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Increased mass-specific maximal fat oxidation rate with small vs large muscle mass exercise

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Abstract

Introduction: Skeletal muscle perfusion and oxygen (O_2) delivery are restricted during whole-body exercise due to a limited cardiac output ($\dot{Q}$). This study investigated the role of reducing central limitations to exercise on the maximal fat oxidation rate (MFO) by comparing mass-specific MFO (per kg active lean mass) during one-legged (1L) and two-legged (2L) cycling. We hypothesized that the mass-specific MFO would be higher during 1L- than 2L cycling.

Methods: Twelve male subjects ($\dot{V}O_{2peak}$: 59.3±8.4 mL·kg^{-1}·min^{-1}; mean±SD) performed step-incremental 2L- (30-80% of $\dot{V}O_{2peak}$) and 1L (50% of 2L power output, i.e., equal power output per leg) cycling (counterbalanced) while steady-state pulmonary gas exchanges, $\dot{Q}$ (pulse-contour analysis), and skeletal muscle (vastus lateralis) oxygenation (near-infrared spectroscopy) were determined. MFO and the associated power output (Fat_{Max}) were calculated from pulmonary gas exchanges and stoichiometric equations. A counterweight (10.9kg) was added to the contralateral pedal arm during 1L cycling. Leg lean mass was determined by DEXA.

Results: The absolute MFO was 24% lower (0.31±0.12 vs 0.44±0.20 g·min^{-1}; P=0.018) while mass-specific MFO was 52% higher (28±11 vs 20±10 mg·min^{-1}·kg^{-1}; P=0.009) during 1L- than 2L cycling. Fat_{Max} was similar expressed as power output per leg (60±28 vs 58±22 W; P=0.649). $\dot{Q}$ increased more from rest to exercise during 1L- than 2L cycling when expressed per active leg (ANOVA main effect: P=0.003). The tissue oxygenation index and Δ[deoxy(Hb+Mb)] were not different between exercise modes (ANOVA main effects: P≥0.587), indicating similar skeletal muscle fractional O_2 extraction.

Conclusion: Mass-specific MFO is increased by exercising a small muscle mass, potentially explained by increased perfusion and more favorable conditions for O_2 delivery than during whole-body exercise.

Keywords: Fatmax, maximal oxygen uptake, metabolism, one-legged cycling, single-leg, substrate

Alphabetic list of abbreviations

CHO: carbohydrate
Δ[deoxy(Hb+Mb)]: concentration change of deoxygenated hemoglobin and myoglobin
Fat_{Max}: the exercise intensity at the maximal fat oxidation rate
HR: heart rate
HR_{max}: maximal heart rate
HR_{peak}: peak heart rate
MFO: maximal fat oxidation
$\dot{Q}$: cardiac output
$\dot{Q}_{max}$: maximal cardiac output
RER: respiratory exchange ratio
SD: standard deviation
SEM: standard error of the mean
SV: stroke volume
TOI: tissue oxygenation index
VE_{peak}: peak ventilation
$\dot{V}O_2$: oxygen uptake
$\dot{V}O_{2max}$: maximal oxygen uptake
$\dot{V}O_{2peak}$: peak oxygen uptake
$\dot{W}_{peak}$: peak power output

Introduction

During aerobic exercise, carbohydrate (CHO) and fat are the primary energy sources fueling oxidative metabolism, and their relative contribution is influenced by several factors such as training status (1), nutrition (2), muscle glycogen content (3), sex (4), type of exercise (5), and exercise duration and -intensity (6). With increasing exercise intensity, the absolute CHO oxidation ($g \cdot min^{-1}$) increases gradually up to the maximal oxygen uptake ($\dot{V}O_{2max}$), whereas the maximal fat oxidation rate (MFO) is reached at low-moderate exercise intensities and undergoes a marked reduction at higher exercise intensities (6). The exercise intensity at MFO is defined as Fat_{Max} (7) and is elicited from ~30% of $\dot{V}O_{2max}$ in untrained individuals to ~70% of $\dot{V}O_{2max}$ in endurance-trained athletes (1, 7, 8). Fuel shifts are controlled by a complex interplay between the intracellular and extracellular metabolic environments (9), is influenced by hormonal control (3) and may occur solely by changing the availability of substrates without any change in metabolic rate. For instance, by increasing plasma glucose (CHO ingestion) or plasma free fatty acids (FFA; lipid-heparin infusion) concentrations during exercise, a reduced and increased fractional reliance on fat are detected, respectively (10, 11). In connection, it is shown that the MFO can be increased with both prior fasting and prior exercise (12), meaning that MFO is not a constant and can be manipulated by altering skeletal muscle endogenous and exogenous environments.

During whole-body exercise, a limited maximal cardiac output ($\dot{Q}_{max}$) restrain skeletal muscle perfusion and, thus, O_2 and substrate deliveries (13-16). By reducing the competition for blood flow by exercising a smaller muscle mass, such as during one-legged (1L) cycling (~10 kg active muscle mass) or dynamic 1L knee-extensions (~2.5 kg active muscle mass), larger mass-specific (i.e., per kg active muscle mass) blood flows are achieved when compared to conventional two-legged (2L) cycling (~20 kg active muscle mass) (14, 16-22). Consequently, 1L cycling elicits peak O_2 uptake ($\dot{V}O_{2peak}$) and peak power output ($\dot{W}_{peak}$) values that are ~75-85% and 55-60% of the maximal values achieved during 2L cycling, respectively (19, 23, 24), despite only recruiting half of the leg muscle mass. This clearly illustrates large muscle performance-related benefits from reducing the competition for blood flow, but whether this strategy also affects fuel selection during exercise is not elucidated. Fat is the least O_2 efficient fuel producing only ~4.6 mole ATP per mole O_2 consumed compared to ~5 mole for CHO (25), which may contribute to the lower fractional reliance on fat under O_2 delivery-limited conditions such as high exercise intensities (9). Therefore, it is conceivable that the higher mass-specific O_2 delivery during small muscle mass exercise may induce a higher reliance on fat oxidation. Indeed, Helge et al. (26) found sustained fat oxidation rates at 65% and 85% of $\dot{W}_{peak}$ when compared to 25% of $\dot{W}_{peak}$ during 2L knee-extension (5-6 kg active muscle mass), which contrast finding from whole-body exercise where fat oxidation rates are markedly reduced at such high exercise intensities. Moreover, MacInnis et al. (24) found a significantly larger fraction of the total energy coming from fat during 1L- than 2L cycling at 50% of $\dot{W}_{peak}$. Fat oxidation rates need to be established over a wide range of exercise intensities (30-80% of $\dot{V}O_{2max}$) to establish the curves needed to identify MFO and Fat_{Max} precicely on an individual basis (7, 27). Since the studies presented above only assessed one submaximal power output (24) or only assessed small muscle mass exercise without measurements on those intensities commonly associated with MFO (40-60% of $\dot{V}O_{2peak}$) (26), no study has investigated whether MFO differs between small and large muscle mass exercises within the same subjects.

The primary objective of this study was to compare MFO, mass-specific MFO (i.e., per kg active lean mass), and Fat_{Max} calculated from a wide range of power outputs during 2L cycling (30-80% of $\dot{V}O_{2max}$) and 1L cycling (50% of the 2L power output, i.e., identical power output per leg). We hypothesized that 1) the mass-specific MFO would be higher during 1L-

than 2L cycling attributed to lower competition for skeletal muscle blood flow, and 2) that Fat_{Max}, defined as power output per leg, would be highest during 1L cycling.

Methods

Ethical approval

This study was approved by the Ethics Committee of the Norwegian School of Sport Sciences (ref. nr.: 40-191217) and reported to The Norwegian Centre for Research Data (ref. nr.: 858276). Oral and written informed consents were obtained from all subjects before the start of this investigation, which was carried out in accordance with the Declaration of Helsinki.

Subjects

Twelve moderately-trained men were recruited and completed all tests (mean ± standard deviation; age: 27.6 ± 4.1 years; weight: 77.8 ± 6.5 kg; height: 1.84 ± 0.05 m; $\dot{V}O_{2max}$: 59.3 ± 8.4 mL · kg^{-1} · min^{-1}; lean body mass: 62.8 ± 4.9 kg). Only men were recruited since many studies find an impact of the menstrual cycle on the metabolism in women, with increased relative fat oxidation in the presence of high estrogen and low progesterone levels (around ovulation) (28). All subjects were non-smokers and reported no contraindications to maximal exercise testing.

Experimental design

The subjects visited the laboratory on four occasions over two weeks: for familiarization and initial testing during 2L cycling (day 1) and 1L cycling (day 2). On day 3 and 4, the subjects arrived at the laboratory in the morning after overnight fasting and carried out graded exercise tests for the determination of MFO, Fat_{Max} and $\dot{V}O_{2max\ /\ peak}$ during 2L or 1L cycling (counterbalanced order of exercise test days). The body composition was assessed by dual-energy X-ray absorptiometry (DEXA; Lunar iDXA, enCORE software version 17; GE Healthcare, Chicago, IL, USA) on day 3 or 4 (fasted, before the exercise test). The subjects were instructed to eat identical meals the day before test days 3 and 4, and abstain from exercise and caffeine consumption during the last 24 h and 12 h before lab visits, respectively. Leg lean mass was defined as the lean mass distally to collum femoris. Specifically, this segment was constructed by creating a midline between the two legs and a triangle around the pelvis. In the triangle, the horizontal line transversed just above the two iliac crests, and the two angled lines crossed the two collum femoris at 90 degrees and met below the genital area.

Measurements and procedures

Initial testing (day 1 and 2)

On day 1, the subjects carried out a graded exercise test during 2L cycling (Excalibur Sport; Lode B.V., Groningen, The Netherlands) commencing at 50-150 W with increases of 25 W every 5th minute until exceeding a blood lactate concentration ([La]) of 4 mmol · L^{-1} (Biosen C-line; EKF Diagnostic, Cardiff, UK). The cadence was set to 80 revolutions per minute (RPM) for all submaximal power outputs (all test days). The subjects then cycled at 50 W for 5 min, followed by 5 min rest. A second graded exercise test was carried out starting 25 W below the power output producing a blood lactate concentration of 4 mmol · L^{-1} with increases of 25 W every minute until exhaustion for determination of $\dot{W}_{peak}$ and $\dot{V}O_{2max}$. Exhaustion was defined as the point where the subject was unable to keep the cadence above 60 RPM. Lastly, the subjects were familiarized with 1L cycling for 15 min after placing a counterweight on the contralateral pedal arm (10.9 kg; Toppfysik AB, Grycksbo, Sweeden) on the same ergometer. The counterweight rotates from top to bottom and assists the active

limb in the upward phase, thus minimizing the need to recruit the ipsilateral hip flexor muscles more than during 2L cycling. The passive leg rested on a chair beside the ergometer, and the hip was flexed at approximately 90°. On day 2, an identical protocol was carried out during 1L cycling (the use of the right or the left leg was counterbalanced among the subjects), except that the submaximal power outputs were half of those carried out with 2L cycling, and the graded exercise test to exhaustion had steps of 13 W · min^{-1}. During the exercise tests, respiratory variables were measured by open-circuit indirect calorimetry with a mixing chamber (Oxycon Pro; Jaeger Instrument, Friedberg, Germany), as validated by Foss and Hallén (29). These two tests were used for familiarization and to establish the linear relationship between power output and $\dot{V}O_2$ that was used, together of $\dot{V}O_{2max}$, to calculate individual relative power outputs for test days 3-4 (explained below). No data from test days 1 and 2 will be presented in the results.

Maximal fat oxidation trials (day 3 and 4)

The graded exercise test to assess MFO and Fat_{Max} during 2L cycling began with a 3-min resting measurement while seated on the cycle ergometer, followed by seven 3-min power outputs corresponding to 25%, 30%, 40%, 50%, 60%, 70%, and 80% of $\dot{V}O_{2max}$ (calculated from the linear relationship between power output and $\dot{V}O_2$ on test day 1), where respiratory variables were continuously measured. This test was followed by 5 min cycling at the power output corresponding to 30% of $\dot{V}O_{2max}$ and 5 min rest. Lastly, an identical graded exercise test as day 1 was carried out with increases of 25 W (13W for 1L cycling) every minute until exhaustion for determination of $\dot{W}_{peak}$ and $\dot{V}O_{2max}$, except that the first power output was adjusted (± 0-50 W) to induce a time to exhaustion of ~8 min on the test. The first power output was adjusted from the familiarization tests (day 1 and 2) to meet this demand: if the subject completed the incremental test to exhaustion with a shorter/longer duration than 8 min, the first power output was adjusted accordingly (30). This caused a similar time to exhaustion of 8:38±1:03 and 8:31±1:12 (min:seconds) for 2L- and 1L cycling, respectively (P = 0.826). Peak blood lactate concentration was measured 1 min after exhaustion, followed by 5 min warm-down at the power output corresponding to 30% of $\dot{V}O_{2max}$. During 1L cycling, the submaximal power outputs were half of those carried out with 2L cycling, with the protocol otherwise identical. The mean power output during the last 60 s was defined as $\dot{W}_{peak}$. The highest average 30-s $\dot{V}O_2$ was taken as $\dot{V}O_{2max}$ (2L cycling) or $\dot{V}O_{2peak}$ (1L cycling). The coefficient of variation (the standard deviation of the difference scores/$\sqrt{2}$) of duplicate VO_{2max} (2L) and VO_{2peak} (1L) determinations in the present study (days 1 & 2 vs. days 3 and 4) were 2.3% and 3.4%, respectively. The heart rate (HR) was measured continuously (Polar RS800; Polar Electro Oy, Kempele, Finland), and the highest 10-s average was defined as HR_{max} (2L cycling) and HR_{peak} (1L cycling). Substrate oxidation was calculated from $\dot{V}O_2$ and CO_2 production ($\dot{V}CO_2$) measurements (60 s averages at the end of each power output), using standard stoichiometric equations, neglecting protein oxidation (31):

$$\text{Fat oxidation } (g \cdot min^{-1}) = (1.67 \cdot \dot{V}O_2) - (1.67 \cdot \dot{V}CO_2)$$

The relationship between fat oxidation and power output was fitted individually for each subject (3rd order polynomial) using all submaximal power outputs where the respiratory exchange ratio (RER) < 1.0, except the first power output (25% of $\dot{V}O_{2max}$) that was carried out for warm-up. MFO and Fat_{Max} were defined as the highest fat oxidation rate (the peak of the curve) and the power output (W) and relative intensity (% of $\dot{V}O_{2max}$) where MFO occurred, respectively (7).

Oxygenation changes in the vastus lateralis muscle were evaluated by continuous wavelength near-infrared spectroscopy (NIRS; PortaMon; Artinis Medical Systems, Elst, The Netherlands). The oxygenation parameters were obtained using three light-emitting diodes (wavelengths: 760 and 850 nm) spaced 30, 35, and 40 mm from the detector. Concentration changes of oxygenated Hb+Mb, Δ[oxy(Hb+Mb)], and deoxygenated Hb+Mb, Δ[deoxy(Hb+Mb)], were calculated from an initial value arbitrary set equal to zero. A "physiological calibration" was applied; one minute after the termination of exercise, an 8.5 cm-wide cuff (Zimmer Biomet, Warsaw, IN, USA) was inflated (VBM Medizintechnik, Sulz am Neckar, Germany) to 300 mmHg to occlude the arterial blood flow for 6 min (prolonged up to 8 min if necessary) until the Δ[deoxy(Hb+Mb)] increase reached a plateau. After cuff-release, the minimum Δ[deoxy(Hb+Mb)] was recorded during hyperemia, and the range during ischemia was used to standardize the Δ[deoxy(Hb+Mb)] obtained during exercise. The tissue oxygenation index (TOI) was calculated as the ratio of oxy(Hb+Mb) to total tissue Hb+Mb. TOI and Δ[deoxy(Hb+Mb)] served as substitute measures of skeletal muscle fractional O_2 extraction, an important parameter expressing the relative balance between O_2 delivery and O_2 consumption in skeletal muscle (32-34). The subjects had a skin- and subcutaneous tissue thickness of 5.1 ± 1.7 mm (range: 2.7-9.0 mm) below the optode, assessed using an ultrasound device (Vivid E95; GE Vingmed Ultrasound AS, Horten, Norway). During the submaximal power outputs (i.e., not during the maximal tests), arterial pressure was measured with the volume-clamp method (35) using a finger-cuff at the third middle phalanx of the (pre-warmed) left hand connected to a hemodynamic monitor (Finometer Pro; Finapres Medical Systems, Enschede, The Netherlands). To stabilize body position and to minimize movement in the upper torso and arms, the handlebars of the cycle ergometer were placed high, and on it, connected armrest/aero bars. The forearms rested passive, and the left hand was fully immobilized to minimize movement-related pressure artifacts. The subjects were told to minimize the use of the right arm, the inactive leg (1L cycling), and the torso during submaximal exercise to avoid that these tissues contributed significantly to the pulmonary $\dot{V}O_2$. The finger artery pressure waveform was used to reconstruct the brachial artery pressure waveform after an upper-arm cuff calibration (36) conducted at rest. A heart-reference sensor was included to report values at the level of the heart. Stroke volume (SV) was derived from the Modelflow pulse-contour analysis method incorporated in the Finometer Pro (37), allowing for the calculation of $\dot{Q}$. The Modelflow pulse-contour method estimates the aortic flow (SV) from arterial pressure waveforms by applying a three-element model using aortic characteristic impedance, arterial compliance, and total peripheral resistance (37). The aortic characteristic impedance and arterial compliance are derived from age- and sex-dependent arctangent aortic pressure-area relationships (37). As the aortic area may differ substantially between individuals of the same age and sex, a bias in the derived SV may occur if not calibrated with a reference method. Since no calibration against gold-standard methods (e.g., thermodilution, the direct Fick method) was carried out, SV and $\dot{Q}$ were expressed as percent changes from the control situation (rest, seated on the ergometer), which offers sufficient precision for tracking relative changes (35). Irrespectively, the method agrees well with reference methods such as thermodilution (37) and Doppler echocardiography (38).

Statistical analyses

Data in text and tables are presented as mean ± standard deviation (SD), and in graphs as mean ± standard error of the mean (SEM). Maximal responses during incremental exercise, MFO, and Fat_{Max} were compared between 2L- and 1L cycling using a paired sample t-test and calculating Cohen's *d* effect size (ES) by dividing their difference score by their pooled SD. The submaximal data were analyzed using a two-way repeated measures ANOVA

(exercise mode - power output per leg). When a significant main effect or interaction was observed, pairwise comparisons were carried out using the Fisher's least significant difference post hoc test when appropriate. Regression between power output and physiological responses was analyzed using simple linear regression ($\dot{V}O_2$, RER, RPE, HR, $\dot{Q}$, MAP), second-order- (ventilatory equivalent for O_2, SV, total peripheral resistance) and third-order (fat oxidation, lactate concentration) polynomials, and exponential growth models ($y = Y0 \cdot e^{K \cdot x}$) (ventilation), all using least squares as the fitting method. The alpha-level was set to ≤ 0.05, and values between > 0.05 and ≤ 0.10 were considered to indicate trends. GraphPad Prism 9 (v.9.0.0; GraphPad Software, CA, USA) and Microsoft Office Excel 2016 (Microsoft Corporation, WA, USA) were used for statistical analysis.

Results

Maximal exercise

The subjects reached a significantly lower $\dot{W}_{peak}$ ($P < 0.001$; ratio: 0.56 ± 0.05), $\dot{V}O_{2peak}$ ($P < 0.001$; ratio: 0.80 ± 0.08), peak ventilation ($P = 0.008$; ratio: 0.88 ± 0.12) and HR_{peak} ($P < 0.001$; ratio: 0.94 ± 0.02) during 1L- than 2L cycling (Table 1). There were strong correlations between exercise modes for $\dot{W}_{peak}$ ($r = 0.89$; $P < 0.001$) and $\dot{V}O_{2peak}$ ($r = 0.84$; $P < 0.001$).

<<Table 1 here>>

Fat oxidation

MFO was 24 ± 22% lower during 1L- than 2L cycling when expressed as $g \cdot min^{-1}$ ($P = 0.018$; Fig. 1A and 1D). In contrast, when related to the active muscle mass (1L lean mass: 11.1 ± 1.1 kg; 2L lean mass: 22.2 ± 2.3 kg), the mass-specific MFO was 52 ± 44% higher during 1L- than 2L cycling ($P = 0.009$; Fig. 1B, 1C and 1E). Fat_{Max} occurred at 36 ± 9% of $\dot{V}O_{2max}$ during 2L cycling and at 29 ± 11% of $\dot{V}O_{2peak}$ during 1L cycling (difference, $P = 0.032$). Fat_{max} was also larger during 2L cycling when expressed as whole-body power output (117 ± 44 W vs. 60 ± 28 W; $P < 0.001$), but similar when expressed as power output per leg ($P = 0.649$; Fig. 1F).

<<Figure 1 here>>

Pulmonary gas exchanges and metabolic responses

The submaximal $\dot{V}O_2$ rose by 11.9 $mL \cdot min^{-1} \cdot W^{-1}$ during 1L cycling, which tended to be a larger slope than during 2L cycling (10.1 $mL \cdot min^{-1} \cdot W^{-1}$; $P = 0.058$; Fig. 2A). The pulmonary ventilation, ventilatory equivalent for O_2 ($V_E / \dot{V}O_2$), RER, blood lactate concentration, and rating of perceived exertion (RPE) increased more with power output during 1L- than 2L cycling (Fig. 2 B-F). However, when expressed relative to power output per leg, no differences were found between exercise modes for RPE (ANOVA main effect of exercise mode: $P = 0.670$; ANOVA interaction effect: $P = 0.975$) and RER (ANOVA main effect of exercise mode: $P = 0.234$; ANOVA interaction effect: $P = 0.398$). The blood lactate concentration was similar at low power outputs per leg, but increased steeper during 2L cycling (ANOVA interaction effect: $P = 0.002$; ANOVA main effect of exercise mode: $P = 0.063$), leading to a significant difference at the two highest power outputs per leg (post-hoc test, $P \leq 0.050$). The ventilatory equivalent for O_2 was higher for 1L- than 2L cycling when expressed relative to power output per leg (ANOVA main effect of exercise mode: $P = 0.047$; ANOVA interaction effect: $P = 0.093$).

<<Figure 2 here>>

Cardiovascular responses

The HR increased more with power output during 1L- than 2L cycling (difference slope: P < 0.001; Fig. 3A), whereas ΔSV (the percent change from rest) showed an opposite pattern (Fig. 3B). Therefore, $\Delta\dot{Q}$ (the percent change from rest) had a similar slope with power output for the two exercise modes (P = 0.461; Fig. 3C). However, $\Delta\dot{Q}$ increased more during 1L- than 2L cycling when expressed per active leg (ANOVA main effect of exercise mode: P = 0.003; Fig. 3D). Absolute values of $\dot{Q}$ ($L \cdot min^{-1}$) is presented in Supplemental Fig. 1. MAP increased steeper with power output during 1L- than 2L cycling (difference slope: P = 0.002; Fig. 3E), but this difference disappeared when expressed relative to power output per leg (ANOVA main effect of exercise mode: P = 0.942; ANOVA interaction effect: P = 0.122). Δ[deoxy(Hb+Mb)] and TOI did not differ between exercise modes when expressed relative to power output per leg (Fig. 4A-B).

<<Figure 3 and 4 here>>

Discussion

The present investigation provides novel insight into the role of active muscle mass on fat oxidation during exercise. We show that the mass-specific MFO is enhanced when exercising a small (1L cycling) compared to a large (2L cycling) muscle mass. $\Delta\dot{Q}$ per active leg was larger during 1L- than 2L cycling, which supports that the difference in mass-specific MFO may be attributed to enhanced skeletal muscle perfusion and O_2 delivery.

Maximal responses

$\dot{W}_{peak}$ and $\dot{V}O_{2peak}$ during 1L cycling were 56% and 80% of the values obtained during 2L cycling, respectively, in agreement with previous research (19, 23, 24). The enhanced performance and $\dot{V}O_{2peak}$ standardized per active leg indicate a larger leg blood flow at maximal exercise during 1L- than 2L cycling (19), which is further supported by a similar skeletal muscle fractional O_2 extraction (Δ[deoxy(Hb+Mb)] and TOI) at exhaustion that cannot explain the higher mass-specific $\dot{V}O_2$.

Maximal fat oxidation

Our values of MFO during 2L cycling (0.44 $g \cdot min^{-1}$) agree with previous studies assessing moderately trained men (27, 39). A smaller MFO was found during 1L- than 2L cycling, which is not surprising given the involvement of half of the leg muscle mass. However, MFO was reduced by only 24% during 1L cycling (to 0.31 $g \cdot min^{-1}$), and when standardized to the lean mass of the active leg(s), the mass-specific MFO was higher in 11 out of 12 subjects and significantly higher on average during 1L cycling. This is supported by the findings of MacInnis et al. (24) and Helge et al. (26) and strengthens our hypothesis of enhanced mass-specific MFO when exercising a small muscle mass. Therefore, altered metabolism during small muscle mass exercise must be considered when findings from such models (1L knee extension, 1L cycling, arm cranking etc.) are transferred to whole-body exercise, both in research- and applied settings.

Fat oxidation is altered by manipulating plasma- (10, 11) and intramuscular- (2, 3) fat and CHO availability during exercise. The present study aimed to manipulate skeletal muscle perfusion and O_2 delivery and test its impact on MFO. Although not directly measured in the present study, earlier work indicates increased leg blood flow during 1L- than 2L cycling (18, 19, 40), which is further supported by others' and our findings of a larger increase in $\dot{Q}$ from rest during submaximal 1L cycling when standardized to the active muscle mass (20). Therefore, at a given power output per leg, increased O_2 delivery and higher partial pressure of O_2 within the skeletal muscles (17) may have facilitated a higher reliance on aerobic

metabolism and fewer glycogenolysis-stimulating disturbances in the intracellular environment, in favor of β-oxidation flux (26, 41). It is previously shown that acute hypoxia decreases fat oxidation when measured on the same absolute power output as in normoxia, likely due to reduced O_2 delivery, increased relative exercise intensity, and increased plasma catecholamines (42). Therefore, due to the higher $\dot{W}_{peak}$ and $\dot{V}O_{2peak}$ standardized per active leg during 1L than 2L cycling, the submaximal power outputs represented lower relative exercise intensities that, together with a possible increased mass-specific O_2 delivery, may have caused the increased mass-specific MFO. Although the capillary blood lactate concentration was similar between 1L and 2L cycling at Fat_{Max} (0.8 ± 0.3 vs. 0.9 ± 0.5 mmol $\cdot$ L^{-1}, respectively), we cannot exclude local intracellular perturbation in the exercising legs disfavoring fat oxidation during 2L cycling.

The plasma catecholamine concentration increases gradually with the amount of recruited muscle mass (43, 44) and exercise intensity (45), caused by a larger sympathetic nervous system activation. Lower concentrations of plasma catecholamines during 1L than 2L cycling may have reduced the glycolytic activation, reduced the acetyl CoA production from glucose, and caused a reduced buffering of acetyl CoA by carnitine to form acylcarnitine. Consequently, this may have caused a smaller reduction in free carnitine availability, increased palmitoyltransferase 1 (CPT1) activity, and increased long-chain fatty acid transport into the mitochondria (46) during 1L than 2L cycling. Although a higher plasma catecholamine concentration during 2L than 1L cycling may have stimulated adipose tissue lipolysis and led to a larger FFA release during prolonged exercise, the protocol in the present study was designed short not to induce any increase in the plasma FFA concentration (6, 47). Therefore, the leg blood flow likely decided the FFA delivery and may, therefore, even have been higher during 1L- than 2L cycling. Moreover, it is shown, by graded epinephrine infusion, that FFA re-esterification is elevated, and the fatty acid oxidation in muscle is reduced at moderate and high infusion rates, caused by catecholamine-stimulated glycogenolysis that inhibits β-oxidation (45, 48). Therefore, a likely lower catecholamine concentration during 1L- than 2L cycling (43) may have favored a larger uptake and utilization of plasma FFA, reduced glycogenolysis, and ultimately increased β-oxidation flux and contributed to the larger mass-specific MFO during 1L cycling. Unfortunately, we did not obtain venous blood samples to measure FFA- and catecholamine concentrations, which could have strengthened this series of arguments.

Contrary to our second hypothesis, MFO occurred at a similar power output per leg (i.e., Fat_{Max} was the same), and the shape of the fat oxidation curves during 1L- and 2L cycling were similar (Fig. 1C). This was surprising since Helge et al. found almost identical thigh fat oxidation rates at 25%, 65%, and 85% of $\dot{W}_{peak}$ during 2L knee-extension (5-6 kg active muscle mass) when measured over the exercising legs using catheters (26). If transferred to 1L cycling, which also engages a small muscle mass, it could have caused a sustained fat oxidation rate at higher exercise intensities, and a flatter curve than during 2L cycling, potentially with the peak shifted to the right (i.e., a higher Fat_{Max} defined as power output per leg). The different findings from Helge et al. (26) may have been caused by choice of an exercise model engaging more muscle mass in the present study (11 kg vs. 5-6 kg), which likely induced a lower mass-specific O_2 delivery and a higher concentration of circulating catecholamines (43). Another potential reason may be differences between isolated measurements for the thighs using catheters and indirect whole-body measurements via pulmonary gas exchanges (49).

Methodological considerations

A limitation to our experimental design is that we obtained systemic measurements of gas exchanges, hemodynamics, and fat oxidation and calculated the mass-specific responses

instead of measuring in isolation for the leg(s) using catheterization techniques. It is some uncertainty in the magnitude of the active muscle mass, despite using DEXA, and to what extent the energy expenditure of non-exercising muscles and other metabolically active tissues contributed to the differences in the physiological responses to exercise between 1L- and 2L cycling (MFO, $\dot{V}O_2$, ventilation, $\dot{Q}$, etc.). However, after calculating the "net" fat oxidation, by subtracting the fat oxidation measured at rest from the fat oxidation during exercise, the calculated net mass-specific MFO was still significantly higher for 1L- than 2L cycling (25±11 vs. 19±9 mg · min^{-1} · kg^{-1}, respectively; P=0.046). In connection, despite finding a significantly larger $\Delta\dot{Q}$ (the percent change from rest) per active leg during 1L- than 2L cycling, we cannot exclude the possibility that a larger fraction of $\dot{Q}$ was distributed to non-exercising tissue (torso, arms, the non-exercising leg) during 1L- than 2L cycling (19). However, care was taken to "immobilize" the use of the arms and torso by placing the handlebars of the cycle ergometer high, using armrest bars, and instructing the subjects carefully not to use their arms, torso, and non-exercising leg actively during submaximal exercise. Moreover, our findings are supported by studies assessing leg blood flow directly using indicator dilution-, thermodilution-, or ultrasound Doppler techniques, demonstrating larger leg blood flow during 1L- than 2L cycling (18, 19, 40).

Conclusions

The mass-specific MFO is higher when exercising a smaller muscle mass, potentially explained by more favorable conditions for O_2 and substrate delivery than during whole-body exercise.

Additional information

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Author Contributions

Conception and design of the experiment: Ø.S., D.P., E.I.J., J.J.
Data collection: Ø.S., D.P., M.C.,
Analysis of data: Ø.S., D.P.
Interpretation of data: Ø.S., D.P., J.J.
Writing the first draft: Ø.S.
Revising the manuscript: All authors.
All authors have read and approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no conflicts of interest regarding the publication of this paper. There are no financial conflicts of interest to disclose. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute an endorsement by ACSM.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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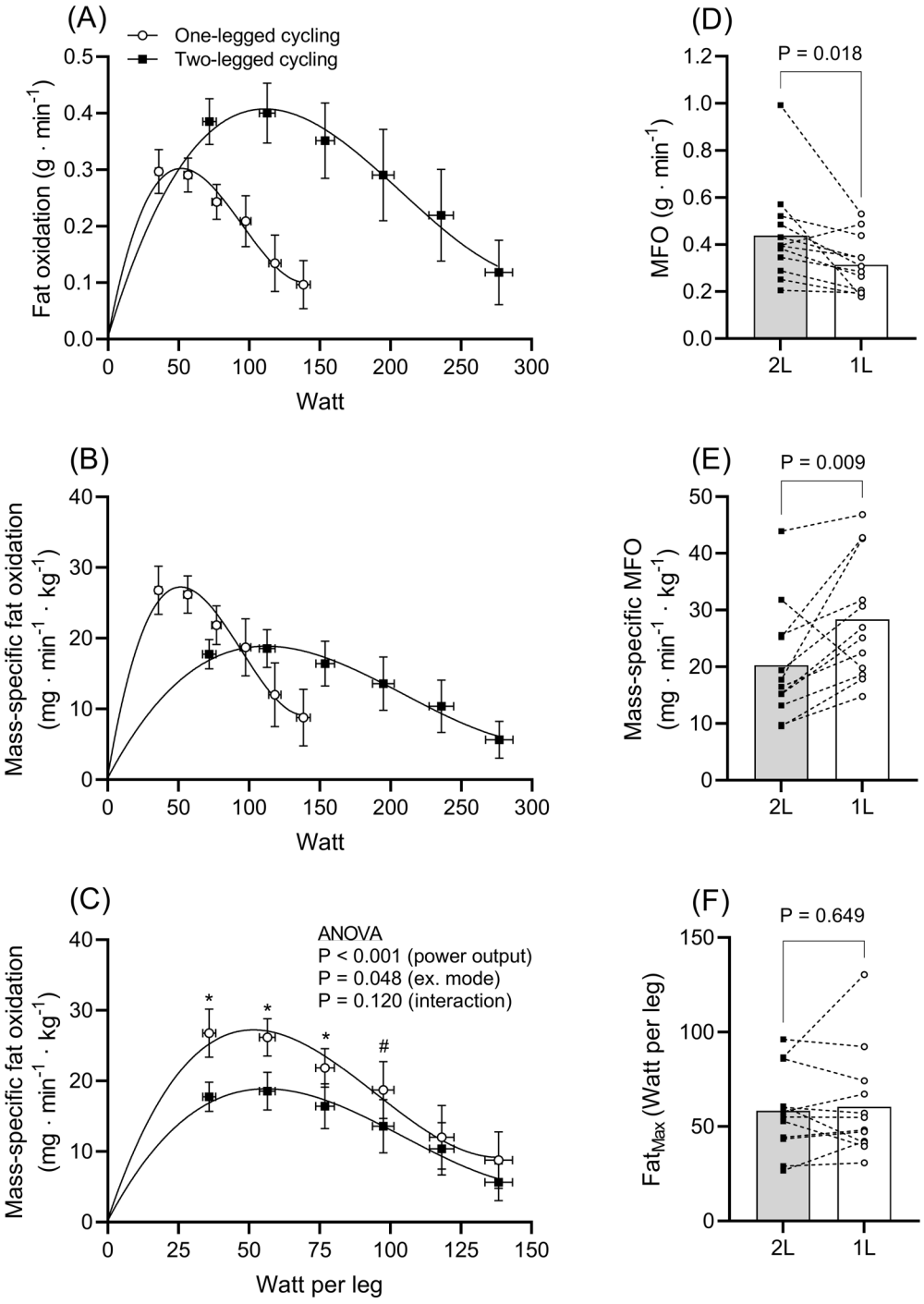


Fig. 1: Mean (± SEM) values of fat oxidation and mass-specific fat oxidation (fat oxidation divided by the lean mass of the exercising leg(s)) as a function of power output (A-B) and power output per leg (C). X-axis error bars are ± SEM in power output. In Fig. D and E, the individual (symbols and lines) and mean (bars) data of the maximal fat oxidation (MFO) and mass-specific MFO are compared between exercise modes, respectively. Fig. F displays the power output per exercising leg at which MFO occurred (Fat_{Max}). Results are from test days 3 and 4. Significant (*; $P < 0.05$) and a trend towards (#; $P < 0.1$) difference between exercise modes (post hoc test). N = 12.

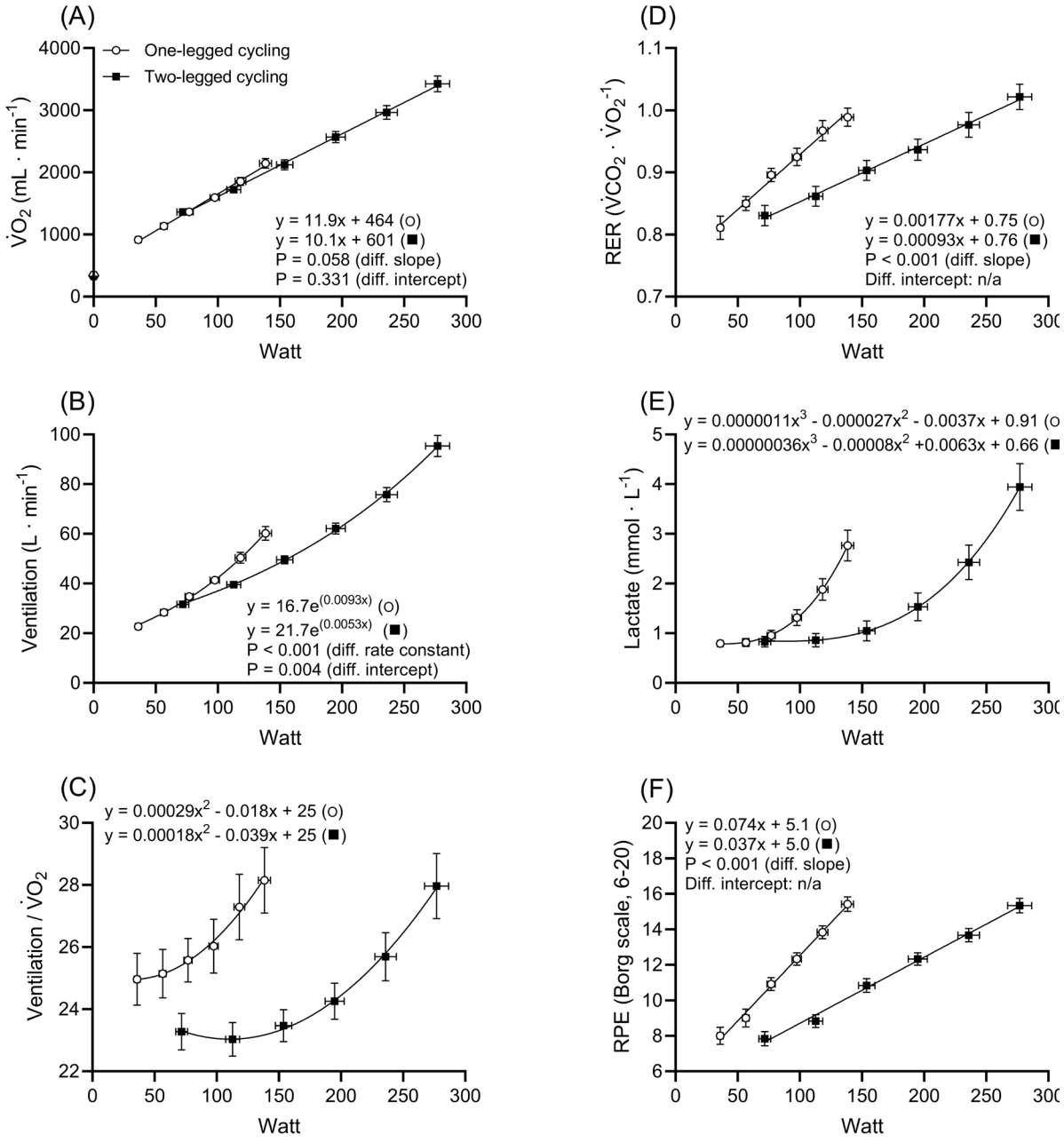


Fig. 2: Mean (± SEM) values of the oxygen uptake ($\dot{V}O_2$; A), ventilation (B), ventilatory equivalent for O_2 (C), respiratory exchange ratio (RER; D), capillary blood lactate concentration (E), and rating of perceived exertion (RPE; F) as a function of power output during one- and two-legged cycling. X-axis error bars are ± SEM in power output. Results are from test days 3 and 4. Inserted are the equations describing the associated lines/curves (solid) and the P-values describing whether slopes, rate constants, or intercepts were significantly different between exercise modes. n/a: the difference in intercepts was not possible to analyze since the slopes differed too much. N = 12.

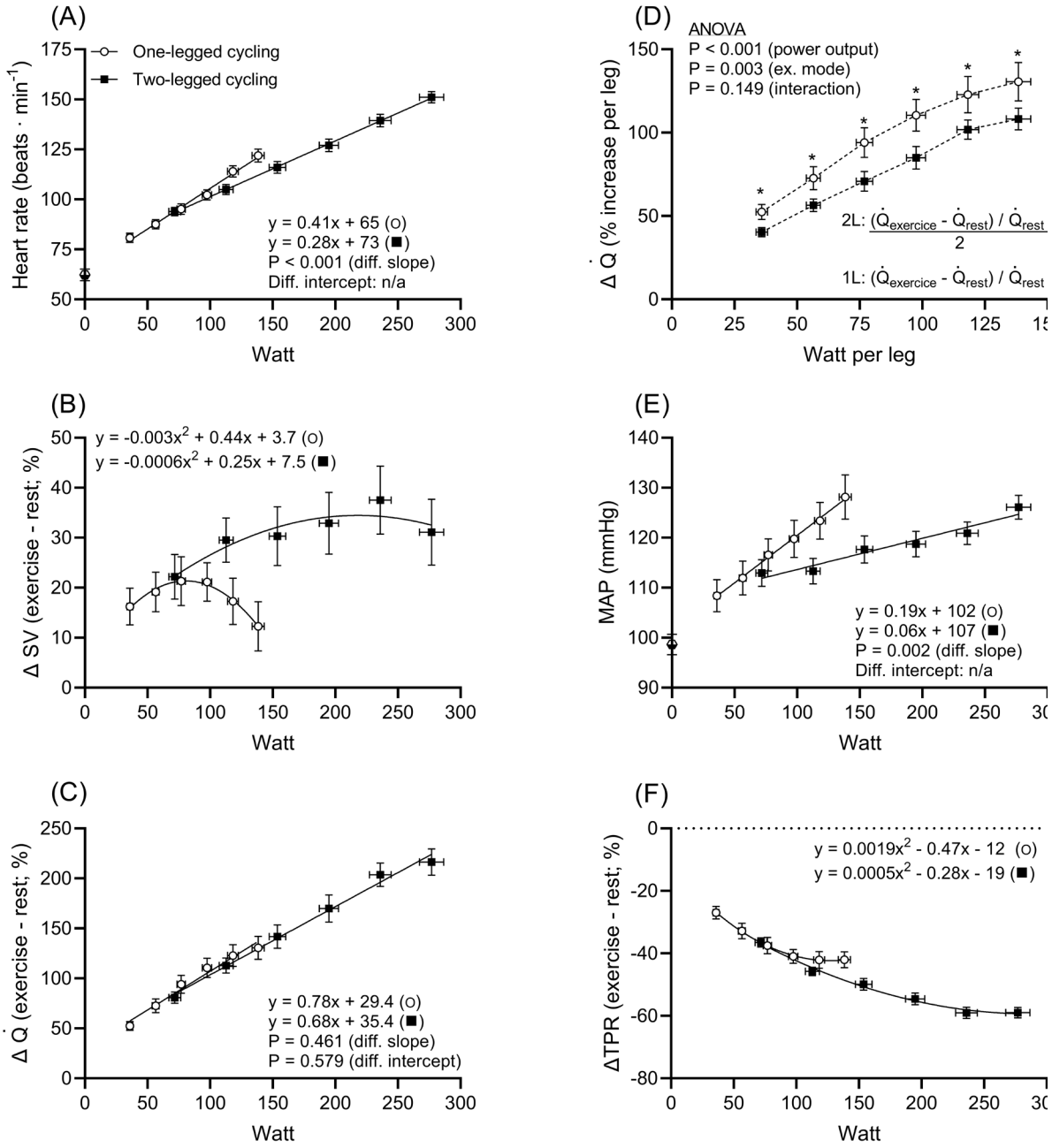


Fig. 3: Mean (± SEM) values of the heart rate (A), change in stroke volume from rest (ΔSV; B), change in cardiac output from rest ($\Delta\dot{Q}$; C), mean arterial pressure (MAP; E), and change in total peripheral resistance from rest (ΔTPR; F) as a function of power output during one- and two-legged cycling. In graph D, $\Delta\dot{Q}$ is expressed per exercising leg (i.e., divided on 2 for two-legged cycling) as a function of power output per leg. The absolute values for cardiac output ($L \cdot min^{-1}$) are presented in Supplemental Fig. 1. X-axis error bars are ± SEM in power output. Results are from test days 3 and 4. Inserted in Fig. 3D are the P-values from two-way repeated measures analysis of variance (ANOVA). Significant difference (*; $P < 0.05$) between exercise modes (post hoc test). See Fig. 2 for the remaining statistical information. N = 12.

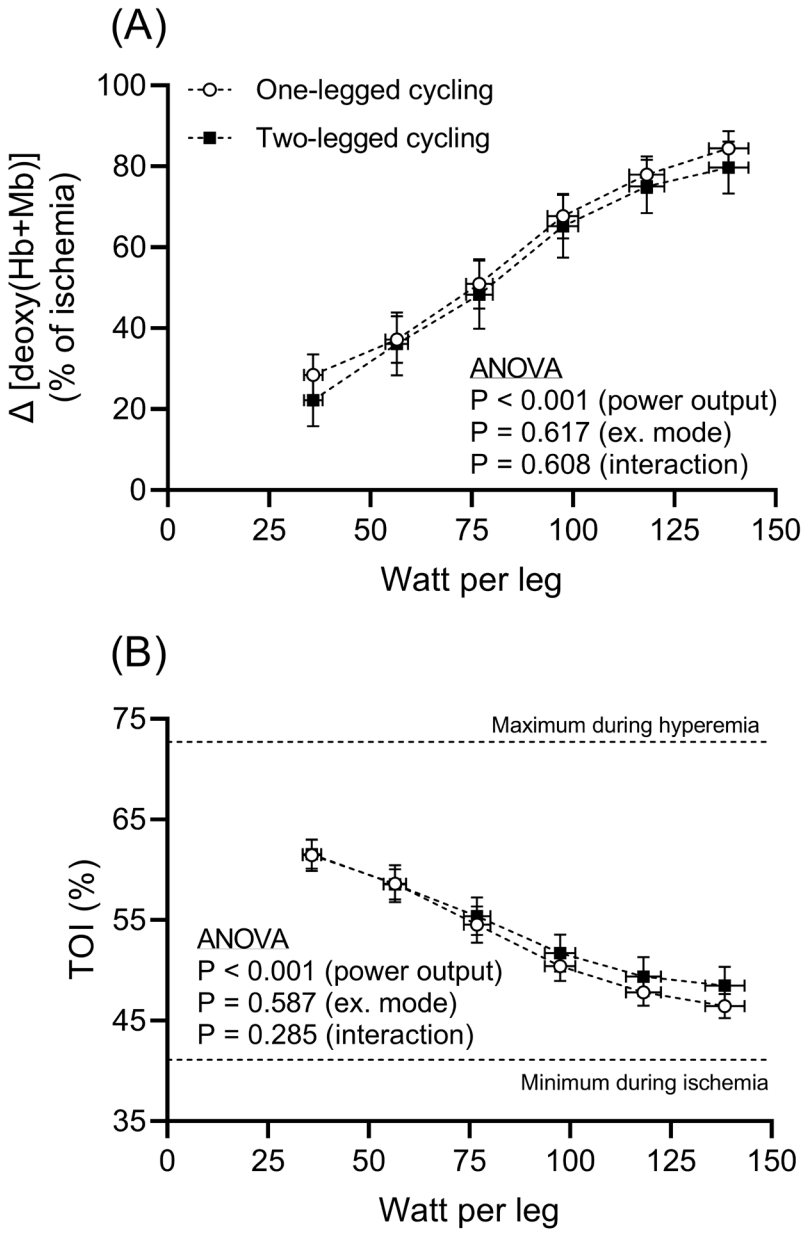


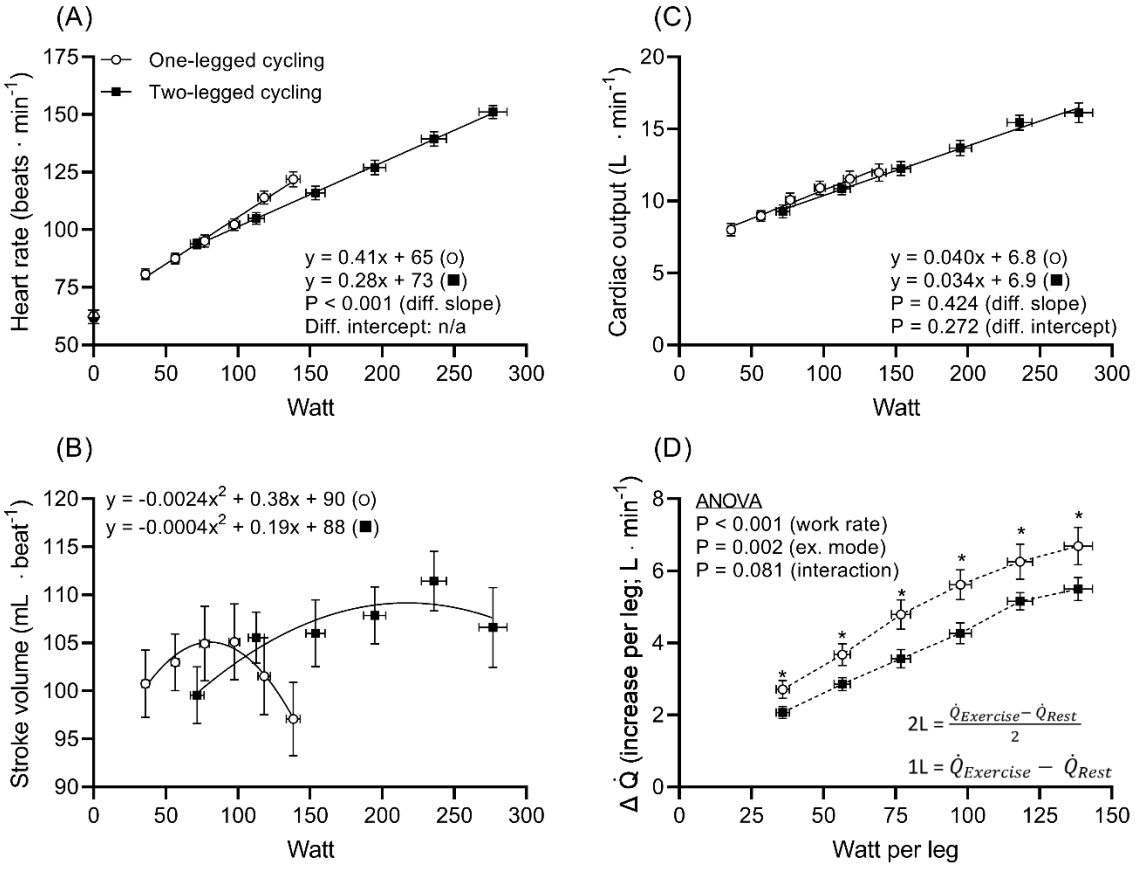
Fig. 4: Mean (± SEM) values of near-infrared spectroscopy (NIRS)-determined oxygenation in forms of Δ[deoxy(Hb+Mb)] (A) and tissue oxygenation index (TOI; B) as a function of power output per leg. The Δ[deoxy(Hb+Mb)] data was expressed as a percentage of those obtained during post-exercise limb ischemia and served, together with TOI, as substitute measures of skeletal muscle fractional O_2 extraction. X-axis error bars are ± SEM in power output. Results are from test days 3 and 4. Inserted are the P-values from two-way repeated measures analysis of variance (ANOVA). N = 12.

Table 1. Measurements obtained at maximal exercise (test days 3 and 4).

	Two-legged cycling (mean ± SD)	One-legged cycling (mean ± SD)	ES
Peak power output (watt)	420 ± 60	235 ± 32***	-3.86
$\dot{V}O_{2max\ /\ peak}$ (mL · min^{-1})	4638 ± 665	3704 ± 517***	-1.57
VE_{peak} (L · min^{-1})	190 ± 26	168 ± 31**	-0.79
BF (breaths · min^{-1})	62 ± 10	65 ± 13	0.27
Tidal volume (L)	3.1 ± 0.5	2.6 ± 0.5***	-1.01
RER_{peak}	1.18 ± 0.04	1.15 ± 0.05*	-0.74
VE / $\dot{V}O_2$	41.3 ± 4.1	45.6 ± 7.0*	0.75
VE / $\dot{V}CO_2$	35.0 ± 3.4	39.8 ± 5.9***	0.99
$HR_{max\ /\ peak}$ (beats · min^{-1})	186 ± 10	176 ± 12***	-0.99
Δ[Deoxy(Hb+Mb)], (% of ischemia)	75.4 ± 18.0	82.1 ± 16.2	0.30
TOI Vastus Lateralis (%)	50.9 ± 6.0	49.1 ± 3.9	-0.22
$[La]_{peak}$ (mmol · L^{-1})	12.9 ± 1.8	10.3 ± 1.7***	-1.34

N = 12. ES, Cohen's *d* effect size; ΔDeoxy(Hb+Mb) (% of ischemia), concentration change of deoxygenated hemoglobin and myoglobin in Vastus Lateralis at exhaustion; $HR_{max\ /\ peak}$, maximal and peak heart rate during two- and one-legged cycling, respectively; $[La]_{peak}$, peak blood lactate concentration; RER_{peak}, peak respiratory exchange ratio; TOI, tissue oxygenation index at exhaustion; $\dot{V}CO_2$, carbon dioxide production; VE_{peak}, peak ventilation; $\dot{V}O_{2max\ /\ peak}$, maximal and peak oxygen uptake during two- and one-legged cycling, respectively. *, ** and *** Significantly different from two-legged cycling ($P \leq 0.05$, $P < 0.01$ and $P < 0.001$, respectively). # Trend towards being different from two-legged cycling ($0.05 < P \leq 0.10$).

Supplements
Increased mass-specific maximal fat oxidation rate with small vs large muscle mass exercise



Supplemental Fig. 1: Mean (± SEM) values of the heart rate (A), stroke volume (B), and cardiac output ($\dot{Q}$; C) as a function of power output during one- and two-legged cycling. In Fig. D, the change in cardiac output from rest to exercise ($\Delta\dot{Q}$) is expressed per exercising leg (i.e., divided on 2 for two-legged cycling) as a function of power output per leg. X error bars are ± SEM in power output. Results are from test days 3 and 4. Inserted in Figs. A-C are the equations describing the associated lines/curves (solid), and the P-values describing whether slopes or intercepts were significantly different between exercise modes. n/a: the difference in intercepts was not possible to analyze since the slopes differed too much. Inserted in Fig. D are the P-values from two-way repeated measures analysis of variance (ANOVA). Significant difference (*; $P < 0.05$) between exercise modes (post hoc test). N = 12.