there is a surplus of O_2 delivery during both transitional phases of exercise thereby elevating/maintaining microvascular O_2 pressures (P_{O_2}) and therefore O_2 availability across the exercise transients (Behnke *et al.* 2001; McDonough *et al.* 2001).

Capillary gas exchange

The variables examined in this study (F_{RBC}, V_{RBC} and Hct_{CAP}) are key components of the gas exchange properties of the microcirculation (Federspiel & Popel, 1986; Tsai & Intaglietta, 1989). F_{RBC} provides an excellent representation of convective O_2 delivery (Berg & Sarelius, 1996) having a crucial role in determining the microvascular P_{O_2}, which is the pressure head driving O_2 movement from blood to muscle. V_{RBC} will determine the RBC transit time in muscle capillaries and in the presence of fast V_{RBC} there is an increased likelihood of RBCs with transit times that are short enough to compromise gas exchange (Sarelius, 1986; Piiper & Scheid, 1999). On the other hand, slow V_{RBC} in the microcirculation may be associated with arteriolar O_2 loss (or venular oxygenation) (Swain & Pittman, 1989; Pittman, 2000) that will result in a diminished mean capillary P_{O_2}. Therefore, assuming that capillary and arteriolar V_{RBC} are related variables the ~15 s where capillary V_{RBC} remained elevated after cessation of contractions (Figs 4 and 5) may favourably affect gas exchange by maintaining an increased mean capillary P_{O_2}, although the possibility for short transit times counterbalancing this effect cannot be ignored.

The low diffusivity of O_2 in plasma determines that the principal pathway for myocyte O_2 delivery is in close

proximity to the RBC. In this context, blood–myocyte O_2 transfer is thought to be dependent upon the number of RBCs within the capillaries adjacent to the myocytes. Consistent with this notion, Hct_{CAP} (or RBC number per capillary length) is an important determinant of the O_2 diffusing capacity (D_{O_2} from Fick's law of diffusion) and therefore O_2 transport (Federspiel & Popel, 1986; Tsai & Intaglietta, 1989). The decrease in Hct_{CAP} within 5 s post-contractions, consequent to a faster initial decrease in F_{RBC} than V_{RBC}, may contribute to a lower D_{O_2} early into recovery. Although the changes in Hct_{CAP} appear to be small they become relevant when considering the heterogeneity of changes in Hct_{CAP} within the microvascular field (e.g. Fig. 6). It is worth noting that the D_{O_2} of capillaries with high haematocrits (or small decrease in Hct_{CAP} after contractions) may not compensate for the decreased D_{O_2} of capillaries with low haematocrits (or large changes in Hct_{CAP} post-contraction). Therefore, it is crucial to understand what factors determine Hct_{CAP} and the possible causes of Hct_{CAP} heterogeneity within muscles.

Relationship between microvascular variables (F_{RBC}, V_{RBC} and Hct_{CAP})

The mechanisms determining a Hct_{CAP} that is lower than systemic values have not been fully elucidated. There are studies demonstrating the partial roles of (1) the network Fahraeus effect (i.e. at bifurcations the increase in haematocrit of high-flow branches is smaller than the decrease in Hct_{CAP} of low-flow branches) (Pries *et al.* 1986), and (2) the presence of the glycocalyx on the capillary endothelial surface creating a stationary or relatively slow-moving RBC-free layer (Desjardins & Duling, 1990) that has been termed the capillary Fahraeus effect (V_{RBC} faster than plasma velocity).

The network Fahraeus effect can be altered according to V_{RBC} heterogeneity, where less heterogeneity would lead to higher Hct_{CAP} (Frisbee, 1998). At bifurcations RBCs tend to enter high-flow branches increasing the Hct_{CAP}. However, the decrease in Hct_{CAP} of low-flow branches is not compensated by the increased Hct_{CAP} of capillaries with fast velocities leading to a lower microvascular than systemic haematocrit (Fahraeus network effect; Pries *et al.* 1986). Thus, lower V_{RBC} heterogeneity would be accompanied by less difference between high- and low-flow branches causing an overall increase in Hct_{CAP}. Some studies have shown a decrease in V_{RBC} heterogeneity with muscle contractions (Tyml & Cheng, 1995) while others suggest no change (Damon & Duling, 1985) or an increase (Kindig *et al.* 2002). In the present study we observed no significant change in the CV (used as an index of heterogeneity) for V_{RBC} when comparing R, EC and ER while Hct_{CAP} at EC was ~15% greater than at R and ER (up to 49% for individual capillaries). Collectively these observations disagree with suggestions that the network Fahraeus effect is an important determinant of the increase in Hct_{CAP} induced by muscle contractions (Frisbee, 1998).

It has been proposed that the thickness of the slow-moving RBC-free layer can be affected by V_{RBC}, with a decrease in thickness resulting from increases in V_{RBC} (i.e. shear rate) (Duling *et al.* 1982; Pries *et al.* 1997). This implies that there is an association between ΔV_{RBC} and ΔHct_{CAP}; however, in the present investigation such a relationship was not found (Fig. 6). Therefore, the mechanisms determining changes in Hct_{CAP} with muscle contraction remain to be elucidated. In this context, we must consider whether Hct_{CAP} is an independent or a dependent variable that changes as a consequence of alterations in other variables (possibly F_{RBC} and V_{RBC}).

A remarkable feature of capillary haemodynamics is the close-to-linear relationship between V_{RBC} and F_{RBC} (Fig. 7; Kindig *et al.* 1998, 1999; Kindig & Poole, 2001; Russell *et al.* 2003). Analysing the mathematical description of Hct_{CAP} where

$$Hct_{CAP} = \frac{Vol_{RBC}\, F_{RBC}}{\pi\, (Dia_{CAP}/2)^2\, V_{RBC}}$$

(see definition of variables above), it becomes apparent that for constant Vol_{RBC} and Dia_{CAP}, Hct_{CAP} is proportional to the F_{RBC}/V_{RBC} ratio. The relationship between V_{RBC} and F_{RBC} has an intercept different from zero, meaning that upon alterations in F_{RBC} and V_{RBC} the ratio of F_{RBC}-to-V_{RBC}, and therefore Hct_{CAP}, does not remain constant. As mentioned above, early into exercise the increase in V_{RBC} is faster than the dynamics of F_{RBC} such that the relationship between V_{RBC} and F_{RBC} is temporarily disrupted (i.e. increase in V_{RBC} and F_{RBC} with no change in Hct_{CAP} in the first ~20 s of contractions), which may reflect the transient muscle pump effect on capillary haemodynamics. Interestingly, the relationship between V_{RBC} and F_{RBC} in resting muscles is similar in health and disease. Specifically, conditions that perturb muscle microvascular control such as type I diabetes (Kindig *et al.* 1998), heart failure (Kindig *et al.* 1999) and ageing (Russell *et al.* 2003) do not appear to alter Hct_{CAP} substantially. Thence, changes in Hct_{CAP} with muscle contractions (Fig. 3) appear to be a consequence of disproportionate alterations in the control of F_{RBC} and V_{RBC}. We observed a similar relationship between V_{RBC} and F_{RBC} across steady-state conditions (EC, ER; Fig. 7, upper panel), which resembles those from previous studies (Kindig *et al.* 1998, 1999; Russell *et al.* 2003), and recovery from muscle contractions (Fig. 7, lower panel). Therefore, changes in Hct_{CAP} during the steady state and recovery from exercise may occur as a consequence of mechanisms affecting V_{RBC} and F_{RBC} (e.g. shear stress and vasodilatation) rather than Hct_{CAP} *per se*.

Methodological aspects

Kindig *et al.* (2002) presented a detailed discussion of the methodological considerations relevant for extrapolation of our results to the microcirculation of conscious spontaneously exercising animals and muscles other than the spinotrapezius. Briefly, these relate to the effects of anaesthesia, muscle recruitment during electrical stimulation and the anatomical characteristics of the spinotrapezius muscle. In our study, anaesthesia could have blunted a sympathetic vasoconstriction and prolonged the recovery of cardiovascular responses after cessation of muscle contractions. However, the stimulation protocol employed in the current study does not have a direct effect on sympathetic nerves (Honig, 1979) and elicits contractions with metabolic rates similar to exercise of moderate intensity where there is negligible sympathetic activation (Saito *et al.* 1993). Moreover, the $t_{0.5}$ of F_{RBC} was similar to that seen in conscious humans (Ferreira *et al.* 2005*a*). Thus, anaesthesia had little or no effects on our results.

Despite differences in muscle recruitment patterns of spontaneous *versus* electrically induced contractions, the temporal profile of muscle hyperaemia represented by F_{RBC} following the onset (Kindig *et al.* 2002) and recovery from contractions (present study) is similar to that reported for moderate exercise in humans (Shoemaker *et al.* 1996; Lutjemeier *et al.* 2005). Although differences may exist when compared to estimated responses (Ferreira *et al.* 2005*a*), these are not thought to result from the muscle recruitment pattern itself (see above). Finally, the temporal profile of capillary haemodynamics following the onset of exercise (Kindig *et al.* 2002), which indicates the presence of the muscle pump, suggests that the spinotrapezius muscle is useful for examining the effects of the muscle pump or vasodilatation/vasoconstriction of very rapid onset on capillary hyperaemia.

Conclusion

In summary, the present investigation demonstrates that the decrease in red blood cell velocity becomes evident only 20 s after the end of contractions while red blood cell flux and capillary haematocrit decrease within 5 s of cessation of muscle contractions, but only a small percentage of the final response. Based on the close-to-monoexponential response of recovery of red blood cell flux and the 'delay' to the decrease in red blood cell velocity we consider that the muscle pump does not contribute substantially to the steady state of capillary hyperaemia in the spinotrapezius muscle. The slower dynamics of red blood cell flux during recovery ($t_{0.5} \sim 26$ s) compared to the onset of contractions ($t_{0.5} \sim 11$ s) confirms theoretical (Barstow *et al.* 1990) and empirical data (McDonough *et al.* 2001; Ferreira *et al.* 2005*a*) indicating on–off asymmetry for the kinetics of O_2 delivery in the microcirculation. With regard to the mechanisms determining capillary haematocrit, our findings suggest that changes in capillary haematocrit during muscle contractions may not be mechanistically linked to changes in red blood cell velocity.

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Acknowledgements

We would like to thank K. Sue Hageman for invaluable technical assistance and Drs Thomas Barstow, Brad Behnke and Paul McDonough for insightful conversations. L. F. Ferreira was supported by a Fellowship from the Ministry of Education/Capes, Brazil. This investigation was supported, in part, by grants from the NIH (HL-69739, HL-67619, HL-50306 and AG-19228) and a grant-in-aid from AHA, Heartland Affiliate, to D. C. Poole.