Eur J Appl Physiol (2006) 96: 558–563
DOI 10.1007/s00421-005-0107-3

ORIGINAL ARTICLE

**Leonardo F. Ferreira · Barbara J. Lutjemeier
Dana K. Townsend · Thomas J. Barstow**

Effects of pedal frequency on estimated muscle microvascular O_2 extraction

Accepted: 10 November 2005 / Published online: 21 December 2005

Abstract An increase in muscle contraction frequency could limit muscle blood flow $(\dot{Q}_M)$, compromising the matching of $\dot{Q}_M$ and muscle oxygen uptake $(\dot{V}_{O_{2M}})$. This study examined the effects of pedal cadence on skeletal muscle oxygenation at low, moderate and peak exercise. Nine healthy subjects [24.7 ± 6.3 years (SD)] performed incremental cycling exercise at 60 and 100 rpm. Pulmonary $\dot{V}_{O_2}$ $(\dot{V}_{O_{2P}})$ was measured breath-by-breath and vastus lateralis oxygenation was determined by near-infrared spectroscopy (NIRS). The deoxyhemoglobin signal ([HHb]) from NIRS was used to estimate microvascular O_2 extraction (i.e., [HHb] ∝ $\dot{V}_{O_{2M}}/\dot{Q}_M$). The $\dot{V}_{O_{2P}}$ and [HHb] for low, moderate and at peak exercise were determined. The $\dot{V}_{O_{2P}}$ at 60 rpm (low = 0.64 ± 0.13, moderate = 2.03 ± 0.38 and peak = 3.39 ± 0.84 l/min) were lower ($P < 0.01$) than at 100 rpm (1.29 ± 0.23, 2.14 ± 0.39 and 3.54 ± 0.88 l/min, respectively). There was a progressive increase in [HHb] from low to peak exercise. However, there was no significant difference (ANOVA, $P = 0.94$) for the 60 (in μM, low = 24.0 ± 9.5, moderate = 30.5 ± 13.8 and peak = 36.7 ± 16.5) and 100 contractions/min (in μM, low = 25.7 ± 11.6, moderate = 32.1 ± 14.0 and peak = 35.4 ± 16.5). We conclude that vastus lateralis O_2 extraction was similar at 60 and 100 cpm, suggesting that the $\dot{V}_{O_{2M}}/\dot{Q}_M$ in the microcirculation was not altered and, presumably, no impairment of $\dot{Q}_M$ occurred with the increase in pedal frequency.

Keywords Exercise · Blood flow · Contraction frequency · Muscle oxygenation · Near-infrared spectroscopy

L. F. Ferreira · B. J. Lutjemeier · D. K. Townsend · T. J. Barstow
Departments of Kinesiology and Anatomy and Physiology, Kansas State University, Manhattan, KS 66506-0302, USA

T. J. Barstow (✉)
Department of Kinesiology, Kansas State University, 1A Natatorium, Manhattan, KS 66506-0302, USA
E-mail: tbarsto@ksu.edu
Tel.: +1-785-5320712
Fax: +1-785-5326486

Introduction

To perform exercise for more than a few minutes skeletal muscle blood flow $(\dot{Q}_M)$ must meet the metabolic demand. Importantly, muscle contraction has direct effects on muscle blood flow that depend on exercise mode, intensity and frequency of contractions (Bellemare et al. 1983; Buchler et al. 1985; Walloe et al. 1988; Lutjemeier et al. 2005). Even with low-to-moderate force development the intramuscular pressure generated during contraction exceeds arterial pressure, such that blood flows through the muscle in the relaxation phase (Walloe et al. 1988). Because of the shorter period of time spent in the relaxation phase at high contraction frequencies and duty cycles blood flow may become limited during such contractions, as is indeed observed in the dog diaphragm (Bellemare et al. 1983; Buchler et al. 1985). Specifically, there is an interaction between duty cycle and frequency such that for constant duty cycle $\dot{Q}_M$ becomes limited at frequencies above 80 contractions/min (cpm) (Buchler et al. 1985).

Hoelting et al. (2001) extended the findings from the diaphragm to human muscles to show that shortening the relaxation phase by increasing both the contraction frequency and duty cycle results in a smaller increase in $\dot{Q}_M$ during incremental knee-extension exercise than expected from the increased power output. However, others find that when knee-extension exercise is performed at a constant duty cycle, elevations in contraction frequency do not impair $\dot{Q}_M$ (Sjogaard et al. 2002; Osada et al. 2002). In fact, the greater muscle oxygen consumption $(\dot{V}_{O_2})$ during heavy exercise performed at 100 than at 60 cpm is matched by an increase in $\dot{Q}_M$ such that leg O_2 extraction is similar at 60 and 100 cpm (Ferguson et al. 2001).

The primary concern regarding the adequacy of $\dot{Q}_M$ during muscle contractions is capillary O_2 exchange. Near-infrared spectroscopy (NIRS) uses the optical properties of light-absorbing chromophores (predominantly hemoglobin and myoglobin) to noninvasively

estimate tissue O_2 saturation (St_{O_2}) or O_2 extraction in the skeletal muscle microcirculation. During cycling (Takaishi et al. 2002) and plantar flexion exercise (Quaresima et al. 2001) St_{O_2} is similar at contraction rates ranging from 40 to 80–85 cpm suggesting that $\dot{Q}_M$ in the microcirculation is not limited during exercise performed $\leq$ 85 cpm. However, a shortcoming of St_{O_2} measurements is that they are affected by changes in blood volume under the NIRS probe (Ferrari et al. 1997). Moreover, these studies (Quaresima et al. 2001; Takaishi et al. 2002) did not evaluate the effects of increasing contraction frequency on the $\dot{V}_{O_{2M}}/\dot{Q}_M$ balance across a wide range of exercise intensities. The deoxyhemoglobin concentration ([HHb]) signal from NIRS is less sensitive to blood volume changes, and thus is considered a better estimate of capillary O_2 extraction (Kowalchuk et al. 2002; Grassi et al. 2003; Ferreira et al. 2005), reflecting the $\dot{V}_{O_{2M}}/\dot{Q}_M$ in the microcirculation.

The current investigation examined the effects of pedal cadence (60 cpm vs. 100 cpm) on [HHb] at low, moderate and peak exercise. We anticipated one of the following outcomes: (a) If the increase in $\dot{V}_{O_2}$ at elevated contraction frequencies is matched by an increase in $\dot{Q}_M$, [HHb] will be similar at 60 and 100 rpm; conversely (b) if $\dot{Q}_M$ during moderate (~lactate threshold) and peak exercise is limited at elevated contraction frequencies when $\dot{V}_{O_2}$ is expected to be similar (Jones et al. 2004), [HHb] will be greater at 100 rpm compared to 60 rpm. Since increasing pedal cadence during cycling exercise is similar to knee-extension exercise where increases in contraction frequency are accomplished with relatively constant duty cycles (Ferguson et al. 2001; Osada et al. 2002), we hypothesized that [HHb] would be similar at 60 and 100 cpm.

Methods

This study involved healthy, physically active subjects (seven males, two females; 24.7 ± 6.3 years; 67.9 ± 12.2 kg, mean ± SD) who volunteered to participate after receiving detailed explanation of the experimental protocol, which was approved by the Institutional Review Board and followed the principles outlined in the Declaration of Helsinki. Each subject provided written informed consent prior to performance of any procedure.

The protocol involved two visits to the laboratory, separated by at least 48 h, but no longer than 1 week, for incremental cycling exercise on an electronically braked cycle ergometer (Corival 400, Lode, The Netherlands). Incremental exercise involved 4 min of baseline cycling (20 W) at the desired cadence (60 or 100 cpm) followed by a progressive (ramp) increase in exercise intensity (15–30 W/min) until volitional fatigue or until the cadence was lower than 55 or 95 cpm, respectively, for 5–10 s despite strong verbal encouragement. The incremental work rate, seat height and handlebar position on the cycle ergometer were reproduced on the second test. Pulmonary gas exchange and skeletal muscle O_2 extraction, estimated by the deoxy-hemoglobin concentration ([HHb]) determined with NIRS, were measured during both exercise tests. Peak oxygen uptake ($\dot{V}_{O_{2peak}}$) was defined as the highest $\dot{V}_{O_2}$ achieved during the test averaged over a 15-s interval. The LT was estimated from gas exchange measurements using the V-slope method (i.e., the change in the slope of the pulmonary $\dot{V}_{CO_2}-\dot{V}_{O_2}$ relationship), ventilatory equivalents and end-tidal gas tensions (Wasserman et al. 1973; Beaver et al. 1986).

Pulmonary gas exchange ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$) and minute expired ventilation ($\dot{V}_E$) were measured breath-by-breath (CardiO$_2$, Medical Graphics Corporation, St. Paul, MN). Before each test, the volume signal was calibrated by pumping a 3-l syringe at flow rates spanning the range expected during the exercise studies, while the O_2 and CO_2 analyzers were calibrated with gases of known concentration. Heart rate was recorded from the electrocardiogram using a modified lead I configuration and stored in the breath-by-breath file.

Skeletal muscle [HHb] was evaluated by a frequency-domain multidistance (FDMD) NIRS system (OxiplexTS, ISS, Champaign, IL). The NIRS probe consists of eight light-emitting diodes (LED), operating at wavelengths of 690 (four LEDs) and 830 nm (four LEDs), and one detector fiber bundle. Source–detector separations were 2.0, 2.5, 3.0 and 3.5 cm. The modulation frequency of the light-source intensity was 110 MHz and the cross-correlation frequency for heterodyne detection was 5 KHz. The NIRS data were stored at a frequency of 31.25 Hz. The probe was positioned longitudinally on the belly of the muscle vastus lateralis, bonded to the skin (Skin-Bond, Smith & Nephew, Largo, FL) and secured with Velcro® straps around the thigh. The probe position was pen-marked and in the second visit the probe was placed at the same site. The NIRS was calibrated on each test day following the manufacturer's recommendations.

The FDMD NIRS provides measurement of absolute concentration (expressed in μM) of oxy-([HbO$_2$]) and deoxy-hemoglobin ([HHb]). [HHb] and [HbO$_2$] were calculated incorporating the dynamic measurement of reduced scattering coefficients made throughout the exercise test. Total hemoglobin concentration ([THb]) was the sum of [HHb] and [HbO$_2$], while St_{O_2} = [HbO$_2$]/[THb] (%). The NIRS data were averaged in 15-s bins and the [HHb], [HbO$_2$], [THb] and St_{O_2} corresponding to the baseline cycling (60 s average), LT and $\dot{V}_{O_{2peak}}$ (15 s average for both) were determined throughout exercise.

The [HHb] signal is less sensitive to changes in blood volume under the area of the probe than St_{O_2} (Ferrari et al. 1997) and is considered an estimate of O_2 extraction in the microcirculation (Kowalchuk et al. 2002; Grassi et al. 2003; Ferreira et al. 2005). The NIRS–FDMD provides absolute measurements of [HHb]; however, the day-to-day reproducibility is unclear. To examine the reproducibility of [HHb] one subject visited

the laboratory on four different days and [HHb] was measured during 4 min of baseline pedaling (20 W) at 60 cpm. Sixty seconds of data were averaged to determine [HHb] each day.

Data analysis

To determine differences between two means, a two-tailed Student's paired *t* test was performed. A repeated-measures analysis of variance was performed to compare more than two means and the Bonferroni post hoc test was used for pairwise comparisons. Statistical significance was accepted when $P < 0.05$ (NCSS 2000—Statistical Software, Kaysville, UT). Values were reported as mean ± SD, unless otherwise specified.

Results

The coefficient of variation for [HHb] on one subject during baseline cycling at different days was 7.0% ([HHb]; Day 1 = 16.8 μM, Day 2 = 18.5 μM, Day 3 = 18.3 μM and Day 4 = 20.0 μM).

The responses of pulmonary $\dot{V}_{O_2}$ and [HHb] for a representative subject are shown in Fig. 1, while the results are summarized in Fig. 2. Baseline and peak $\dot{V}_{O_2}$ were greater at 100 cpm compared with 60 cpm. However, the lactate threshold at 60 and 100 cpm was not significantly different, either as absolute value or as percent of $\dot{V}_{O_{2peak}}$ ($LT_{60rpm} = 56.9 \pm 7.8\%$ and $LT_{100rpm} = 57.2 \pm 7.0\%$; $P = 0.89$). The [HHb] increased from baseline pedaling to peak exercise. However, there was no significant difference for the 60- and 100-cpm trials.

The St_{O_2} decreased from BSL to Peak and St_{O_2} at 100 cpm was slightly, but significantly greater than for the 60-cpm trial (Table 1). Similarly, [HbO_2] was greater at 100 than at 60 cpm; however, [HbO_2] did not change significantly from baseline to peak exercise. The [THb] tended to be higher at 100 cpm compared to 60 cpm. In addition, [THb] increased from baseline to the lactate threshold but did not change further at peak exercise. Peak work rate was not significantly different between 60 and 100 cpm.

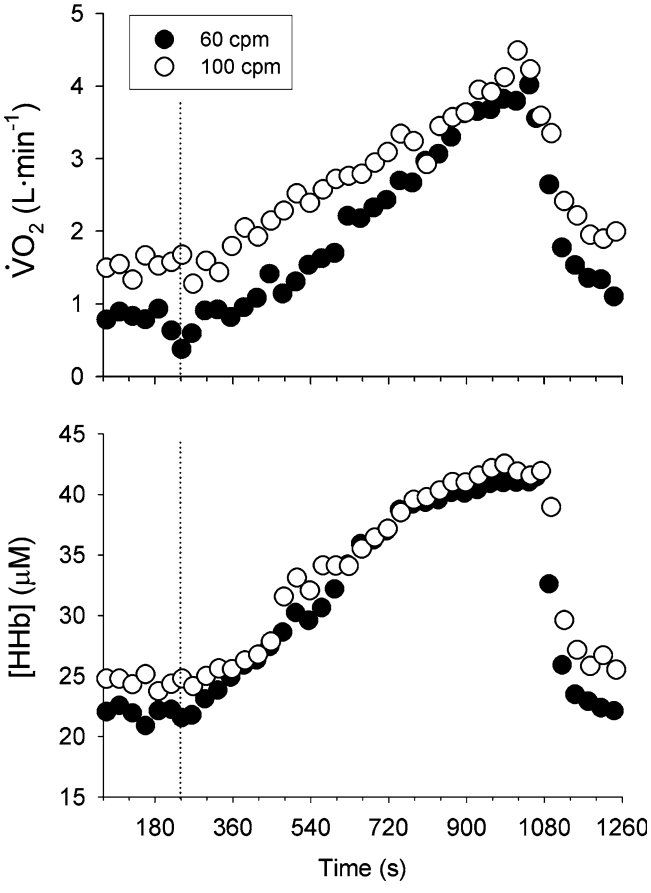

Fig. 1 Pulmonary $\dot{V}_{O_2}$ (*Top*) and [HHb] (*Bottom*) from a representative subject during incremental exercise performed at 60 and 100 cpm. *Dotted line* depicts the onset of incremental exercise

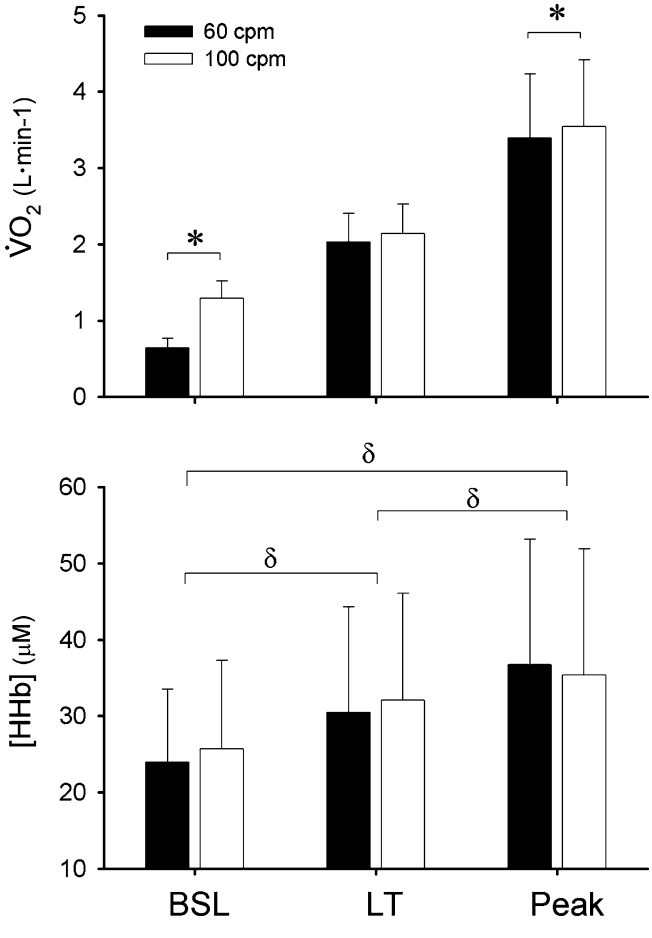

Fig. 2 Pulmonary $\dot{V}_{O_2}$ (*Top*) and Deoxy-hemoglobin concentration ([HHb], *Bottom*) at Baseline (*BSL*), estimated lactate threshold (*LT*) and peak exercise (*Peak*) during cycling at 60 and 100 cpm. Values are mean ± SD (*error bars*). Significant difference between pedal cadences (*) and exercise intensities (δ)

Table 1 Muscle oxygenation and power during incremental cycling exercise at 60 and 100 cpm

	Baseline		Lactate threshold		Peak	
	60 cpm	100 cpm	60 cpm	100 cpm	60 cpm	100 cpm
[HbO_2] (μM)	52.4 ± 16.4[a]	56.1 ± 16.7	51.7 ± 16.5[a]	58.4 ± 16.6	49.0 ± 15.9[a]	53.6 ± 17.6
[THb] (μM)	76.3 ± 24.9[b,c]	80.6 ± 25.2[b,c]	82.4 ± 28.3	90.4 ± 27.2	85.7 ± 31.2	89.1 ± 32.2
St_{O_2} (%)	69.0 ± 4.2[a–c]	70.1 ± 6.2[b,c]	63.9 ± 5.4[a,c]	65.1 ± 7.1[c]	58.3 ± 5.4[a]	61.4 ± 6.9
WR (W)	20	20	162 ± 32	132 ± 37[a]	273 ± 72	287 ± 77

Values are mean ± SD. The results can be summarized as follows: [HbO_2] main effect of pedal cadence (100 cpm > 60 cpm) but no main effect of exercise intensity (BSL≈LT≈Peak), [THb] main effect of exercise intensity (BSL < LT≈Peak) and tendency for 100 cpm > 60 cpm, St_{O_2} main effect for exercise intensity (BSL > LT > Peak) and pedal cadence (100 cpm > 60 cpm)
[HbO_2] Oxyhemoglobin concentration, *[THb]* total hemoglobin concentration, St_{O_2} tissue oxygen saturation, *WR* work rate
[a]Significantly different ($P < 0.05$) from 100 cpm at corresponding intensity
[b]Significantly different ($P < 0.05$) from lactate threshold at corresponding cpm
[c]Significantly different ($P < 0.05$) from Peak at corresponding cpm

Discussion

The principal new finding was the similar [HHb], assumed to reflect capillary O_2 extraction, at baseline (20 W), estimated lactate threshold and $\dot{V}_{O_{2peak}}$ during incremental cycling exercise performed at 60 and 100 cpm. This was so even though $\dot{V}_{O_{2peak}}$ was higher at 100 cpm compared to 60 cpm. These data suggest that during cycling exercise at 100 cpm $\dot{Q}_M$ was not limited any further than during cycling at 60 cpm. Thus, our data indicate that the likely greater (internal) work performed at 100 cpm was 'matched' by a higher $\dot{Q}_M$ compared to 60 cpm at a given external power.

The NIRS is a noninvasive estimate of muscle oxygenation that has limitations which cannot be circumvented (Ferrari et al. 2004). The main concerns regarding NIRS are an inability to separate light absorption by hemoglobin from that by myoglobin, and the relative contribution of arterioles, capillaries and venules to the signal at rest and during exercise. We assumed that changes in [HHb] provide an estimate of O_2 extraction, and that the relative contribution of vascular compartments to the NIRS signal was similar and unchanging across conditions and during exercise. Myoglobin deoxygenation is relatively unchanged from 50–60% WR_{peak} to peak work rate (Richardson et al. 1995) while [HHb] continued to increase above 50% WR_{peak}, which is qualitatively similar to leg O_2 extraction (Knight et al. 1992; Richardson et al. 1993), suggesting that changes in the NIRS signal originated primarily from changes in hemoglobin. The validity of the assumption regarding the relative contribution of arterioles, capillaries and venules to the NIRS signal is not clear; nor is it clear if these relative proportions remain constant across exercise intensities. However, as capillaries comprise the majority of the muscle microvascular volume (Poole et al. 1995) it appears reasonable to consider that the changes in [HHb] will primarily reflect capillary O_2 extraction.

We observed excellent reproducibility (CV = 7.0%) for measurements during cycling at 20 W. We did not specifically test the reproducibility of measurements at the lactate threshold and $\dot{V}_{O_{2peak}}$. However, previous data from our laboratory suggest a CV of ~16% for changes in [HHb] during cycling (60 cpm) from 20 W to work rates representing ~50% and 80% $\dot{V}_{O_{2peak}}$ (Ferreira et al. 2005).

There is an interaction between pedal frequency and power that can affect muscle recruitment during cycling (MacIntosh et al. 2000). We studied the oxygenation profile of the vastus lateralis, as this muscle is a predominant contributor to the power, and thus pulmonary $\dot{V}_{O_2}$, during cycling (Li et al. 1998). However, a possible increase in recruitment of other muscles, which would contribute to the elevated pulmonary $\dot{V}_{O_2}$ at 100 cpm, could not be assessed with the current protocol. Nevertheless, muscle-shortening velocity is the main determinant of metabolic cost of cycling at increased pedal cadence (McDaniel et al. 2002). Therefore, at least part of the increase in pulmonary $\dot{V}_{O_2}$ and presumably muscle $\dot{V}_{O_2}$ at 100 cpm was due to elevated vastus lateralis metabolism.

We observed that [HHb] during the baseline pedaling, estimated LT and $\dot{V}_{O_{2peak}}$ were not different at the 100 and 60 cpm trials, indicating a similar $\dot{V}_{O_{2M}}/\dot{Q}_M$ in the microcirculation. These results are in agreement with investigations showing that O_2 extraction (Ferguson et al. 2001) or muscle oxygen saturation by NIRS (Quaresima et al. 2001; Takaishi et al. 2002) are not affected by alterations in the contraction frequency. Thus, our data suggest that during cycling exercise performed at a duty cycle of ~1/3, muscle blood flow in the microcirculation was not mechanically impeded by increasing pedal cadence from 60 to 100 cpm. In fact, two aspects of exercise at higher contraction frequencies that might optimize muscle perfusion are the lower intramuscular pressures developed during the contraction period and a possibly greater contribution of the muscle pump (Gotshall et al. 1996; Sheriff et al. 2001). However, the effectiveness of the muscle pump as an important mechanism to increase $\dot{Q}_M$ during exercise in humans may be confined to light work rates (Lutjemeier et al. 2005) while in animals its effect has been disputed (Hamann et al. 2004; Valic et al. 2005). In addition,

higher mean arterial pressure during exercise at 100 cpm would aid to maintain (or increase) muscle blood flow at levels similar to those observed at 60 cpm (Gotshall et al. 1996), although this is only a partial explanation since systemic vascular resistance also decreases when pedal frequency is increased from 70 to 110 cpm (Gotshall et al. 1996).

The [HHb] determined with NIRS is less sensitive to changes in blood volume during exercise than St_{O_2} (Ferrari et al. 1997; Grassi et al. 2003) and is considered a reliable estimate of fractional capillary O_2 extraction (Kowalchuk et al. 2002; Grassi et al. 2003; Ferreira et al. 2005). Based on the Fick principle, O_2 extraction [C(a–v)O_2] is equal to $\dot{V}_{O_{2M}}/\dot{Q}_M$ (assuming constant CaO_2). $\dot{Q}_M$ is linearly related to $\dot{V}_{O_{2M}}$ and has a positive intercept on the $\dot{Q}_M$ axis (Andersen et al. 1985; Richardson et al. 1993). This predicts that [C(a–v)O_2] increases hyperbolically as a function of work rate, or more precisely $\dot{V}_{O_{2M}}$, considering that CaO_2 remains relatively constant in normal healthy subjects (Richardson et al. 1993; Whipp 1994). However, in subjects with low exercise capacity the plateau region of C(a–v)O_2 seen at a higher $\dot{V}_{O_2}$ may not be achieved. In seven subjects the [HHb] response followed a hyperbolic profile (e.g., Fig. 1). Therefore, these observations suggest that the [HHb] response approximates predictions of the O_2 extraction profile in the skeletal muscle microcirculation, assuming that the linear relationship between $\dot{Q}_M$ and $\dot{V}_{O_{2M}}$ measured in larger vessels holds true for the microcirculation.

There is debate surrounding the limiting factors of maximal $\dot{V}_{O_2}$ (di Prampero 2003). In healthy subjects, performing large muscle mass exercise under normoxia, $\dot{V}_{O_{2peak}}$ is determined primarily by the capacity to deliver O_2 to the contracting muscle (Andersen et al. 1985; Knight et al. 1993; Richardson et al. 1993; Cardus et al. 1998; di Prampero 2003). We found an approximately 4% greater $\dot{V}_{O_{2peak}}$ at 100 cpm than at 60 cpm. However, an increase in $\dot{V}_{O_{2peak}}$ when exercise is performed at higher pedal frequencies is not a universal finding. In other studies (Zoladz et al. 1995; Jones et al. 2004) similar $\dot{V}_{O_{2peak}}$ was found for pedal cadences ranging from 40 to 120 cpm in subjects similar to those presently studied. It appears that in our study the greater $\dot{V}_{O_{2peak}}$ at 100 cpm was caused by an increased muscle O_2 delivery, and not by an increased extraction as reflected by the similar [HHb] at $\dot{V}_{O_{2peak}}$ at 60 and 100 cpm.

Conclusion

We demonstrate that vastus lateralis oxygenation is similar during cycling exercise at 60 and 100 cpm, suggesting no impairment of muscle blood flow with the increase in contraction frequency. The similar muscle oxygenation and greater muscle metabolism at 100 cpm than at 60 cpm indicates a higher blood flow at 100 cpm, so that the ratio between O_2 uptake and blood flow was similar during exercise at 60 and 100 cpm. In addition, a greater $\dot{V}_{O_{2peak}}$ was achieved during the 100 cpm trials, further suggesting increased muscle O_2 delivery compared to the 60 cpm trial. Therefore, we conclude that vastus lateralis O_2 extraction was similar at 60 and 100 cpm, suggesting that the $\dot{V}_{O_{2M}}/\dot{Q}_M$ in the microcirculation was not altered and, presumably, no impairment of $\dot{Q}_M$ occurred with the increase in pedal frequency.

Acknowledgments This work was supported in part by American Heart Association, Grant-in-Aid # 0151183Z to T.J. Barstow. L.F. Ferreira was supported by a Fellowship from the Ministry of Education/CAPES - Brazil.

References

Andersen P, Saltin B (1985) Maximal perfusion of skeletal muscle in man. J Physiol 366:233–249

Beaver WL, Wasserman K, Whipp BJ (1986) A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol 60:2020–2027

Bellemare F, Wight D, Lavigne CM, Grassino A (1983) Effect of tension and timing of contraction on the blood flow of the diaphragm. J Appl Physiol 54:1597–1606

Buchler B, Magder S, Roussos C (1985) Effects of contraction frequency and duty cycle on diaphragmatic blood flow. J Appl Physiol 58:265–273

Cardus J, Marrades RM, Roca J, Barbera JA, Diaz O, Masclans JR, Rodriguez-Roisin R, Wagner PD (1998) Effects of FIO_2 on leg $\dot{V}_{O_2}$ during cycle ergometry in sedentary subjects. Med Sci Sports Exerc 30:697–703

Di Prampero PE (2003) Factors limiting maximal performance in humans. Eur J Appl Physiol 90:420–429

Ferguson RA, Ball D, Krustrup P, Aagaard P, Kjaer M, Sargeant AJ, Hellsten Y, Bangsbo J (2001) Muscle oxygen uptake and energy turnover during dynamic exercise at different contraction frequencies in humans. J Physiol 536:261–271

Ferrari M, Binzoni T, Quaresima V (1997) Oxidative metabolism in muscle. Philos Trans R Soc Lond B Biol Sci 352:677–683

Ferrari M, Mottola L, Quaresima V (2004) Principles, techniques, and limitations of near infrared spectroscopy. Can J Appl Physiol 29:463–487

Ferreira LF, Townsend DK, Lutjemeier BJ, Barstow TJ (2005) Muscle capillary blood flow kinetics estimated from pulmonary O_2 uptake and near-infrared spectroscopy. J Appl Physiol 98:1820–1828

Gotshall RW, Bauer TA, Fahrner SL (1996) Cycling cadence alters exercise hemodynamics. Int J Sports Med 17:17–21

Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C, Cerretelli P (2003) Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions in humans. J Appl Physiol 95:149–158

Hamann JJ, Buckwalter JB, Clifford PS (2004) Vasodilatation is obligatory for contraction-induced hyperaemia in canine skeletal muscle. J Physiol 557:1013–1020

Hoelting BD, Scheuermann BW, Barstow TJ (2001) Effect of contraction frequency on leg blood flow during knee extension exercise in humans. J Appl Physiol 91:671–679

Jones AM, Campbell IT, Pringle JS (2004) Influence of muscle fibre type and pedal rate on the $\dot{V}_{O_2}$-work rate slope during ramp exercise. Eur J Appl Physiol 91:238–245

Knight DR, Poole DC, Schaffartzik W, Guy HJ, Prediletto R, Hogan MC, Wagner PD (1992) Relationship between body and leg $\dot{V}_{O_2}$ during maximal cycle ergometry. J Appl Physiol 73:1114–1121

Knight DR, Schaffartzik W, Poole DC, Hogan MC, Bebout DE, Wagner PD (1993) Effects of hyperoxia on maximal leg O_2 supply and utilization in men. J Appl Physiol 75:2586–2594

Kowalchuk JM, Rossiter HB, Ward SA, Whipp BJ (2002) The effect of resistive breathing on leg muscle oxygenation using near-infrared spectroscopy during exercise in men. Exp Physiol 87:601–611

Li L, Caldwell GE (1998) Muscle coordination in cycling: effect of surface incline and posture. J Appl Physiol 85:927–934

Lutjemeier BJ, Miura A, Scheuermann BW, Koga S, Townsend DK, Barstow TJ (2005) Muscle contraction-blood flow interactions during upright knee extension exercise in humans. J Appl Physiol 98:1575–1583

MacIntosh BR, Neptune RR, Horton JF (2000) Cadence, power, and muscle activation in cycle ergometry. Med Sci Sports Exerc 32:1281–1287

McDaniel J, Durstine JL, Hand GA, Martin JC (2002) Determinants of metabolic cost during submaximal cycling. J Appl Physiol 93:823–828

Osada T, Radegran G (2002) Femoral artery inflow in relation to external and total work rate at different knee extensor contraction rates. J Appl Physiol 92:1325–1330

Poole DC, Wagner PD, Wilson DF (1995) Diaphragm microvascular plasma PO_2 measured in vivo. J Appl Physiol 79:2050–2057

Quaresima V, Homma S, Azuma K, Shimizu S, Chiarotti F, Ferrari M, Kagaya A (2001) Calf and shin muscle oxygenation patterns and femoral artery blood flow during dynamic plantar flexion exercise in humans. Eur J Appl Physiol 84:387–394

Richardson RS, Poole DC, Knight DR, Kurdak SS, Hogan MC, Grassi B, Johnson EC, Kendrick KF, Erickson BK, Wagner PD (1993) High muscle blood flow in man: is maximal O_2 extraction compromised? J Appl Physiol 75:1911–1916

Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD (1995) Myoglobin O_2 desaturation during exercise. Evidence of limited O_2 transport. J Clin Invest 96:1916–1926

Sheriff DD, Hakeman AL (2001) Role of speed vs. grade in relation to muscle pump function at locomotion onset. J Appl Physiol 91:269–276

Sjogaard G, Hansen EA, Osada T (2002) Blood flow and oxygen uptake increase with total power during five different knee-extension contraction rates. J Appl Physiol 93:1676–1684

Takaishi T, Ishida K, Katayama K, Yamazaki K, Yamamoto T, Moritani T (2002) Effect of cycling experience and pedal cadence on the near-infrared spectroscopy parameters. Med Sci Sports Exerc 34:2062–2071

Valic Z, Buckwalter JB, Clifford PS (2005) Muscle blood flow response to contraction: influence of venous pressure. J Appl Physiol 98:72–76

Walloe L, Wesche J (1988) Time course and magnitude of blood flow changes in the human quadriceps muscles during and following rhythmic exercise. J Physiol 405:257–273

Wasserman K, Whipp BJ, Koyl SN, Beaver WL (1973) Anaerobic threshold and respiratory gas exchange during exercise. J Appl Physiol 35:236–243

Whipp BJ (1994) The bioenergetic and gas exchange basis of exercise testing. Clin Chest Med 15:173–192

Zoladz JA, Rademaker AC, Sargeant AJ (1995) Non-linear relationship between O_2 uptake and power output at high intensities of exercise in humans. J Physiol 488:211–217