©Journal of Sports Science and Medicine (2024) **23,** 46-55
http://www.jssm.org DOI: https://doi.org/10.52082/jssm.2024.46

Research article

The Improvement in Exercise Performance during Reduced Muscle Mass Exercise is Associated with an Increase in Femoral Blood Flow in Older and Younger Endurance-Trained Athletes

Toni Haddad [1,2]✉, Angela L. Spence [3,4], Jeremiah Peiffer [5], Gregory M. Blain [2], Jeanick Brisswalter [2] and Chris R. Abbiss [1]

[1] Centre for Human Performance, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia; [2] Université Côte d'Azur, LAMHESS, France; [3] Curtin School of Allied Health, Exercise and Sport Science Discipline, Curtin University, WA, Australia; [4] Curtin Health Innovation Research Institute, Curtin University, WA, Australia; [5] Centre for Healthy Ageing, Murdoch University, Murdoch, WA, Australia

Abstract

This study investigated whether the improved performance observed with maximal self-paced single-leg (SL), compared with double-leg (DL) cycling, is associated with enhanced femoral blood flow and/or altered tissue oxygenation. The hyperaemic response to exercise was assessed in younger and older athletes. Power output was measured in 12 older (65 ± 4 y) and 12 younger (35 ± 5 y) endurance-trained individuals performing 2 x 3 min maximal self-paced exercise using SL and DL cycling. Blood flow (BF) in the femoral artery was assessed using Doppler ultrasound and muscle oxygenation was measured using near-infrared spectroscopy on the vastus lateralis. SL cycling elicited a greater power output (295 ± 83 *vs* 265 ± 70 W, $P < 0.001$) and peak femoral BF (1749.1 ± 533.3 *vs* 1329.7 ± 391.7 ml/min, $P < 0.001$) compared with DL cycling. Older individuals had a lower peak BF in response to exercise (1355.4 ± 385.8 *vs* 1765.2 ± 559.6 ml/min, $P = 0.019$) compared with younger individuals. Peak BF in response to exercise was correlated with power output during SL ($r = 0.655$, $P = 0.002$) and DL ($r = 0.666$, $P = 0.001$) cycling. The greater exercise performance during SL compared with DL cycling may be partly explained by a greater hyperaemic response when reducing active muscle mass. Despite regular endurance training, older athletes had a lower femoral BF in response to maximal self-paced exercise compared with younger athletes.

Key words: Blood flow; muscle oxygenation; muscle mass; endurance; ultrasound; ageing.

Introduction

Skeletal muscle blood flow increases in response to exercise to match metabolic demand until maximal cardiac output is reached (Richardson et al., 1993). When exercise is performed using a large muscle mass and at high intensity, some degree of peripheral vasoconstriction is observed and thus vascular conductance is reduced to maintain blood pressure (Calbet et al., 2004; Volianitis and Secher, 2002). As a result, cardiac output is thought to be a significant liming factor to maximal exercise involving a large muscle mass (e.g., double-leg cycling, rowing, cross-country skiing) (Saltin and Calbet, 2006). In contrast, greater levels of peripheral vasodilation are observed when a smaller amount of muscle mass (e.g., arm cranking, knee-extension, single-leg cycling) is exercised (Volianitis and Secher, 2002) since central blood pressure is not compromised. This allows blood flow to increase linearly with exercise intensity (Richardson et al., 1993) and may potentially explain the greater exercise performance observed during small compared with large muscle mass exercise. Indeed, it has been shown that power output per leg is generally 5 to 10% greater during single-leg (SL) compared with double-leg (DL) cycling (Abbiss et al., 2011; Gordon et al., 2018; Iannetta et al., 2019). Yet, blood flow during maximal SL and DL cycling has only been assessed in a small clinical population (LeJemtel et al., 1986) or during submaximal exercise (Burns et al., 2014; Draper et al., 2022). Therefore, the improved capacity to increase blood flow and enhance oxygen delivery during a maximal cycling exercise performed using SL compared to DL remains to be demonstrated.

In addition to being affected by active muscle mass, the hyperaemic response to exercise may also be influenced by age. An attenuation of the blood flow response in active muscles is sometimes observed in older compared with younger individuals (Beere et al., 1999; Donato et al., 2006; Poole et al., 2003; Proctor et al., 2004). This may be due to a reduction in vasodilatory capacity (e.g., reduced vascular conductance), an increase in sympathetically mediated vasoconstriction (e.g., increased diaphragm metaboreceptor activity) and/or an impairment of "functional sympatholysis" (e.g., reduced ability to blunt sympathetic vasoconstriction in the locomotor muscles) with ageing (Hearon et al., 2016). Yet, blood flow values to the active muscles were similar in older (Magnusson et al., 1994) and younger (Andersen et al., 1985) individuals during small muscle mass exercise (i.e., single-leg knee extension). It is possible that the age-related decrease in local blood flow is less pronounced or prevented during such exercise modality given a greater cardiac output is available during such exercise compared with a maximal effort involving large muscle mass (Davies and Sargeant, 1974; Iannetta et al., 2019; Klausen et al., 1982). However, a lower blood flow during small muscle mass exercise was also reported in older compared with younger individuals (Donato et al., 2006; Mortensen et al., 2012). The discrepancies between these studies may partly be explained by the differences in fitness status of the participants since no

Received: 09 October 2023 / Accepted: 01 December 2023 / Published (online): 01 March 2024

age-related difference in leg blood flow were observed between younger and older individuals engaged in short- (i.e., 3 months, Beere et al. (1999)) or long-term (i.e., 30 years, Mortensen et al., 2012)) endurance training. Nonetheless, a lower blood flow was also reported in older trained individuals compared with younger trained individuals (Proctor et al., 1998). Further studies are therefore needed to clarify the effects of ageing on the hyperaemic response to exercise in older-trained individuals, and better understand the age-related changes in vascular function without the confounding effect of sedentariness.

The purpose of this study was to investigate peripheral blood flow in response to maximal exercise involving a small and a large muscle mass. A secondary aim was to assess the effect of ageing on the hyperaemic response to exercise. Therefore, we compared femoral blood flow in response to double- and single-leg cycling in younger (<40 years) and older (>60 years) endurance-trained individuals. We hypothesised that 1) femoral blood flow would be greater in response to maximal self-paced single-leg when compared with double-leg cycling but 2) attenuated in older compared to younger endurance-trained individuals.

Methods

Participants

This study was conducted in a subgroup of individuals recruited for a larger study previously conducted in the same laboratory and involving participants with varying levels of fitness. Twenty-four endurance-trained participants were recruited for this study and were classified as over 60 years old (O60, n = 12, n = 1 female and n = 11 males) or under 40 years old (U40, n = 12, n = 4 females and n = 8 males) (Table 1). Participants were participating in aerobic exercise at least 5 hours a week for at least 5 years. Smoking and presenting any musculoskeletal injury, neuromuscular disease, lower limb injury within the past 6 months were set as exclusion criteria. Prior to all visits, participants were requested to refrain from stimulants or depressants (including caffeine or alcohol) and strenuous exercise for 24 h while replicating the same dietary intake. Throughout the duration of the study, participants were asked to maintain regular training commitments. Prior to data collection, participants provided written informed consent in accordance with the Edith Cowan University Human Research Ethics Committee (REF: 2020-01267- HADDAD). Procedures used within this investigation conformed to the Declaration of Helsinki.

Experimental design

All participants completed three laboratory-based cycling sessions separated by at least 48 h, and no more than 10 days. During the first visit, participants performed an incremental cycling test to determine peak oxygen consumption followed by a familiarisation to maximal self-paced cycling exercise (described below). During visits two and three, participants performed experimental trials using either double-leg (DL) or single-leg (SL) cycling in a randomised and counterbalanced order (described below). All visits were completed under standard laboratory conditions (20 - 21°C, 40 - 50% relative humidity) and at a similar time of day (±1 h) to avoid any possible effect of the circadian rhythm.

Incremental cycling test

Participants completed the incremental cycling test on an electromagnetically braked SRM cycle ergometer (Schoberer Rad Messtechnik, Jülich, Germany). After three stages (each 3 min) performed at 75, 100 and 125 W, intensity was further increased by 15 W every minute until volitional exhaustion or a cadence ≥ 60 rpm could not be maintained. Verbal encouragement was given to the participants. Gas exchange was measured via a verified respiratory gas analyser (TrueOne, ParvoMedics, Sandy, UT, USA), sampled at 1 Hz and averaged into 30 sec intervals. Peak oxygen consumption ($\dot{V}O_{2peak}$) was defined as the highest $\dot{V}O_2$ value recorded over a 30 sec moving average during the incremental test. Heart rate (HR) was sampled at 1Hz (Polar, S610, Polar Electro Oy, Finland) and peak heart rate (HR_{peak}) was defined as the highest value recorded during the incremental test. Power output was sampled at a rate of 2 Hz and peak power output (PPO) was defined as the power output that elicited $\dot{V}O_{2peak}$. After recovery (≥ 20min), participants were familiarised to maximal self-paced cycling exercise using double-leg cycling (DL) and single-leg cycling (SL) on a randomly chosen leg. The familiarisation included 4 repeated bouts of 30 sec of cycling from a stationary start where participants progressively increased power at self-selected intensity, followed by a 3 min maximal self-paced effort.

Experimental trials

Prior to the maximal DL and SL self-paced cycling test, participants performed 5 min of passive rest on the bike in the cycling position (PRE), followed by a short duration (3min) submaximal exercise to allow participants to briefly be accustomed to the ergometer and task (i.e., SL

Table 1. Anthropometric characteristics, blood flow in response to exercise, and peak exercise values during the incremental test in older (O60) and younger (U40) endurance-trained individuals.

	O60	U40	*P* value
Age (y)	65 ± 4	35 ± 5	< .001*
Body mass (kg)	69.7 ± 11.2	69.8 ± 12.7	0.985
Height (m)	1.73 ± 0.07	1.73 ± 0.08	0.864
$\dot{V}O_{2\,peak}$ (L/min)	3.21 ± 0.63	3.98 ± 0.71	0.017*
PPO (W)	276 ± 52	325 ± 60	0.042*
HR_{peak} (bpm)	167 ± 10	185 ± 9	< .001*
Training volume (min/week)	838 ± 368	637 ± 256	0.140
Peak BF after DL exercise (ml/min)	1160.4 ± 307.2	1520.2 ± 422.7	0.039*
Peak BF after SL exercise (ml/min)	1550.4 ± 366.9	2022.4 ± 625.8	0.044*

$\dot{V}O_{2\,peak}$, peak oxygen consumption; PPO, peak power output; HR_{peak}, peak heart rate; peak BF, peak blood flow; DL, double-leg; SL, single-leg. Values are presented as mean ± SD. *main effect of age O60 vs U40, $P < 0.05$.

vs DL cycling). During the SL session, the submaximal exercise was performed single-legged at 25% PPO whereas during the DL session, it was performed double-legged at 50% PPO. After some passive rest (>15 min), the participants then performed a maximal self-paced cycling test involving two intervals of 3 min interspersed by 90 s of passive recovery (i.e., two double-leg intervals during DL or four single-leg intervals during SL cycling). Such duration was chosen to spend time ≥ 95% $\dot{V}O_{2peak}$ during DL therefore increasing the likelihood that performance during double-leg cycling would be limited by the cardiovascular system (Abbiss et al., 2011). The order of conditions (i.e., SL *vs* DL cycling), and the first leg during SL cycling (i.e., left *vs* right) was randomised and counterbalanced across participants. During SL cycling, at least 15 minutes of recovery were given after testing the first leg and prior to testing the second leg. A 10 kg counterweight was used during SL to reduce the differences in flexion and extension compared to the bilateral cycling pattern (Bini et al., 2015). Continuous feedback was provided for time only and participants were instructed and encouraged to perform each interval at the highest average power output they could sustain (Abbiss et al., 2011). The individuals were free to manipulate cadence and/or resistance at any time during the effort to maximise their power output. Perceptual responses were assessed within 30 sec after each 3 min interval using 3 different scales: perception of effort (0 - 10: "how much you were trying to"), rating of perceived exertion (Borg's RPE scale, 6 - 20: "your level of whole-body perceived exertion") and pain (0 - 10: "your own subjective definition of pain"). Leg muscle oxygen saturation (SmO_2) and total haemoglobin (THb), and femoral blood flow were assessed pre- and post-exercise using near-infrared spectroscopy (NIRS) and Doppler ultrasound respectively (described below). The first leg used during SL cycling was the leg chosen to measure NIRS and blood flow (BF) since local hemodynamic responses can be affected by the order in which each leg performs sequential SL cycling (Gordon et al., 2018). The same leg was used to measured NIRS and BF during each modality (i.e., DL and SL cycling) and was randomised and counterbalanced among participants.

Power output, HR, $\dot{V}O_2$, and $\dot{V}_E$

Power output (SRM cycle ergometer) was averaged over each interval of the maximal self-paced cycling test and presented for each modality separately (i.e., DL *vs* SL cycling). Oxygen consumption, minute ventilation(metabolic analysis system), and heart rate (Polar, S610) were averaged over 60 sec prior to exercise (i.e., PRE), during each 3-min interval (i.e., INT1 and INT2), immediately post-exercise (POST INT1 and POST INT2) and throughout the passive recovery (i.e., 3 - 4 min, 5 - 6 min, 9 - 10 min post-exercise).

Near-infrared spectroscopy (NIRS)

Tissue oxygenation was measured using a portable NIRS device (Moxy Monitor, Fortiori Design LLC, MN, USA). The NIRS Moxy Monitor calculates total haemoglobin (sum of oxygenated and deoxygenated haemoglobin, THb) and leg muscle oxygen saturation (oxygenated haemoglobin expressed as percentage of the total haemoglobin) based upon absorption and reflection of near-infrared light by oxygenated and deoxygenated haemoglobin (Crum et al., 2017). The sensor was placed on the vastus lateralis, one of the main contributors to power production during cycling. The leg that BF and NIRS was assessed on was equipped with a Moxy Monitor sensor at mid-point between the greater trochanter and lateral epicondyle of the femur following manufacturer guidelines, and its position was marked to ensure consistency between the trials. The sensor was secured with a light shield and athletic tape to block ambient light from interfering with the detectors. SmO_2 and THb were averaged over 60 sec prior to exercise (i.e., PRE), during each interval (i.e., INT1 and INT2), immediately post-exercise (POST INT1 and POST INT2) and throughout the passive recovery (i.e., 3 - 4 min, 5 - 6 min, 9 - 10 min post-exercise). SmO_2 and THb during exercise and recovery were expressed as a percentage of resting values.

Blood flow and ultrasound imaging

Blood flow was assessed in the superficial femoral artery using a high-resolution duplex ultrasound system (T3000, Terason, Burlington, MA, USA). Ultrasound parameters were set to optimize the longitudinal B-mode images of the lumen-arterial wall interface using the lowest possible insonation angle (always <60°). Resting images and Doppler velocity were recorded prior to any exercise in a standardised seated cycling position for 1 min to quantify mean blood flow pre-exercise. After exercise, participants were instructed to immediately extend the measurement leg perpendicular to the ground, while staying seated on the cycle ergometer. It was ensured that the cycling position was standardised for each participant. The hip angle during BF assessment was not significantly different between older and younger participants (113 ± 5 *vs* 116 ± 7°, respectively). Leg blood flow post-exercise was assessed using the same settings and optimal images as pre-exercise. BF was averaged over 60 sec prior to exercise (i.e., PRE) and re-assessed immediately after interval 1 (POST INT1) and interval 2 (POST INT2) and later during the recovery. A qualitative signal was obtained on average 10.3 ± 6.5 and 9.7 ± 5.4 sec after interval 1 (POST INT1) and 12.2 ± 7.9 and 10.8 ± 6.2 sec after interval 2 (POST INT2) using DL and SL cycling respectively (mean ± standard deviation). A signal was deemed appropriate when a region of interest for both the vessel diameter and the velocity trace could be determined to enable offline analysis using a custom edge-detection software for offline analysis (software analysis described below). This implied the ability to clearly identify the anterior and posterior walls of the artery and its intima for a prolonged duration without interruption. To maximise the strength of blood flow analysis, the values recorded immediately post-exercise were analysed only from 15 sec post-exercise where a signal was appropriate for at least ≥ 85% of participants (≥ 90% after 20 sec). Peak blood flow post-exercise (i.e., greatest flow achieved over 1 sec averaged window) was assessed within the window from 15 to 45 sec after each interval separately. Peak blood flow after intervals 1 and 2 was also averaged as a single value for DL and SL cycling respectively to investigate the relationship between femoral blood flow and power output per leg (Pearson's correlation). Mean BF was presenter after each interval and calculated over a 30 sec window from 15 to 45 sec post-exercise. Mean BF was also calculated over a 30 sec window throughout the course of passive recovery after 3 min (3 min to 3 min 30 sec), 5 min (5 min to 5 min 30 sec) and 9 min (9 min to 9 min 30 sec). Peak blood flow post-exercise (i.e., greatest flow achieved over 1 sec averaged window) was assessed within the window from 15 to 45 sec after each interval separately.

Data analysis

Power output during exercise

Power output during DL cycling was the averaged power produced by the two legs simultaneously during intervals 1 and 2, whereas power output during SL cycling was the sum of the averaged power from each leg sequentially (i.e., left plus right leg) during both intervals. Power output during intervals 1 and 2 was also averaged as a single value during DL and SL cycling respectively and expressed in power output per leg (i.e., 50% power during DL and 100% power during SL) to investigate the relationship between peak femoral blood flow and power output per leg (Pearson's correlation).

Blood flow pre- and post-exercise

Vessel diameter and blood velocity were analysed using custom-designed, edge-detection wall-tracking software (FMD/Blood Flow 4.0, LabView 10.0, National Instruments, Austin, Texas, USA) which is validated and investigator-independent with an intra-observer coefficient of 6.7% (Woodman et al., 2001). In addition, a visual inspection of vessel diameter and blood velocity interpolated at 99Hz was performed by two operators to remove any potential outliers. Subsequent analysis was performed over data interpolated at 1Hz. Simultaneous regions of interest were identified on both the B-mode and Doppler images to analyse arterial diameter and blood velocity profile, respectively. Blood flow was calculated using the equation: blood flow = [(mean blood velocity) × (π × (vessel radius^2)) x 60], where mean blood velocity is expressed in cm/s and radius is expressed in cm. Blood flow measured before and after exercise was presented as the mean blood flow (ml/min), which is calculated as a running mean of peak blood flow over three cardiac cycles that shifts by one cardiac cycle each time, and then multiplied by the constant of 0.5 and the angle correction factor based on the insonation angle (i.e., 60°). An additional analysis was performed over the time-course of recovery (i.e., after 15 - 45 sec, 3 to 3 min 30, 5 to 5 min 30, and 9 to 9 min 30 of recovery) where mean blood flow was expressed as a % of the peak value measured after interval 2. Blood flow data were excluded for one participant since the signal recorded in the first leg that exercised during SL cycling did not satisfy analysis requirements and using data from the second leg may have led to misinterpretation due to the influence of leg order.

Statistical analysis

Participants characteristics (i.e., age, body mass, height, peak oxygen consumption, peak power output, peak heart rate, weekly training volume, peak blood flow in response to exercise and hip angle during BF assessment were compared between older (O60) and younger (U40) individuals using independent samples *t*-tests. Normality of the distribution was tested and confirmed using a Shapiro-Wilk test. A linear mixed model was used to compare peak blood flow post-exercise between older (O60) and younger (U40) (age was set as a fixed effect and participants were set as random effect).

A linear mixed model was used to determine the effects of modality (i.e., DL or SL) and age (i.e., O60 or U40) on the physiological (i.e., power output, cadence, heart rate, oxygen consumption, minute ventilation, SmO_2, THb, femoral blood flow) and perceptual (i.e., effort, exertion, pain) responses during single-leg and double-leg cycling. Modality (i.e., DL or SL) and age (i.e., O60 or U40) were set as fixed effects and participants were set as random effect. Assumptions of residuals normality and homoscedasticity were confirmed by inspecting residuals plots. Bivariate associations were determined by Pearson's correlation coefficients between femoral blood flow and power output and peak oxygen consumption. 95% confidence intervals and the equations for each linear regression were calculated. Statistical significance was set at $P < 0.05$. All data are presented as means ± SD. Statistical analysis was performed using Jasp 0.16 software.

Results

Performance during double-leg and single-leg cycling

A main effect for power output was observed between SL (sum of both legs) and DL cycling (295 ± 83 *vs* 265 ± 70 W respectively, $P < 0.001$). Power output was not significantly different ($P = 0.053$) between older *vs* younger endurance-trained individuals when SL and DL were considered together, although a trend was observed. Power output was 268 ± 72 *vs* 233 ± 52 W in older individuals and 322 ± 87 *vs* 296 ± 74 W in younger individuals during SL *vs* DL cycling, respectively.

Physiological response to double-leg and single-leg cycling

Before exercise

A main effect for mean pre-exercise blood flow was observed between older compared with younger individuals (138.5 ± 62.4 *vs* 197.4 ± 81.5 ml/min respectively, $P = 0.045$, Figure 1, A and B).

During and after exercise

$\dot{V}O_2$, HR and $\dot{V}_E$ were significantly lower at all-time points during and after intervals 1 and 2 performed with SL compared with DL cycling (Table 2). SmO_2 was significantly lower at all-time points (except 5 min post-exercise) during DL compared with SL cycling (Table 3). THb was not different between modalities (Table 3). Mean BF was greater after intervals 1 and 2 performed using SL compared with DL cycling until 3 min post-exercise and was not significantly different thereafter (i.e., after 5 and 9 min post-exercise (Figure 1). Peak BF was greater immediately after interval 1 (1821.7 ± 632.2 *vs* 1322.6 ± 478.5 ml/min, $P < 0.001$) and after interval 2 (1667.0 ± 500.6 *vs* 1367.4 ±

344.8 ml/min, $P < 0.001$) performed using SL compared with DL cycling. When expressed as a % of peak, BF post interval 2 was not significantly different between modalities throughout the course of recovery. $\dot{V}O_2$, HR and $\dot{V}_E$ were significantly greater in younger compared with older groups at some but not all time points during and after exercise (Table 2). SmO_2 and THb did not differ significantly between age groups throughout exercise and recovery (Tables 2 and 3). Peak BF and mean BF at every time point were significantly lower in older compared with younger individuals (Figure 1). When expressed as a % of peak, mean BF post interval 2 was significantly lower in older compared with younger individuals throughout the course of recovery after 15-45 sec (81 ± 7 *vs* 88 ± 5 %, $P = 0.011$), 3-3.5 min (31 ± 9 *vs* 52 ± 12 %, $P < 0.001$), 5-5.5 min (24 ± 5 *vs* 37 ± 10 %, $P = 0.005$) and 9-9.5 min (19 ± 8 *vs* 31 ± 13 %, $P < 0.001$).

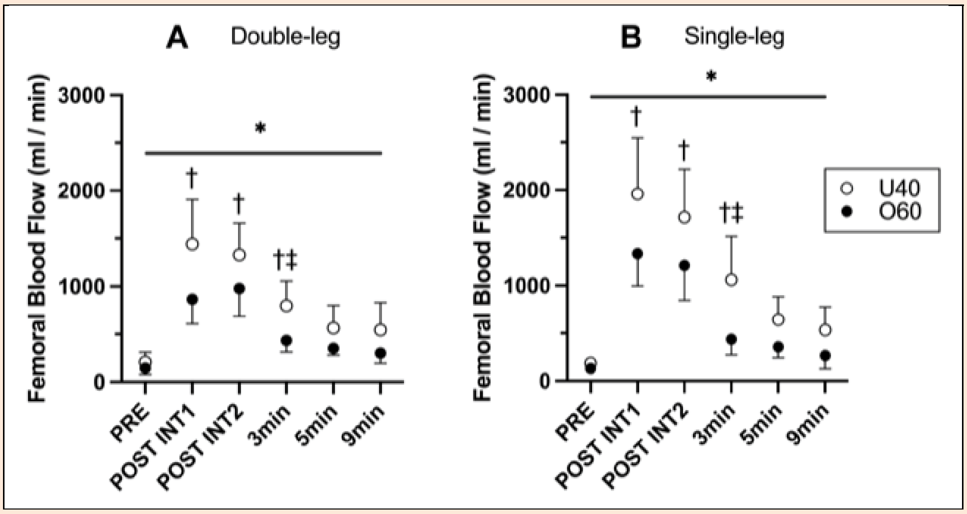

Figure 1. Femoral blood flow before (PRE) and after (POST) double- and single-leg cycling in older (O60) and younger (U40) endurance-trained individuals. *, Main effect of age, comparisons made between all older and all younger subjects, regardless of modality. †, Main effect of modality, comparisons made between DL and SL cycling, regardless of age. ‡, Interaction effect between modality and age Values are presented as mean ± SD.

Table 2. Oxygen consumption ($\dot{V}O_2$), heart rate (HR) and minute ventilation ($\dot{V}_E$) before, during and after exercise in older (O60) and younger (U40) endurance-trained individuals.

	Double-leg cycling		Single-leg cycling		
HR (bpm)	**O60**	**U40**	**O60**	**U40**	**Main effect**
PRE	76 ± 11	78 ± 10	76 ± 10	80 ± 15	
INT 1	139 ± 11	158 ± 7	130 ± 10	144 ± 10	†*
POST INT 1	140 ± 16	161 ± 7	130 ± 12	147 ± 11	†*
INT 2	149 ± 10	167 ± 5	141 ± 9	158 ± 9	†*
POST INT 2	152 ± 12	169 ± 5	141 ± 11	157 ± 8	†*
3 min	93 ± 9	105 ± 11	88 ± 8	98 ± 12	†*
5 min	92 ± 8	102 ± 12	85 ± 8	91 ± 11	†*
9 min	92 ± 8	99 ± 10	87 ± 8	91 ± 10	
$\dot{V}O_2$ (L/min)	**O60**	**U40**	**O60**	**U40**	**Main effect**
PRE	0.38 ± 0.08	0.45 ± 0.1	0.36 ± 0.09	0.39 ± 0.1	†
INT 1	2.35 ± 0.48	3.19 ± 0.71	1.93 ± 0.38	2.24 ± 0.56	†*‡
POST INT 1	2.35 ± 0.63	2.91 ± 0.67	1.89 ± 0.45	2.11 ± 0.62	†‡
INT 2	2.73 ± 0.48	3.56 ± 0.77	2.24 ± 0.45	2.6 ± 0.61	†*‡
POST INT 2	2.55 ± 0.42	3.03 ± 0.67	2.03 ± 0.52	2.29 ± 0.56	†
3 min	0.55 ± 0.11	0.64 ± 0.16	0.45 ± 0.1	0.52 ± 0.13	†
5 min	0.5 ± 0.09	0.63 ± 0.18	0.38 ± 0.1	0.47 ± 0.13	†*‡
9 min	0.39 ± 0.08	0.49 ± 0.15	0.34 ± 0.07	0.41 ± 0.09	†*
$\dot{V}_E$ (L/min)	**O60**	**U40**	**O60**	**U40**	**Main effect**
PRE	14 ± 4	16 ± 5	12 ± 3	13 ± 4	†
INT 1	83 ± 23	112 ± 27	74 ± 19	82 ± 26	†
POST INT 1	89 ± 27	112 ± 27	74 ± 20	79 ± 27	†‡
INT 2	101 ± 22	131 ± 29	91 ± 24	102 ± 27	†‡
POST INT 2	98 ± 22	116 ± 26	82 ± 26	88 ± 25	†
3 min	27 ± 7	32 ± 8	19 ± 5	23 ± 6	†
5 min	20 ± 4	27 ± 6	16 ± 4	18 ± 4	†*
9 min	16 ± 4	20 ± 5	13 ± 3	16 ± 5	†*

$\dot{V}O_2$, oxygen consumption; HR, heart rate; $\dot{V}_E$, minute ventilation. Values are presented as mean ± SD. *, Main effect of age, comparisons made between all older and all younger subjects, regardless of modality. †, Main effect of modality, comparisons made between DL and SL cycling, regardless of age. ‡, Interaction effect between modality and age

Table 3. Leg muscle oxygen saturation (SmO_2) and total haemoglobin (THb) before, during and after exercise in older (O60) and younger (U40) endurance-trained individuals.

	Double-leg cycling		Single-leg cycling		
SmO_2 (% PRE)	**O60**	**U40**	**O60**	**U40**	**Main effect**
PRE	100	100	100	100	N/A
INT 1	37.3 ± 9.8	38.7 ± 10.2	44.9 ± 15.1	51.3 ± 16	†
POST INT 1	59.8 ± 13.4	67.3 ± 18.5	70.7 ± 19.7	74.1 ± 21.6	†
INT 2	36.3 ± 9.2	37.9 ± 10.5	44.4 ± 12.3	51 ± 14.5	†
POST INT 2	57.4 ± 12.8	60.8 ± 19.9	67.6 ± 19.1	74.5 ± 25.5	†
3 min	111.6 ± 13.9	118.6 ± 13.8	130.6 ± 20.3	132.3 ± 14.1	†
5 min	95.6 ± 16.2	101.3 ± 20.6	95.7 ± 17.8	113.4 ± 18.7	
9 min	109.3 ± 10.7	111.9 ± 12.7	116.9 ± 11.6	122.7 ± 14.2	†
THb (% PRE)	**O60**	**U40**	**O60**	**U40**	**Main effect**
PRE	100	100	100	100	N/A
INT 1	100 ± 0.9	100.2 ± 1	99.9 ± 0.6	99.5 ± 1.2	
POST INT 1	100 ± 1.1	100.3 ± 0.7	100 ± 0.4	99.8 ± 1	
INT 2	99.8 ± 0.9	100 ± 0.9	99.8 ± 0.5	99.4 ± 1.3	
POST INT 2	100 ± 1.2	100.3 ± 1	99.9 ± 0.5	99.8 ± 1.1	
3 min	99.7 ± 1	100.3 ± 1.2	99.6 ± 0.9	99.9 ± 0.9	
5 min	99.2 ± 5.2	99.5 ± 4.8	100.4 ± 4.9	101.6 ± 5.1	
9 min	99.7 ± 0.7	99.9 ± 0.9	99.6 ± 0.6	99.6 ± 0.7	

SmO2, leg muscle oxygen saturation; THb, total haemoglobin. Values are presented as mean ± SD. †, Main effect of modality, comparisons made between DL and SL cycling, regardless of age.

An interaction effect (modality x age) was observed during and after exercise for $\dot{V}O_2$ and $\dot{V}_E$. Younger individuals had a higher $\dot{V}O_2$ ($\dot{V}O_2$ during SL was 72% *vs* 82% DL in U40 compared with O60) and a higher $\dot{V}_E$ ($\dot{V}_E$ during SL was 76% *vs* 89% DL in U40 compared to O60) during SL relatively to DL (Table 2). An interaction effect (modality x age) was also observed for mean BF 3 min post-exercise. Mean BF 3 min post-exercise after SL was lower in O60 but greater in U40 compared with DL cycling (90% DL in O60 *vs* 130% DL in U40, $P = 0.022$, Figure 1).

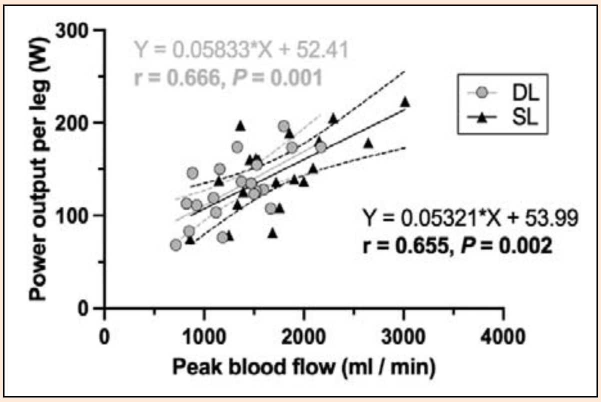

Figure 2. Pearson's correlation coefficients between peak femoral blood flow and power output during double- and single-leg cycling. Pearson's correlation coefficients are presented for all participants (i.e., O60 and U40) considered as a single group. Double- (circles, grey) and single-leg (triangles, black) cycling is presented separately. The equations for each linear regression line are presented separately during double- (grey) and single-leg (black) cycling. Continuous lines represent mean slopes. Dashed lines represent 95% confidence intervals.

Perceptual response to double-leg and single-leg cycling

Effort (8.7 ± 1.1 *vs* 9.3 ± 0.7 a.u., $P < 0.001$), exertion (16.7 ± 1.6 *vs* 18.2 ± 1.1 a.u.), and pain (6.0 ± 3.1 *vs* 6.9 ± 3.0 a.u., $P < 0.001$) were significantly lower after SL compared with DL cycling. Perceptual responses did not differ between older and younger individuals.

Relationship between peak femoral blood flow and power output

A significant relationship (SL: r = 0.655, $P = 0.002$; DL: r = 0.666, $P = 0.001$) between peak femoral blood flow post-exercise and power output per leg was observed, when all participants were analysed as a single group and both intervals averaged as a single measure (Figure 2). When analysing the two age groups separately, the relationship between Peak BF and power output per leg was significant during SL but not DL in the older sub-group (SL: r = 0.615, $P = 0.044$; DL: r = 0.514, $P = 0.106$) and no significant relationship was observed in the younger sub-group (SL: r = 0.618, $P = 0.076$; DL: r = 0.617, $P = 0.077$).

Discussion

To better understand the effect of ageing on the hyperaemic response to maximal exercise when various amount of muscle mass is engaged, femoral blood flow in response to maximal single-leg and double-leg cycling exercise was compared in younger (<40 years) and older (>60 years) trained individuals. The major findings were that:

i) Single-leg cycling elicited a greater femoral blood flow compared with double-leg cycling in both older and younger endurance-trained individuals, which may partly explain the greater power output observed during SL compared to DL.
ii) Femoral blood flow in response to maximal exercise was lower in older compared with younger endurance-trained individuals regardless of the amount of muscle mass engaged.

In the present study, single-leg cycling elicited a greater femoral blood flow compared with double-leg cycling (Figure 1). Indeed, peak blood flow measured following SL cycling corresponded to ~132 and ~130% of DL cycling in older and younger endurance-trained individuals, respectively. To our knowledge, this is the first study to show that femoral blood flow was greater in response to maximal SL compared with DL cycling in both older and younger trained populations. Yet, these results are in agreement with observations of greater femoral blood flow during SL cycling in a limited cohort of healthy (n = 4) and clinical (patients with heart failure, n = 5) individuals (LeJemtel et al., 1986) and after submaximal SL cycling exercise (Burns et al., 2014; Draper et al., 2022). As such, this study supports the finding that a transition from large to small muscle mass exercise may allow for a greater peripheral hyperaemic response (Calbet et al., 2009; Cardinale et al., 2019; Klausen et al., 1982). In turn, this greater femoral blood flow may partly explain the greater power output during SL compared with DL cycling observed here and in previous studies (Abbiss et al., 2011; Gordon et al., 2018; Iannetta et al., 2019). Indeed, during exercise involving a small muscle mass, blood pressure is not threatened to the same extent as maximal exercise involving a large muscle mass where a decrease in vascular conductance is required to avoid hypotension (Calbet et al., 2004; Davies and Sargeant, 1974; Klausen et al., 1982; Saltin and Calbet, 2006; Volianitis and Secher, 2002). As a result, a greater proportion of cardiac output was presumably redistributed to the active muscles during SL compared with DL cycling (Iannetta et al., 2019) although we were not able to measure blood pressure and vascular conductance. Overall, the present study illustrates a dissociation between exercise performance and the level of pulmonary and cardiovascular stress as previously documented (Abbiss et al., 2011; Gordon et al., 2018; Gordon et al., 2020) and indicates that the greater power output during SL cycling is accompanied by a greater blood flow but a lower heart rate, oxygen consumption, and minute ventilation compared with DL cycling (Table 2).

Noteworthy, no change in THb was observed when reducing muscle mass in the present study (Table 3) and previously (Gordon et al., 2018) despite observing a difference in BF (Figure 1). This indicates that THb may have some limitations to appropriately measure BF after a cycling task as previously suggested (Gordon et al., 2020) although others have reported a correlation between THb and blood flow after isolated muscle contractions (Alvares et al., 2020). Since leg dominance was not considered in this cohort, it is plausible that using dominant and non-dominant leg during SL without distinction may have impacted power output and NIRS, and masked some differences compared with double-leg cycling (Iannetta et al., 2019). Yet, single-leg power output differed only by ~2.1% between left and right leg, indicating that randomising the leg used to assess NIRS and BF minimised the effect of leg dominance on tissue oxygenation or blood flow.

Muscle oxygenation was greater during SL compared with DL cycling (Table 3), indicating that oxygen extraction may be attenuated during small compared with large muscle mass exercise, in agreement with some (Cardinale et al., 2019) but in contrast to other (Gordon et al., 2018; Skattebo et al., 2020a) studies. Such discrepancies may partly be explained by the differences in the site of measurement (e.g., vastus lateralis *vs* rectus femoris) and in the exercise protocol (e.g., incremental exercise *vs* intervals). Although greater blood flow and reduced blood transit time may be partly responsible for the lower oxygen extraction (Richardson et al., 1999) observed during small compared with large muscle mass exercise in the present study, there is recent evidence to suggest that it may rather be a consequence of the attainment of maximal mitochondrial capacity (Cardinale et al., 2019). Indeed, since a greater proportion of cardiac output is available during SL compared with DL cycling (Davies and Sargeant, 1974; Iannetta et al., 2019; Klausen et al., 1982), aerobic performance during single-leg cycling seems to be primarily related to the ability to diffuse oxygen from the microvasculature to the mitochondria (Richardson et al., 1999), and the mitochondrial capacity to utilise oxygen for oxidative phosphorylation (Skattebo et al., 2020b). While some authors have previously reported that mitochondrial capacity was greater than the metabolic demand during single-leg knee extension in trained individuals (Gifford et al., 2016), the greater muscle oxygenation observed during SL, compared with DL cycling, indicates that the maximal mitochondrial activity was presumably reached during maximal self-paced single-leg but not double-leg cycling within the present study. Moreover, the strong relationship observed between peak femoral blood flow and power output during both DL and SL cycling (Figure 2) indicates that blood flow was appropriately regulated during exercise to meet the metabolic demand of the exercise task, as previously observed by others (Calbet et al., 2006), regardless of the amount of active muscle mass.

Reducing muscle mass improved oxygen delivery and exercise performance in both older and younger individuals. This is illustrated by the greater femoral blood flow and power output observed during SL compared with DL cycling in both groups (Figure 1). Moreover, no effect of age was observed on leg muscle oxygen saturation or total haemoglobin during DL and SL cycling (Table 3), indicating that the older individuals were able to accommodate an increase in blood flow in a similar way to younger individuals. It was however not accounted for the possible influence of adipose tissue thickness on the NIRS signal (Hamaoka and McCully, 2019), and these results should be interpreted with caution, although the comparison of leg muscle oxygen saturation in relative values (i.e., % THb) could minimise the risk of error during inter-individual comparison.Femoral blood flow was consistently lower at rest, immediately post-exercise, and over the course of recovery (Figure 1) in response to both large (i.e., DL) and small (i.e., SL) muscle mass exercise in the older compared to the younger group. The ~30% lower peak blood flow observed in older compared to younger individuals (Figure 1) is in agreement with previous findings showing a reduction in leg blood flow during maximal exercise with ageing (Beere et al., 1999; Donato et al., 2006; Poole et al., 2003; Proctor et al., 2004). Although not significantly different, mean power output was slightly lower during the single-leg and double-leg cycling efforts in the older, compared with the younger athletes, which may contribute to explain the lower blood flow observed. Importantly, despite evidence showing that skeletal muscle blood flow increases in response to exercise to match metabolic demand (Richardson et al., 1993), it is hard to distinguish whether the greater blood flow observed during SL cycling (in both older and younger athletes) was responsible for, or the consequence of a greater power output. Since a greater blood flow may also increase muscle glucose uptake (Malone et al., 2021), the possible contribution of nutrient delivery to the greater power output per leg observed during small *vs* large muscle deserves further attention in the future.

The lower femoral blood flow in the older compared with the younger group (Figure 1) observed in the present study contrasts with the findings from others who reported that blood flow was preserved with ageing during submaximal exercise involving a large muscle mass (Poole et al., 2003) and during exercise involving a small muscle mass (Donato et al., 2006; Magnusson et al., 1994; Mortensen et al., 2012). It is possible that the absence of age-related difference in blood flow was due to the intensity and/or nature of the exercise (e.g., submaximal intensity and/or small muscle mass exercise) used in previous studies. Indeed, any age-related reduction in vascular function (e.g., vasodilatory capacity) might be compensated by an increase blood flow to the locomotor muscles in response to an increase in cardiac output (Montero, 2015) during such exercise. Yet, we observed a lower hyperaemic response following exercise involving a small muscle mass in our older participants (Table 2). As a result, it is unlikely that cardiac output limited blood flow in the older group since heart rate, and presumably cardiac output, was not maximal during maximal self-paced SL cycling exercise (Davies and Sargeant, 1974; Iannetta et al., 2019; Klausen et al., 1982). Interestingly, the lower femoral blood flow in older *vs* younger individuals (Figure 1) was observed in the present study despite a consistent participation in aerobic activity in our sample of endurance-trained athletes. This finding extends previous results during submaximal exercise in older trained athletes (Proctor et al., 1998) and indicates that femoral blood flow may be lower in older compared with younger individuals regardless of the amount of muscle mass engaged and their training status. Further research is however needed to understand the longitudinal effects of ageing and training on vascular function.

Conclusion

Femoral blood flow was greater in response to maximal exercise involving a small (SL cycling) compared to a large (DL cycling) muscle mass in both younger and older endurance-trained individuals. This greater hyperaemic response likely highlights an increase in oxygen delivery to the locomotor muscles thereby explaining the observed improvement in performance. Femoral blood flow post-exercise was however lower in the older compared with the younger athletes, despite regular endurance training.

Acknowledgements

The authors would like to sincerely thank the participants and the research support team for their commitment to this study. We would also like to thank Glen Chin for his help during the data collection and Shih Ching Fu for sharing his statistics knowledge. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The experiments comply with the current laws of the country in which they were performed. The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author who was an organizer of the study.

References

Abbiss, C. R., Karagounis, L. G., Laursen, P. B., Peiffer, J. J., Martin, D. T., Hawley, J. A., Fatehee, N. N., & Martin, J. C. (2011). Single-leg cycle training is superior to double-leg cycling in improving the oxidative potential and metabolic profile of trained skeletal muscle. *Journal of Applied Physiology* **110(5),** 1248–1255. https://doi.org/10.1152/japplphysiol.01247.2010

Alvares, T. S., Oliveira, G. V., Soares, R., & Murias, J. M. (2020). Near-infrared spectroscopy-derived total haemoglobin as an indicator of changes in muscle blood flow during exercise-induced hyperaemia. *Journal of Sports Sciences* **38(7),** 751–758. https://doi.org/10.1080/02640414.2020.1733774

Andersen, P., Adams, R. P., Sjøgaard, G., Thorboe, A., & Saltin, B. (1985). Dynamic knee extension as model for study of isolated exercising muscle in humans. *Journal of Applied Physiology* **59(5),** 1647–1653. https://doi.org/10.1152/jappl.1985.59.5.1647

Beere, P. A., Russell, S. D., Morey, M. C., Kitzman, D. W., & Higginbotham, M. B. (1999). Aerobic exercise training can reverse age-related peripheral circulatory changes in healthy older men. *Circulation* **100(10),** 1085–1094. https://doi.org/10.1161/01.cir.100.10.1085

Bini, R. R., Jacques, T. C., Lanferdini, F. J., & Vaz, M. A. (2015). Comparison of kinetics, kinematics, and electromyography during single-leg assisted and unassisted cycling. *Journal of strength and conditioning research*, *29*(6), 1534–1541. https://doi.org/10.1519/JSC.0000000000000905

Burns, K. J., Pollock, B. S., Lascola, P., & McDaniel, J. (2014). Cardiovascular responses to counterweighted single-leg cycling: implications for rehabilitation. *European Journal of Applied Physiology*, **114(5),** 961–968. https://doi.org/10.1007/s00421-014-2830-0

Calbet, J. A., Jensen-Urstad, M., van Hall, G., Holmberg, H. C., Rosdahl, H., & Saltin, B. (2004). Maximal muscular vascular conductances during whole body upright exercise in humans. *The Journal of Physiology* **558(Pt 1),** 319–331. https://doi.org/10.1113/jphysiol.2003.059287

Calbet, J. A., Lundby, C., Sander, M., Robach, P., Saltin, B., & Boushel, R. (2006). Effects of ATP-induced leg vasodilation on VO2 peak and leg O2 extraction during maximal exercise in humans. *American journal of physiology. Regulatory, Integrative and Comparative Physiology* **291(2),** R447–R453. https://doi.org/10.1152/ajpregu.00746.2005

Calbet, J. A., Rådegran, G., Boushel, R., & Saltin, B. (2009). On the mechanisms that limit oxygen uptake during exercise in acute and chronic hypoxia: role of muscle mass. *The Journal of Physiology* **587(2),** 477–490. https://doi.org/10.1113/jphysiol.2008.162271

Cardinale, D. A., Larsen, F. J., Jensen-Urstad, M., Rullman, E., Søndergaard, H., Morales-Alamo, D., Ekblom, B., Calbet, J. A. L., & Boushel, R. (2019). Muscle mass and inspired oxygen influence oxygen extraction at maximal exercise: Role of mitochondrial oxygen affinity. *Acta physiologica (Oxford, England)* **225(1),** e13110. https://doi.org/10.1111/apha.13110

Crum, E. M., O'Connor, W. J., Van Loo, L., Valckx, M., & Stannard, S. R. (2017). Validity and reliability of the Moxy oxygen monitor during incremental cycling exercise. *European Journal of Sport Science*, **17(8),** 1037–1043. https://doi.org/10.1080/17461391.2017.1330899

Davies, C. T., & Sargeant, A. J. (1974). Physiological responses to one- and two-leg exercise breathing air and 45 percent oxygen. *Journal of Applied Physiology* **36(2),** 142–148. https://doi.org/10.1152/jappl.1974.36.2.142

Donato, A. J., Uberoi, A., Wray, D. W., Nishiyama, S., Lawrenson, L., & Richardson, R. S. (2006). Differential effects of aging on limb blood flow in humans. *American Journal of Physiology. Heart And Circulatory Physiology* **290(1),** H272–H278. https://doi.org/10.1152/ajpheart.00405.2005

Draper, S., Singer, T., Dulaney, C., & McDaniel, J. (2022). Single Leg Cycling Offsets Reduced Muscle Oxygenation in Hypoxic Environments. *International Journal of Environmental Research and Public Health* **19(15),** 9139. https://doi.org/10.3390/ijerph19159139

Gifford, J. R., Garten, R. S., Nelson, A. D., Trinity, J. D., Layec, G., Witman, M. A., Weavil, J. C., Mangum, T., Hart, C., Etheredge, C., Jessop, J., Bledsoe, A., Morgan, D. E., Wray, D. W., Rossman, M. J., & Richardson, R. S. (2016). Symmorphosis and skeletal muscle V̇O2 max : in vivo and in vitro measures reveal differing constraints in the exercise-trained and untrained human. *The Journal of Physiology*, *594*(6), 1741–1751. https://doi.org/10.1113/JP271229

Gordon, N., Abbiss, C. R., Ihsan, M., Maiorana, A. J., & Peiffer, J. J. (2018). Active and Inactive Leg Hemodynamics during Sequential Single-Leg Interval Cycling. *Medicine and Science in Sports and Exercise* **50(6),** 1297–1304. https://doi.org/10.1249/MSS.0000000000001553

Gordon, N., Abbiss, C. R., Maiorana, A. J., & Peiffer, J. J. (2020). Single-leg cycling increases limb-specific blood flow without concurrent increases in normalised power output when compared with double-leg cycling in healthy middle-aged adults. *European Journal of Sport Science* **20(2),** 202–210. https://doi.org/10.1080/17461391.2019.1617789

Hamaoka, T., & McCully, K. K. (2019). Review of early development of near-infrared spectroscopy and recent advancement of studies on muscle oxygenation and oxidative metabolism. *The Journal of Physiological Sciences* **69(6),** 799–811. https://doi.org/10.1007/s12576-019-00697-2

Hearon, C. M., Jr, & Dinenno, F. A. (2016). Regulation of skeletal muscle blood flow during exercise in ageing humans. *The Journal of Physiology*, *594*(8), 2261–2273. https://doi.org/10.1113/JP270593

Iannetta, D., Passfield, L., Qahtani, A., MacInnis, M. J., & Murias, J. M. (2019). Interlimb differences in parameters of aerobic function and local profiles of deoxygenation during double-leg and counterweighted single-leg cycling. *American journal of physiology. Regulatory, Integrative and Comparative Physiology* **317(6),** R840–R851. https://doi.org/10.1152/ajpregu.00164.2019

Klausen, K., Secher, N. H., Clausen, J. P., Hartling, O., & Trap-Jensen, J. (1982). Central and regional circulatory adaptations to one-leg training. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, ***52*(4),** 976–983. https://doi.org/10.1152/jappl.1982.52.4.976

LeJemtel, T. H., Maskin, C. S., Lucido, D., & Chadwick, B. J. (1986). Failure to augment maximal limb blood flow in response to one-leg versus two-leg exercise in patients with severe heart failure. *Circulation* **74(2),** 245–251. https://doi.org/10.1161/01.cir.74.2.245

Magnusson, G., Kaijser, L., Isberg, B., & Saltin, B. (1994). Cardiovascular responses during one- and two-legged exercise in middle-aged men. *Acta Physiologica Scandinavica* **150(4),** 353–362. https://doi.org/10.1111/j.1748-1716.1994.tb09699.x

Malone, J. J., Hulton, A. T., & MacLaren, D. P. M. (2021). Exogenous carbohydrate and regulation of muscle carbohydrate utilisation during exercise. *European Journal of Applied Physiology* **121(5),** 1255–1269. https://doi.org/10.1007/s00421-021-04609-4

Montero D. (2015). Arterial dilator function in athletes: present and future perspectives. *Frontiers in Physiology* **6,** 163. https://doi.org/10.3389/fphys.2015.00163

Mortensen, S. P., Nyberg, M., Winding, K., & Saltin, B. (2012). Lifelong physical activity preserves functional sympatholysis and purinergic signalling in the ageing human leg. *The Journal of Physiology*, *590*(23), 6227–6236. https://doi.org/10.1113/jphysiol.2012.240093

Poole, J. G., Lawrenson, L., Kim, J., Brown, C., & Richardson, R. S. (2003). Vascular and metabolic response to cycle exercise in sedentary humans: effect of age. *American journal of physiology. Heart and Circulatory Physiology* **284(4),** H1251–H1259. https://doi.org/10.1152/ajpheart.00790.2002

Proctor, D. N., Koch, D. W., Newcomer, S. C., Le, K. U., Smithmyer, S. L., & Leuenberger, U. A. (2004). Leg blood flow and VO2 during peak cycle exercise in younger and older women. *Medicine and Science in Sports and Exercise* **36(4),** 623–631. https://doi.org/10.1249/01.mss.0000121951.10417.b5

Proctor, D. N., Shen, P. H., Dietz, N. M., Eickhoff, T. J., Lawler, L. A., Ebersold, E. J., Loeffler, D. L., & Joyner, M. J. (1998). Reduced leg blood flow during dynamic exercise in older endurance-trained men. *Journal of Applied Physiology* **85(1),** 68–75. https://doi.org/10.1152/jappl.1998.85.1.68

Richardson, R. S., Grassi, B., Gavin, T. P., Haseler, L. J., Tagore, K., Roca, J., & Wagner, P. D. (1999). Evidence of O2 supply-dependent VO2 max in the exercise-trained human quadriceps. *Journal of Applied Physiology* **86(3),** 1048–1053. https://doi.org/10.1152/jappl.1999.86.3.1048

Richardson, R. S., Poole, D. C., Knight, D. R., Kurdak, S. S., Hogan, M. C., Grassi, B., Johnson, E. C., Kendrick, K. F., Erickson, B. K., & Wagner, P. D. (1993). High muscle blood flow in man: is maximal O2 extraction compromised?. *Journal of Applied Physiology* 75(4), 1911–1916. https://doi.org/10.1152/jappl.1993.75.4.1911

Saltin, B., & Calbet, J. A. (2006). Point: in health and in a normoxic environment, VO2 max is limited primarily by cardiac output and locomotor muscle blood flow. *Journal of Applied Physiology* **100(2),** 744–745. https://doi.org/10.1152/japplphysiol.01395.2005

Skattebo, Ø., Calbet, J. A. L., Rud, B., Capelli, C., & Hallén, J. (2020). Contribution of oxygen extraction fraction to maximal oxygen uptake in healthy young men. *Acta Physiologica* **230(2),** e13486. https://doi.org/10.1111/apha.13486

Skattebo, Ø., Capelli, C., Rud, B., Auensen, M., Calbet, J. A. L., & Hallén, J. (2020). Increased oxygen extraction and mitochondrial protein expression after small muscle mass endurance training. *Scandinavian journal of Medicine & Science in Sports* **30(9),** 1615–1631. https://doi.org/10.1111/sms.13707

Volianitis, S., & Secher, N. H. (2002). Arm blood flow and metabolism during arm and combined arm and leg exercise in humans. *The Journal of Physiology*, **544(3),** 977–984. https://doi.org/10.1113/jphysiol.2002.023556

Woodman, R. J., Playford, D. A., Watts, G. F., Cheetham, C., Reed, C., Taylor, R. R., Puddey, I. B., Beilin, L. J., Burke, V., Mori, T. A., & Green, D. (2001). Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *Journal of Applied Physiology* **91(2),** 929–937. https://doi.org/10.1152/jappl.2001.91.2.929

Key points

- A transition from large to small muscle mass exercise (i.e., single-leg compared with double-leg cycling) allowed for a greater peripheral hyperaemic response in the locomotor muscles.
- The ability to increase femoral blood flow during single-leg compared with double-leg cycling may partly explain the improvement in power output per leg observed in both, younger and older athletes.
- Femoral blood flow in response to maximal exercise was lower in older compared with younger endurance-trained individuals regardless of the amount of muscle mass engaged.

AUTHOR BIOGRAPHY

Toni HADDAD

Employment

PhD Candidate at Edith Cowan University (Western Australia) and Université Côte d'Azur (France); Sports Physiologist at the South Australian Sports Institute.

Degree

MSc

Research interests

Applied human physiology and exercise performance, with a focus on the effects of ageing and training.

E-mail: t.haddad@ecu.edu.au

Angela L. SPENCE

Employment

Senior Lecturer in Exercise and Sport Science at Curtin University, Western Australia

Degree

PhD

Research interests

The integrative physiological response to different types of exercise in humans.

E-mail: angela.spence@curtin.edu.au

Jeremiah PEIFFER
Employment
Professor of Exercise Physiology at Murdoch University, Western Australia.
Degree
PhD
Research interests
Exploring the role of exercise, specifically what impact the method of delivery has in enhancing physical and cognitive performance within healthy individuals and those with chronic diseases.
E-mail:
jeremiah.peiffer@murdoch.edu.au

Gregory M. BLAIN
Employment
Professor of Exercise Physiology at Université Côte d'Azur, France
Degree
PhD
Research interests
The interaction between neuro-muscular fatigue, central command, cardio-respiratory response, and performance during exercise (integrated physiology).
E-mail:
Gregory.blain@univ-cotedazur.fr

Jeanick BRISSWALTER
Employment
Professor of Exercise Physiology at Université Côte d'Azur, France
Degree
PhD
Research interests
Energetics of human locomotion, neuromuscular fatigue, and interaction between physiological demand and cognition.
E-mail: Jeanick.brisswalter@univ-cotedazur.fr

Chris R. ABBISS
Employment
Professor of Exercise Physiology at Edith Cowan University, Western Australia
Degree
PhD
Research interests
Applied human physiology and exercise performance, with a focus on cycling, fatigue, thermoregulation, pacing strategies, training modalities and recovery.
E-mail: c.abbiss@ecu.edu.au

✉ **Toni Haddad**
Centre for Human Performance, School of Medical and Health Sciences Edith Cowan University, 270 Joondalup Drive, Joondalup, WA, Australia 6027