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Functional reserve and sex differences during exercise to exhaustion revealed by post-exercise ischaemia and repeated supramaximal exercise

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Key points

- Females have lower fatigability than males during single limb isometric and dynamic contractions, but whether sex-differences exist during high-intensity whole-body exercise remains unknown.
- This study shows that males and females respond similarly to repeated supramaximal whole-body exercise, and that at task failure a large functional reserve remains in both sexes.
- Using post-exercise ischaemia with repeated exercise, we have shown that this functional reserve depends on the glycolytic component of substrate-level phosphorylation and is almost identical in both sexes.
- Metaboreflex activation during post-exercise ischaemia and the O_2 debt per kg of active lean mass are also similar in males and females after supramaximal exercise.
- Females have a greater capacity to extract oxygen during repeated supramaximal exercise and reach lower P_{ETCO_2}, experiencing a larger drop in brain oxygenation than males, without apparent negative repercussion on performance.
- Females had no faster recovery of performance after accounting for sex differences in lean mass.

Abstract The purpose of this study was to ascertain what mechanisms explain sex differences at task failure and to determine whether males and females have a functional reserve at exhaustion. Exercise performance, cardiorespiratory variables, oxygen deficit, and brain and muscle oxygenation were determined in 18 males and 18 females (21–36 years old) in two sessions consisting of three bouts of constant-power exercise at 120% of $\dot{V}_{O_2max}$ until exhaustion interspaced by 20 s recovery periods. In one of the two sessions, the circulation of both legs was occluded instantaneously (300 mmHg) during the recovery periods. Females had a higher muscle O_2 extraction during

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fatiguing supramaximal exercise than males. Metaboreflex activation, and lean mass-adjusted O_2 deficit and debt were similar in males and females. Compared to males, females reached lower P_{ETCO_2} and brain oxygenation during supramaximal exercise, without apparent negative consequences on performance. After the occlusions, males and females were able to restart exercising at 120% of $\dot{V}_{O_2max}$, revealing a similar functional reserve, which depends on glycolytic component of substrate-level phosphorylation and its rate of utilization. After ischaemia, muscle O_2 extraction was increased, and muscle $\dot{V}_{O_2}$ was similarly reduced in males and females. The physiological response to repeated supramaximal exercise to exhaustion is remarkably similar in males and females when differences in lean mass are considered. Both sexes fatigue with a large functional reserve, which depends on the glycolytic energy supply, yet females have higher oxygen extraction capacity, but reduced P_{ETCO_2} and brain oxygenation.

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Introduction

During whole-body high-intensity exercise to exhaustion, it is likely that task failure occurs due to a higher energy demand than energy supply. The supply of energy may be curtailed by a reduction in the rate of ATP resynthesis due to partial blockade of the critical reactions in the metabolic energy pathways or by exhaustion of critical substrates. We define 'functional reserve' as the capacity to produce power at the same level or higher than reached at exhaustion. This functional reserve has been demonstrated in males by the capacity to perform sprint exercise at the end of an incremental exercise test to exhaustion followed by 10–60 s ischaemia, which impedes metabolic recovery (Morales-Alamo *et al.* 2015; Gelabert-Rebato *et al.* 2018, 2019*a*,*b*) as well as after 3–5 s recovery without occlusion (Coelho *et al.* 2015). It remains unknown, however, whether this functional reserve is also present in females after an exercise bout supposedly utilizing the totality of the anaerobic capacity (Medbo *et al.* 1988; Medbo & Tabata, 1989). Moreover, the physiological factors determining the nature and magnitude of the functional reserve and whether sex differences exist have not been established.

Classically, during high-intensity exercise, i.e. in the severe exercise-intensity domain (Poole & Jones, 2012), task failure has been attributed to the accumulation of metabolites (AMP, ADP, lactate, H^+ and inorganic phosphate (P_i)), particularly during exercise with marked recruitment of substrate-level phosphorylation (PCr and glycolysis) (Cady *et al.* 1989; Fitts, 1994; Allen *et al.* 2008; Black *et al.* 2017), and the associated production of reactive oxygen and nitrogen species (RONS) (Westerblad & Allen, 2011). At task failure in the severe intensity domain, the level of metabolite accumulation and peripheral fatigue is similar despite marked differences in relative intensity (Thomas *et al.* 2016). However, a study in humans indicates that task failure is not due to the accumulation of energy metabolites or blockade of glycolysis (Morales-Alamo *et al.* 2015), in agreement with previous studies showing that the capacity to generate force is rapidly recovered despite a high concentration of lactate and H^+ (Sahlin & Ren, 1989).

Whether males and females fatigue similarly during whole-body exercise in the severe exercise-intensity domain remains unknown. Previous studies have reported lower fatigability in females during isometric contractions at low (20% of the maximal voluntary contraction (MVC)) but not at high (80% of MVC) intensities (Yoon *et al.* 2007; Hunter, 2014, 2016*a*; Ansdell *et al.* 2017). During single limb dynamic contractions, females fatigue less than males depending on the contraction velocity and the muscle groups recruited (Hunter, 2016*b*). When present, the sex differences are also observed during the first 45 min of recovery after task failure (Ansdell *et al.* 2019). At task failure during isometric intermittent quadriceps muscle contractions in the severe exercise-intensity domain, neuromuscular function is similar in both sexes (Ansdell *et al.* 2019). Consequently, it has been suggested that the lower fatigability and faster recovery of females should be due to less metabolic derangement (Senefeld *et al.* 2018; Ansdell *et al.* 2019). The latter could be due to the fact that females have a lower anaerobic capacity than males (Green *et al.* 1984). In support, near-infrared spectroscopy (NIRS) measurements at task failure and during recovery show lower muscle deoxygenation in females than males (Ansdell *et al.* 2019). Whether females have better muscle oxygenation and lower metabolic muscle impairment at task failure during whole-body exercise in the severe-intensity domain remains unknown (Ansdell *et al.* 2019). Recent work indicates that females have superior mitochondrial respiration than males

(Cardinale *et al*. 2018), which may facilitate O_2 extraction. There is a paucity of data relating to sex differences in recovery (Ansdell *et al*. 2019) and no previous study has determined whether there is a sex-difference in whole-body performance recovery between males and females, after task failure in the severe exercise-intensity domain.

Input from type III and IV muscle afferents may also influence the rate of perceived exertion and contribute to task failure by a central mechanism (Sidhu *et al*. 2014, 2017; Hureau *et al*. 2019), although their exact role during whole-body exercise is poorly understood (Marcora, 2010; Torres-Peralta *et al*. 2016). Muscle afferent input during exercise may be higher in males than females, as suggested by the enhanced sympathetic and pressor response to metaboreflex activation in males compared to females (Jarvis *et al*. 2011; Welch *et al*. 2018; Joshi & Edgell, 2019; Samora *et al*. 2019), as well as the activation of chemoreflex responses secondary to metaboreflex activation (Edgell & Stickland, 2014; Wan *et al*. 2020). Metaboreflex activation may also directly modulate medullary respiratory centre output via relay through the nucleus tractus solitarii (Sander *et al*. 2010), resulting in increased pulmonary ventilation (Lam *et al*. 2019). The latter could contribute to an earlier task failure in males than females. However, most data have been obtained in males lying supine by eliciting the metaboreflex in the forearm muscles (Jarvis *et al*. 2011; Joshi & Edgell, 2019; Samora *et al*. 2019). Thus, if males have a higher metaboreflex sensitivity than females, the application of ischaemia after exercise should result in a higher heart rate (HR) and ventilatory responses during ischaemia in males than females.

Therefore, the main aim of this investigation was to determine whether males and females have a functional reserve at exhaustion following supramaximal exercise at 120% of $\dot{V}_{O_2max}$, and to ascertain what mechanisms may explain potential between-sex differences in task failure and functional reserve.

For these purposes, we applied a new experimental protocol which allows assessment of substrate-level phosphorylation for ATP re-synthesis non-invasively by the application of ischaemia at the end of exercise to impede the metabolic recovery (Harris *et al*. 1975; Morales-Alamo *et al*. 2015). Since muscle fatigue is task-specific (Gandevia, 2001), it is crucial to test the existence of a functional reserve by using the same pattern of movement. This can be accomplished by setting the ergometer in hyperbolic mode at a constant supramaximal power, as for example 120% of maximal oxygen consumption ($\dot{V}_{O_2max}$), such that the subjects will only be able to resume the exercise if they have a functional reserve in substrate-level phosphorylation at exhaustion sufficient to reach the power output corresponding to the supramaximal load.

We hypothesized that (1) females would have a lower anaerobic capacity than males, even when normalized to the lean mass of the lower extremities; (2) following high-intensity exercise to exhaustion, females would have a lower functional reserve than males; (3) during repeated fatiguing high-intensity exercise, females would recover from fatigue faster than males; (4) during repeated fatiguing high-intensity exercise females would achieve greater O_2 extraction than males; and (5) post-exercise ischaemia would reveal higher metaboreflex-induced heart rate and ventilatory responses in males than females.

Methods

Ethical approval

The study was approved by the Ethical Committee of the University of Las Palmas de Gran Canaria (Ref.: CEIH-2017-13) and performed in accordance with the standards set by the latest revision of the *Declaration of Helsinki*, except for registration in a database. All subjects signed a written informed consent before the start of the study.

Subjects

Eighteen males and eighteen females, all healthy and physically active, agreed to participate in this investigation (age = 24.6 ± 4.4 years, height = 170.5 ± 8.1 cm, body mass = 63.8 ± 9.3 kg, body fat = 22.3 ± 5.5%, n = 36, Table 1). The inclusion criteria for participation in this investigation were: age 18–35 years; no chronic diseases or recent surgery; non-smoking; normal resting electrocardiogram; body mass index above 18 and below 30; no medical contraindications to exercise; and no history of disease requiring medical treatments lasting longer than 15 days during the preceding 6 months. Subjects were requested to avoid strenuous exercise 48 h before the laboratory test and not to drink carbonated, caffeinated and alcohol-containing beverages during the 24 h preceding all tests. During the study period, subjects were also requested to abstain from the consumption of drugs, medications and any dietary supplements. Sex and gender of the participants were defined based on self-report during participant recruitment. All participants reported *cis*-gender, and thereafter the terms males and females were applied in the study analysis and reporting (Heidari *et al*. 2016). All females were eumenorrhoeic, and five were taking oral contraceptives. Females not taking contraceptives were tested randomly in different phases of the menstrual cycle (Mattu *et al*. 2020), given the large number of tests performed. This approach is based on the similar sprint and high-intensity responses observed

Table 1. Physical characteristics and ergoespirometric variables (means ± SD)

	Males (n = 18)	Females (n = 18)	P
Age (years)	22.9 ± 2.2	26.3 ± 5.3	0.016
Height (cm)	176.6 ± 4.1	164.4 ± 6.0	<0.001
Weight (kg)	71.3 ± 5.4	56.2 ± 6.0	<0.001
% body fat	18.5 ± 3.5	26.1 ± 4.4	<0.001
LLM (kg)	19.7 ± 2.0	13.8 ± 1.3	<0.001
HR_{max} (beats min^{-1})	190.0 ± 7.9	189.6 ± 10.2	0.98
$\dot{V}_{O_2max}$ (ml kg^{-1} min^{-1})	47.2 ± 6.1	40.3 ± 5.4	<0.001
$\dot{V}_{O_2max}$ (ml kg LLM^{-1} min^{-1})	170.1 ± 16.4	164.4 ± 20.9	0.369
W_{max} (W)	257.2 ± 32.7	173.3 ± 25.1	<0.001
120% $\dot{V}_{O_2max}$ CP (first bout, best performance)			
Time to exhaustion (s)	149.6 ± 39.1	134.9 ± 30.8	0.219
Power (W)	302.8 ± 35.7	209.4 ± 33.4	<0.001
Work (kJ kg LLM^{-1})	2.29 ± 0.58	2.01 ± 0.34	0.090
$\dot{V}_{O_2}$ (ml kg LLM^{-1} min^{-1})	134.8 ± 15.0	134.1 ± 13.2	0.881
O_2 demand (ml)	10 080 ± 3130	6042 ± 1400	0.000
Accumulated O_2 (ml)	6774 ± 2477	4051 ± 1213	0.000
O_2 deficit (ml)	3306 ± 857	1892 ± 454	0.000
O_2 deficit (ml kg^{-1} BW)	59.9 ± 13.6	48.3 ± 9.8	0.006
O_2/work (ml kJ^{-1})	146.7 ± 16.3	148.5 ± 16.6	0.744
O_2 deficit (ml kg LLM^{-1})	166.6 ± 36.9	136.7 ± 27.9	0.010
% anaerobic energy	33.8 ± 6.1	31.6 ± 5.3	0.268
RER	1.16 ± 0.07	1.08 ± 0.06	0.001
$\dot{V}_E$ (l min^{-1})	91.3 ± 18.1	63.3 ± 10.2	0.000
RR (breaths min^{-1})	36.7 ± 6.8	36.2 ± 6.9	0.837
$\dot{V}_E/\dot{V}_{O_2}$	31.5 ± 3.7	31.2 ± 3.6	0.818
$\dot{V}_E/\dot{V}_{CO_2}$	27.3 ± 2.9	28.7 ± 2.5	0.139
P_{ETO_2} (mmHg)	108.9 ± 7.5	108.6 ± 3.3	0.882
P_{ETCO_2} (mmHg)	39.9 ± 4.6	36.2 ± 2.5	0.006

The supramaximal test data correspond to the session with the best performance time in the 120% of VO_2max CP sessions. P-values based on two-tailed unpaired t-test. Accumulated O_2 : total amount of O_2 consumed during the test. HR_{max}, maximal heart rate; LLM, lean mass of the lower extremities; P_{ETCO_2}, end tidal CO_2 pressure; P_{ETO_2}, end tidal O_2 pressure; RER, respiratory exchange ratio; RR, respiratory rate; $\dot{V}_E$, pulmonary ventilation; $\dot{V}_{O_2max}$, maximal oxygen uptake; W_{max}, maximal intensity during the incremental exercise test to exhaustion.

in different phases of the menstrual cycle (Botcazou *et al.* 2006; Bushman *et al.* 2006; Shaharudin *et al.* 2011).

Study overview

The study included the following consecutive phases: (1) recruitment, familiarization and pre-testing; (2) assessment of $\dot{V}_{O_2max}$ and the $\dot{V}_{O_2}$–intensity relationship; and (3) supramaximal exercise tests to determine the functional reserve at exhaustion (Fig. 1).

Recruitment, familiarization and pre-testing

Subjects agreeing to participate underwent a body composition examination and several familiarization sessions. Body composition was assessed by dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Milwaukee, WI, USA) as described elsewhere (Calbet *et al.* 1998). Subjects were familiarized with the experimental procedures by performing an incremental exercise to exhaustion and sprint exercise (30 s Wingate all-out test), which were not used in subsequent analyses. After that, subjects reported to the laboratory to complete different experimental tests on separate days.

Assessment of $\dot{V}_{O_2max}$ and $\dot{V}_{O_2}$–intensity relationship

$\dot{V}_{O_2max}$, maximal heart rate (HR_{max}) and maximal power output (W_{max}) were determined in normoxia (F_{IO_2}: 0.21, P_{IO_2}: 144 mmHg) with an incremental exercise test to exhaustion with verification (Poole & Jones, 2017). For this test, subjects reported to the laboratory at least 4 h after the last ingestion of food. The test started with 3 min at 20 W, followed by 15 W and 20 W

increases every 3 min in females and males, respectively, until the respiratory exchange ratio (RER) was ≥1.00. After that, the load was increased by 10 W and 15 W every minute in females and males, respectively, until exhaustion. The highest intensity attained in the test was taken as the W_{max} of the incremental exercise. At exhaustion, the ergometer was unloaded, and slow pedalling (30–40 rpm) continued for 3 min. At the third minute of active recovery, the verification test was initiated at W_{max} + 5 W during 1 min, followed by a 4 and 5 W increase (females and males, respectively) every 20 s until exhaustion.

Oxygen uptake ($\dot{V}_{O_2}$) during all exercise tests was measured by open-circuit indirect calorimetry with a metabolic cart (Vyntus, Jaeger-CareFusion, Höchberg, Germany) operated in breath-by-breath mode. The gas analysers were calibrated immediately before each test using room air (20.93% O_2 and 0.04% CO_2) and high-grade certified gases provided by the manufacturer containing 16% O_2 and 5% CO_2. The volume flow sensor was calibrated at low (0.2 l s^{-1}) and high (2 l s^{-1}) rates immediately before each test. The validity of this metabolic cart was established by a butane combustion test (Perez-Suarez *et al.* 2018). Respiratory variables

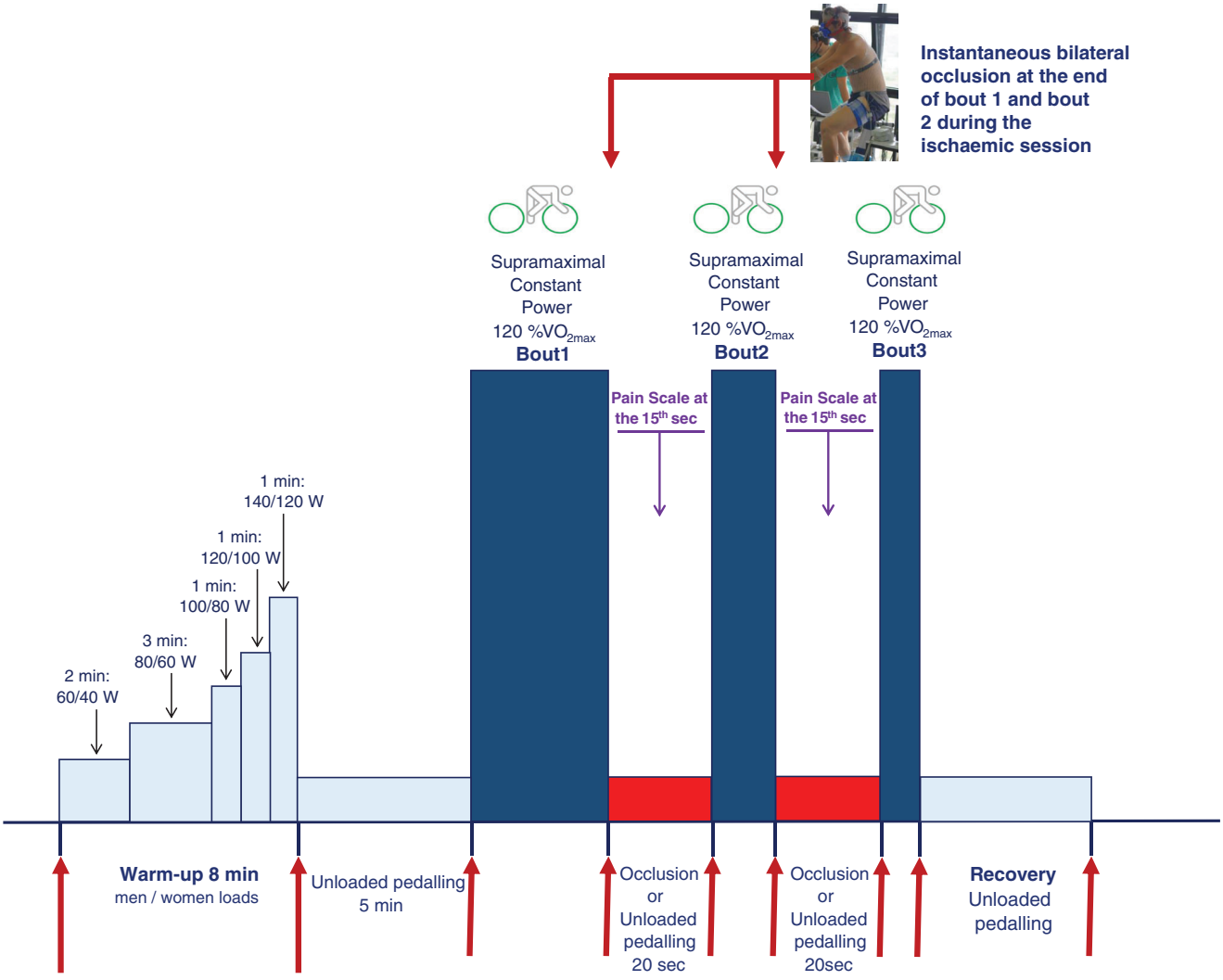

Figure 1. Schematic representation of the experimental protocol
Subjects participated in two experimental sessions. In each of them, after a standardized warm-up, subjects performed three bouts of supramaximal constant intensity exercise at 120% of $\dot{V}_{O_2max}$ until exhaustion, interspersed either with 20 s of recovery with application of immediate post-exercise ischaemia at exhaustion (ischaemic session) or with 20 s of recovery with free circulation (free circulation session), in random order. At the start of the 2nd and 3rd bouts in the ischaemic recovery sessions, the cuffs were deflated instantaneously, to allow for restoration of the circulation during the subsequent bout. The cuffs located around the two thighs were instantaneously inflated at 300 mmHg during the sessions with ischaemic recovery to elicit total occlusion of the circulation of both lower extremities and impede metabolic recovery. [Colour figure can be viewed at wileyonlinelibrary.com]

were analysed breath-by-breath and averaged every 20 s during the incremental exercise tests. The highest 20-s averaged $\dot{V}_{O_2}$ recorded during the whole incremental test (i.e. including the verification phase) was taken as the $\dot{V}_{O_2max}$ (Martin-Rincon *et al.* 2019). HR was recorded continuously with a sampling frequency of 1 s during all exercise tests via short-range radiotelemetry (RS400 and RS800, Polar Electro, Woodbury, NY, USA).

Assessment of the functional reserve at exhaustion

The functional reserve at exhaustion was determined 1–2 weeks after the assessment of $\dot{V}_{O_2max}$. For this purpose, subjects reported to the laboratory on two occasions, 1 or 2 weeks apart, hereafter called ischaemic and free circulation recovery sessions (Fig. 1). Subjects were requested to ingest a similar light breakfast on the experimental days, which should have been ended at least 1 h prior to the scheduled time. One hour after their arrival to the laboratory, the protocol described in Fig. 1 was started. Each of the two sessions consisted of three bouts of constant-power exercise to exhaustion at 120% of $\dot{V}_{O_2max}$ (hereafter referred as 120% CP) interspaced by 20 s recovery periods with (ischaemic session) or without (free circulation session) application of total occlusion of the circulation to the lower extremities. The intensity of the supramaximal exercise bouts was set at 120% of $\dot{V}_{O_2max}$, to facilitate the attainment of the maximal accumulated oxygen deficit during constant-power exercise on the cycle ergometer (Medbo & Tabata, 1989; Calbet *et al.* 1997).

In the ischaemic recovery session, the circulation of both lower extremities was instantaneously occluded at exhaustion after the first and the second 120% CP bouts, and the ischaemia maintained during the two 20 s recovery periods. Right at the restart of the second and third bouts, the cuffs were instantaneously released and the circulation wholly re-established. For the free circulation session, subjects performed unloaded pedalling at low cadence (∼20 rpm) during the two 20 s recovery periods, to minimize the risk of orthostatic post-exercise hypotension (Curtelin *et al.* 2018).

Before the exercise, while the subjects were resting supine, bilateral 10 cm-wide cuffs were placed around the thighs, as close as possible to the inguinal crease, and connected to a rapid cuff inflator (SCD10, Hokanson E20 AG101, Bellevue, WA, USA) as previously reported (Morales-Alamo *et al.* 2015; Torres-Peralta *et al.* 2016). Each session started with a warm-up consisting of 1 min of unloaded pedalling, followed by 2 min at 60 or 40 W, 3 min at 80 or 60 W, 1 min at 100 or 80 W, 1 min at 120 or 100 W and 1 min at 140 or 120 W for males and females, respectively. This was followed by 5 min of unloaded pedalling at low cadence (20–40 rpm). Then the subjects stopped pedalling, and the ergometer was set in hyperbolic mode at the load corresponding to their 120% of $\dot{V}_{O_2max}$ to perform the three 120% CP bouts until exhaustion, interspaced by the corresponding 20 s recovery periods. During the 20 s recovery periods, at the 15th second subjects were given a 5 s reverse countdown and prompted to restart pedalling again at the same intensity (i.e. 120% of $\dot{V}_{O_2max}$) until they were exhausted again. In the sessions with ischaemic recovery, the cuffs were instantaneously inflated at 300 mmHg to occlude completely the circulation of both lower extremities upon exhaustion. The cuffs were deflated instantaneously at the start of the second and third bouts and the circulation re-established during the subsequent exercise.

Cerebral and muscular oxygenation was assessed by NIRS (NIRO-200NX, Hamamatsu, Japan) employing spatially resolved spectroscopy to obtain the tissue oxygenation index (TOI) using a path-length factor of 5.92 (van der Zee *et al.* 1992). The first NIRS optode was placed on the right frontoparietal region at 3 cm from the midline and 2–3 cm above the supra-orbital crest, to avoid the sagittal and frontal sinus areas (Curtelin *et al.* 2018). A second optode was placed in the lateral aspect of the thigh at middle length between the patella and the anterosuperior iliac crest, over the middle portion of the m. vastus lateralis. The vastus lateralis fractional extraction index (TOI O_2 extraction index) was obtained as $TOI_{OBV} - TOI_{MIN}$, where TOI_{OBV} is the mean TOI value registered during exercise and TOI_{MIN} is the minimal 1-s rolling average TOI value registered during ischaemia. The TOI is an indicator of absolute tissue O_2 saturation of the haemoglobin present in arteriolar, capillary and venular beds, combined with the O_2 saturation of myoglobin, and can be assessed using spatially resolved spectroscopy (Boushel *et al.* 2001; Quaresima & Ferrari, 2009). Assuming that blood is distributed similarly in the three vascular beds during supramaximal exercise, and that the TOI during ischaemia represents the maximal levels of deoxygenation reachable, a higher difference between the TOI observed during supramaximal exercise and the minimum reached during ischaemia represents the residual O_2 bound to the haemoglobin and myoglobin that has not been extracted. Recent studies carried out in our laboratory have shown that there is a linear relationship between the raw TOI value recorded by the NIRO-200NX, and the leg O_2 extraction and femoral vein O_2 content (see Appendix).

All exercise tests were performed on the same cycle ergometer (Lode Corival, Lode BV, Groningen, The Netherlands), which maintains the exercise intensity constant despite variations in pedalling rate. During all tests, subjects were requested to keep the pedalling rate at 80 rpm (±3 rpm). In all instances, exhaustion was defined by the incapacity of the subject to maintain a pedalling rate above 50 rpm during 5 s or by the sudden stop of pedalling. Strong verbal encouragement was provided

throughout the entire exercise protocol and particularly approaching task failure. During the ischaemic recovery trials, subjects were requested to rate the level of pain in their thighs with a visual numerical rating scale from 0 to 10, with 10 being the highest muscle pain ever experienced during or after exercise. All subjects were accustomed to performing exercise to exhaustion and post-ischaemia all-out bouts of exercise, as well as to rate their level of pain with the same rating scales due to participation in other projects carried out in our laboratories using similar procedures. The ergometer seat and handlebar configuration were adjusted for comfort during the first visit and replicated in subsequent sessions. Exercise tests took place in an air-conditioned laboratory with an ambient temperature of ∼21°C, a relative humidity of 60–80%, and ∼735 mmHg atmospheric pressure.

The O_2 demand during the supramaximal exercise bouts was estimated from the linear relationship between the last minute averaged $\dot{V}_{O_2}$ of each load, from 20–40 W to the highest intensity with a RER $<$ 1.00. The accumulated oxygen deficit (AOD, an estimate of the energy provided by substrate-level phosphorylation), representing the difference between O_2 demand and accumulated O_2, was determined as previously reported (Calbet *et al*. 1997; Morales-Alamo *et al*. 2015). The contribution of anaerobic energy metabolism to the total energy yield was calculated as AOD $\times$ 100/O_2 demand. Since during the occlusions the myoglobin O_2 stores are depleted and PCr is not resynthesized (Harris *et al*. 1976; Blei *et al*. 1993; Quistorff *et al*. 1993; Morales-Alamo *et al*. 2015), the totality of the O_2 deficit measured during the subsequent bout corresponds to the energy supplied by the glycolytic component of substrate-level phosphorylation. This O_2 deficit was converted into moles of ATP assuming a volume of 22.4 litres per mole of oxygen (STPD), a muscle temperature of 38.5°C, and a phosphorus-to-O_2 ratio (P/O) of 2.5 (Hinkle *et al*. 1991). Lactate production was obtained knowing that 1.5 moles of ATP are produced per mole of lactate.

The oxygen debt incurred during the 20 s recovery by the lower extremities was calculated as the recovery $\dot{V}_{O_2}$ with free circulation minus the $\dot{V}_{O_2}$ during ischaemic recovery. The obtained value was normalized to the lean mass of the lower extremities (LLM) for sex comparisons.

Statistics

To establish the sample size, we first determined the correlation between mean power output in a control sprint of 10 s and the same type of sprints performed following 10 or 60 s occlusions applied at exhaustion (Morales-Alamo *et al*. 2015); the corresponding values ranged between 0.2 and 0.5. In this previous study, the standard deviation of the total work performed after the occlusion represented 22% of the mean value in each sprint. Simulations were run to determine what would be the sample size required to detect a mean difference between sexes of 20% in lean muscle mass normalized power output after the occlusions. This difference was established based on known sex differences in mean power output during supramaximal exercise and $\dot{V}_{O_2max}$ (20–30%, when expressed per kg of body mass) (Perez-Gomez *et al*. 2008; Loe *et al*. 2013). This resulted in a sample size of 24, i.e. 12 males and 12 females, assuming a correlation between repeated measurements of 0.5 (effect size: 0.5, at $\alpha = 0.05$ and $1 - \beta = 0.80$; G*Power v.3.1, *F*-tests, ANOVA for repeated measures between factors). Thirty-six volunteers were recruited to allow for potential dropouts. Since there were no dropouts, the study was powered to detect a 13–16% sex difference, depending on the correlation between repeated measures assumed. Variables were checked for normal distribution by using the Shapiro–Wilks test. A three-way repeated-measures ANOVA was used with two within-subjects factors: exercise bout (with three levels) and occlusion (with two levels: ischaemia and free circulation) and with sex as a between-subjects factor (with two levels: male and female). To test for potential sex differences, the sex contrast was assessed. Besides, the following interactions were evaluated: occlusion by sex (to determine whether males and females responded differently to the occlusions) and bout by sex (to find out whether the repetition of bouts elicited different responses in males and females). The cardiorespiratory responses during the recovery periods were also tested using three-way repeated-measures ANOVAs with two within-subjects factors: exercise bout (with two levels: first and second recovery) and occlusion (with two levels: ischaemia and free circulation), and with sex as a between-subjects factor (with two levels: male and female). Mauchly's test of sphericity was run before the ANOVA, and in the case of violation of the sphericity assumption, the degrees of freedom were adjusted according to the Huynh–Feldt test. When a significant main effect or interaction was observed, specific pairwise comparisons were carried out with the Fisher's least significant difference (LSD) *post hoc* test when appropriate. Besides, Student's paired *t*-test (two-tailed) was used to compare the responses obtained during exercise to resting conditions or between two different bouts at particular time-points. An unpaired two-tailed *t*-test was also used to compared males and females at specific points. The relationship between variables was determined using linear regression analysis. Values are reported as the mean $\pm$ standard deviation (SD), effect size (ES) and 95% confidence intervals (CI). $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS v.15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Sex differences in physical characteristics and performance during incremental exercise to exhaustion and the best 120% constant-power bout (120% CP) are reported in Table 1. Males achieved a 17.2% greater $\dot{V}_{O_2max}$ per kg of body mass than females (ES = 1.18 (CI: 0.4, 1.93)). When expressed per kg of LLM, the difference was reduced to 3.5% and not statistically different (ES = 0.30 (CI: −0.36, 0.96)). No sex differences were observed in time to exhaustion during their best 120% CP (ES = 0.42 (CI: −0.26, 1.08)). Males incurred a greater AOD than females in absolute values (+74.7%, ES = 2.06 (CI: 1.10, 2.99)) and in ml per kg of LLM (+21.8%; ES = 0.91 (CI: 0.18, 1.62)) (Table 1). This increased reliance on anaerobic metabolism in males compared with females was associated with a 0.08 higher RER (ES = 1.20 (CI: 0.42, 1.96)). The ventilatory response to exercise was essentially similar in males and females, except that females had 3.7 mmHg lower mean end-tidal CO_2 pressure (P_{ETCO_2}) values (ES = 0.98 (CI: 0.24, 1.70)). During the exercise at 120% of $\dot{V}_{O_2max}$, both groups obtained one-third of the energy expended through substrate-level phosphorylation (ES = 0.38 (CI: −0.30, 1.04)).

Functional reserve

All subjects were able to resume the exercise at the same intensity in the second and third bouts after ischaemic recovery, demonstrating the existence of a functional reserve after the first and second bouts ($P < 0.001$, occlusion main effect, ES = 1.55 (CI: 1.06, 2.03)). No sex differences were observed in endurance time ($P = 0.35$, ES = −0.31 (CI: −0.97, 0.35), sex contrast; $P = 0.75$, bout by sex interaction) or work per kg of LLM when the exercise was performed after ischaemic recovery ($P = 0.39$, ES = −0.29 (CI: −0.95, 0.37), sex contrast; $P = 0.70$, bout by sex interaction) (Table 2 and Fig. 2*A*). The LLM-normalized accumulated consumption of O_2 ($P = 0.47$, ES = −0.25 (CI: −0.90, 0.42), sex contrast) and AOD ($P = 0.92$, ES = −0.03 (CI: −0.69, 0.62), sex contrast) during the second and third bout after ischaemic recovery were almost identical in males and females (Fig. 2*B* and *C*). The O_2 deficit incurred during the second and third bouts of exercise was almost identical regardless of the recovery mode ($P = 0.28$, ES = −0.18 (CI: −0.51, 0.15), occlusion main effect) (Table 2 and Fig. 2*C*).

No sex differences were observed in the lactate equivalence of the O_2 deficit measured in the bouts performed after ischaemic recovery ($P = 0.80$, ES = −0.08 (CI: −0.74, 0.57), sex contrast; $P = 0.73$, bout × sex interaction, ANOVA). In the second bout, the lactate equivalence of the O_2 deficit after ischaemic recovery was 3.4 ± 1.1 and 3.6 ± 1.7 mmol kg LLM^{-1} for males and females, respectively ($P = 0.72$, ES = −0.12 (CI: −0.78, 0.54), unpaired *t*-test). The corresponding values for the third bout were 2.9 ± 1.0 and 3.0 ± 1.5 mmol kg LLM^{-1} for males and females, respectively ($P = 0.92$, ES = −0.03 (CI: −0.69, 0.62), unpaired *t*-test). Nevertheless, in the third bout, lactate production was 15% lower than in the second ($P = 0.006$, ES = 0.49 (CI: 0.14, 0.83), bout main effect). No sex differences were observed in the glycolytic rates in the bouts performed after ischaemic recovery ($P = 0.30$, ES = 0.35 (CI: −0.32, 1.01), sex contrast, ANOVA; $P = 0.92$ bout × sex interaction, ANOVA). The glycolytic rates of the second and third bouts after occlusion were 0.160 ± 0.057 and 0.174 ± 0.056 mmol kg LLM^{-1} s^{-1}, respectively ($P = 0.107$, ES = −0.28 (CI: −0.61, 0.05), bout effect, ANOVA). In the second bout, the glycolytic rates were 0.169 ± 0.057 and 0.151 ± 0.057 mmol kg LLM^{-1} s^{-1} for males and females, respectively ($P = 0.34$, ES = 0.32 (CI: −0.35, 0.98), unpaired *t*-test), and in the third, 0.183 ± 0.053 and 0.166 ± 0.056 mmol kg LLM^{-1} s^{-1} for males and females, respectively ($P = 0.37$, ES = 0.30 (CI: −0.36, 0.96), unpaired *t*-test).

No sex differences were observed in mean $\dot{V}_{O_2}$ expressed as ml kg LLM min^{-1} in the bouts performed after ischaemic recovery ($P = 0.88$, ES = 0.05 (CI: −0.70, 0.61), sex contrast; $P = 0.92$, bout × sex interaction) (Fig. 2*D*). Compared to the mean values observed during the first bout, the mean $\dot{V}_{O_2}$ in ml kg LLM min^{-1} was reduced by 6.7% during the third bout when preceded by recovery with occlusion ($P = 0.026$, ES = 0.39 (CI: 0.05, 0.72), paired *t*-test), representing 72.0% of the $\dot{V}_{O_2max}$. In contrast, $\dot{V}_{O_2}$ in ml kg LLM min^{-1} was increased by ∼19% during second and third bouts when preceded by recovery with free circulation, to reach ∼93% of $\dot{V}_{O_2max}$ ($P < 0.001$, ES = −1.95 (CI: −2.50, −1.38), first compared to the mean of the last two bouts, paired *t*-test) (Fig. 2*D*). Consequently, the fractional contribution of substrate level phosphorylation to the total energy yield was larger in the bouts preceded by occlusions ($P < 0.001$, ES = 1.74 (CI: 1.21, 2.25), occlusion main effect) with this effect being similar in males and females ($P = 0.62$, ES = 0.17 (CI: −0.49, 0.82), unpaired *t*-test) (Fig. 3*D*). The mean rate of the substrate-level phosphorylation was 1.8 times larger in the bouts preceded by ischaemia ($P < 0.001$, ES = 1.72 (CI: 1.20, 2.24), occlusion main effect) and was similar in both sexes ($P = 0.30$, ES = 0.35 (CI: −0.32, 1.01), sex contrast) (Table 2, see O_2 deficit in ml kg LLM^{-1} s^{-1}).

The O_2 expended per unit of work in the first bout was similar in males and females (142.1 ± 13.7 and 144.5 ± 19.3 ml kJ^{-1}, respectively, $P = 0.67$, ES = −0.14 (CI: −0.80, 0.51), sex contrast, ANOVA) (Table 2). The O_2 expended per unit of work was increased from 144.9 ± 18.7 ml kJ^{-1} in the first bout with free circulation recovery to 171.7 ± 16.7 and 172.5 ± 18.1 ml kJ^{-1}, in

Table 2. Performance and cardiorespiratory variables during three bouts of constant intensity exercise at 120% of $\dot{V}_{O_2max}$ until exhaustion, interspaced with 20 s recovery periods with and without ischaemia in 18 males and 18 females (means ± SD)

		Occlusion during recovery			Free circulation during recovery								
		Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3	Oc	Oc × Sex	Bout	Bout × Sex	Oc × Bout	Oc × Sex × Bout
Time to exhaustion (s)*	M	122.2 ± 24.8	22.6 ± 10.1	17.8 ± 8.6	145.4 ± 42.1	43.0 ± 9.7	32.6 ± 7.6	<0.001	0.62	<0.001	0.34	<0.001	0.88
	W	116.5 ± 29.2	26.5 ± 14.8	20.6 ± 13.6	130.2 ± 33.2	43.8 ± 11.3	35.6 ± 8.5						
Accumulated O_2 (ml)	M	5219 ± 1514	990 ± 584	763 ± 481	6527 ± 2643	2292 ± 720	1744 ± 627	<0.001	0.49	<0.001	0.53	<0.001	0.90
	W	3455 ± 926	854 ± 705	641 ± 606	3909 ± 1278	1518 ± 416	1261 ± 371						
Accumulated CO_2 (ml)*	M	5974 ± 1892	1382 ± 769	1026 ± 626	7722 ± 3312	3000 ± 856	2182 ± 741	<0.001	0.35	<0.001	0.27	<0.001	0.89
	W	3776 ± 1195	1154 ± 815	823 ± 657	4333 ± 1578	1936 ± 497	1542 ± 424						
O_2 deficit (ml)	M	3012 ± 769	508 ± 163	439 ± 153	3282 ± 868	600 ± 165	462 ± 147	0.040	0.92	<0.001	0.062	0.39	0.99
	W	1726 ± 381	378 ± 184	318 ± 179	1847 ± 433	412 ± 123	313 ± 94						
O_2 deficit (ml kg LLM^{-1} s^{-1})	M	1.26 ± 0.22	1.30 ± 0.44	1.40 ± 0.40	1.18 ± 0.26	0.74 ± 0.23	0.75 ± 0.26	<0.001	0.76	<0.001	0.85	<0.001	0.58
	W	1.13 ± 0.30	1.16 ± 0.44	1.27 ± 0.43	1.08 ± 0.30	0.71 ± 0.21	0.67 ± 0.24						
$\dot{V}_{O_2}O_2$/work (ml kJ^{-1})	M	139.7 ± 11.8	137.8 ± 27.0	130.7 ± 28.4	144.5 ± 18.2	173.5 ± 17.6	173.0 ± 18.6	<0.001	0.37	<0.001	0.88	<0.001	0.85
	W	143.9 ± 21.4	141.8 ± 30.5	133.3 ± 27.6	145.2 ± 19.8	169.8 ± 16.0	172.0 ± 18.0						
$\dot{V}_E$ (l min^{-1})	M	91.1 ± 27.0	125.0 ± 32.0	112.0 ± 35.0	89.3 ± 19.4	145.2 ± 22.3	147.8 ± 21.6	<0.001	0.007	<0.001	0.043	<0.001	0.105
	W	64.9 ± 24.3	92.6 ± 12.9	88.4 ± 15.0	60.2 ± 11.6	94.7 ± 14.6	99.2 ± 14.1						
$\dot{V}_E/\dot{V}_{O_2}$	M	30.8 ± 3.5	47.7 ± 7.0	45.6 ± 8.9	31.1 ± 4.1	43.8 ± 5.5	44.6 ± 4.7	0.002	0.078	<0.001	0.19	0.001	0.129
	W	30.5 ± 3.2	50.9 ± 9.7	51.5 ± 10.1	31.0 ± 3.9	42.4 ± 5.9	44.3 ± 5.9						
$\dot{V}_E/\dot{V}_{CO_2}$	M	27.5 ± 2.5	32.5 ± 4.5	32.3 ± 6.4	27.1 ± 2.9	33.1 ± 4.0	35.3 ± 3.8	0.51	0.19	<0.001	0.55	0.071	0.124
	W	28.4 ± 2.2	34.7 ± 3.9	35.7 ± 3.8	28.8 ± 2.8	33.1 ± 4.6	35.9 ± 5.1						
P_{ETO_2} (mmHg)	M	109.1 ± 7.3	118.6 ± 2.8	113.9 ± 20.6	107.4 ± 4.2	117.3 ± 3.1	118.1 ± 2.4	0.50	0.30	<0.001	0.27	0.82	0.62
	W	108.4 ± 3.1	120.3 ± 3.3	121.0 ± 3.8	108.9 ± 3.9	117.2 ± 4.0	118.6 ± 3.6						
P_{ETCO_2} (mmHg)	M	39.6 ± 4.2	33.7 ± 3.8	31.1 ± 7.2	39.0 ± 3.7	32.9 ± 3.6	30.9 ± 3.0	0.81	0.21	<0.001	0.049	0.88	0.88
	W	35.7 ± 3.1	30.8 ± 3.1	29.5 ± 2.9	36.1 ± 3.1	32.4 ± 4.4	30.0 ± 4.1						
RR (breaths min^{-1})	M	36.0 ± 6.2	50.4 ± 10.7	47.0 ± 14.2	36.6 ± 6.7	55.6 ± 10.1	56.8 ± 8.9	0.23	<0.001	<0.001	0.18	<0.001	0.002
	W	36.4 ± 8.0	55.5 ± 6.8	55.2 ± 7.3	34.9 ± 5.6	49.0 ± 7.4	53.5 ± 8.0						
RER	M	1.12 ± 0.10	1.44 ± 0.09	1.35 ± 0.26	1.14 ± 0.07	1.33 ± 0.08	1.26 ± 0.06	<0.001	0.14	<0.001	0.068	0.035	0.017
	W	1.06 ± 0.07	1.45 ± 0.17	1.43 ± 0.19	1.07 ± 0.08	1.29 ± 0.06	1.24 ± 0.05						
HR (beats min^{-1})	M	161.1 ± 11.6	174.2 ± 9.6	172.6 ± 11.2	164.6 ± 9.5	180.2 ± 6.4	181.4 ± 6.8	0.002	0.40	<0.001	0.09	0.038	0.29
	W	166.1 ± 10.0	177.1 ± 9.5	176.3 ± 9.2	169.1 ± 11.8	180.2 ± 9.6	180.9 ± 9.1						

P-values reported in the last six columns. *Statistical analysis with logarithmically transformed values. Accumulated O_2 and CO_2: total amount of O_2 and CO_2 consumed and produced during the bout; Oc: recovery main effect (occlusion *vs.* free circulation); bout: bout main effect. HR, heart rate; LLM, lean mass of the lower extremities; P_{ETCO_2}, end tidal CO_2 pressure; P_{ETO_2}, end tidal O_2 pressure; RER, respiratory exchange ratio; RR, respiratory rate; $\dot{V}_E$, pulmonary ventilation; $\dot{V}_{O_2}$, oxygen uptake; O_2/work, amount of O_2 consumed per work unit.

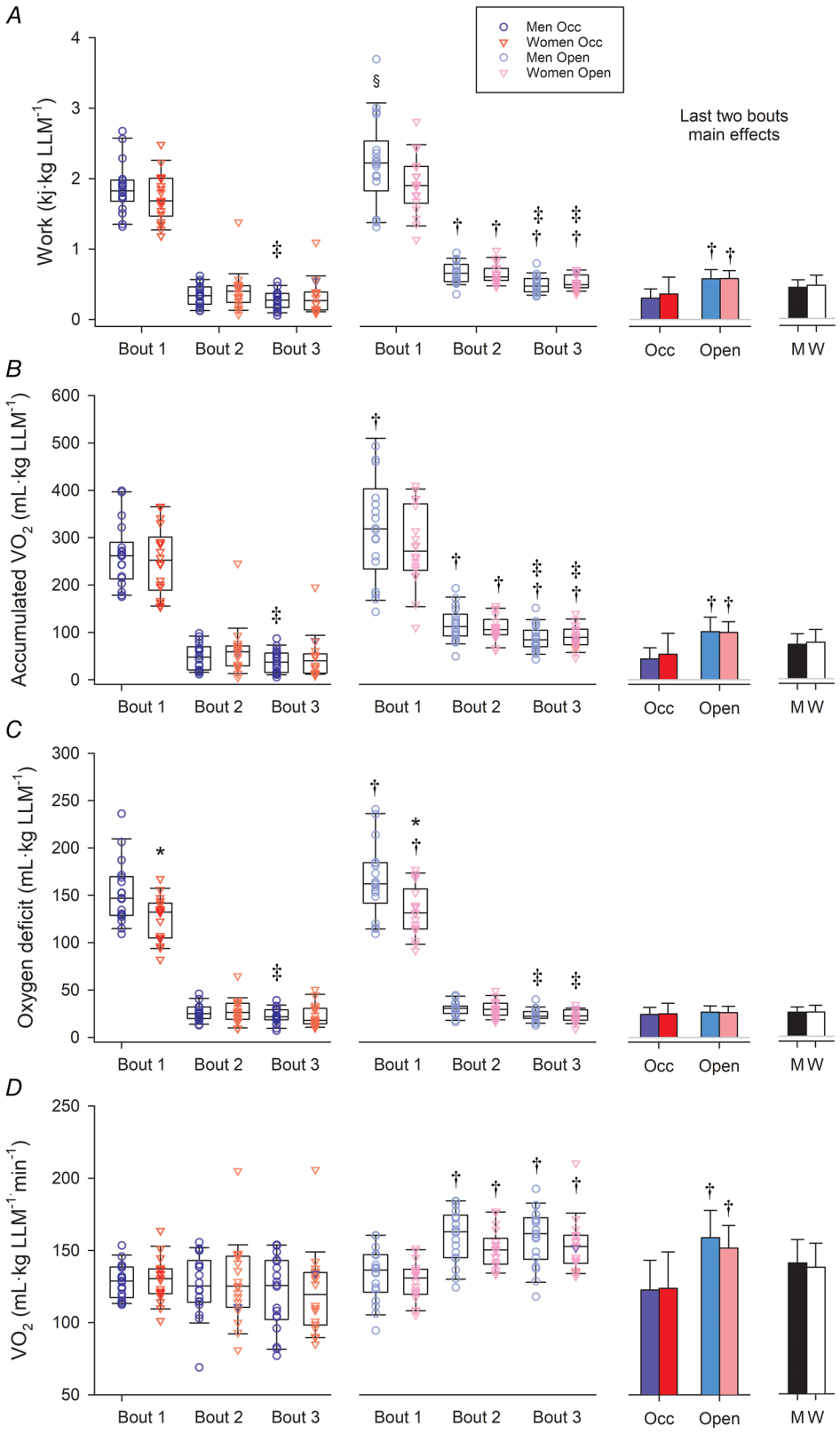

Figure 2. Performance and overall energy expenditure during three bouts of constant power exercise to exhaustion at 120% of $\dot{V}_{O_2max}$, interspaced by 20 s recovery periods with (dark colours) or without (light colours) ischaemia

A, work; *B*, accumulated oxygen uptake; *C*, O_2 deficit; *D*, mean rate of oxygen consumption during the whole exercise bout ($\dot{V}_{O_2}$). Black bars: mean responses for the last two bouts in females; white bars: mean responses for the last two bouts in males. Error bars: standard deviation; *$P < 0.05$ males compared to females; †$P < 0.05$ free circulation compared to occlusion; ‡$P < 0.05$ compared to the second bout. $n = 18$ males and $n = 18$ females. LLM,: lean mass of the lower extremities. [Colour figure can be viewed at wileyonlinelibrary.com]

the second and third bouts, respectively (both $P < 0.001$ compared to the first bout, ES = −2.44 (CI: −3.09, −1.78) and ES = −1.85 (CI: −2.39, −1.30), respectively, paired *t*-test), reflecting a greater reliance on the aerobic metabolism in the last two bouts of exercise when preceded by free circulation recovery. However, the O_2 consumed per work unit was 141.8 ± 17.2, 139.8 ± 28.5 and 132.0 ± 27.6 ml kJ^{-1}, in the first, second and third bouts of the experiments with ischaemic recovery (first compared with second: $P = 0.57$, ES = 0.09 (CI: −0.23, 0.42); first compared with third, $P = 0.020$, ES = 0.41 (CI: 0.06, 0.75), paired *t*-tests) (Table 2).

The mean HR was 5.7 beats min^{-1} lower during the second and third bouts preceded by ischaemia compared to the same bouts preceded by recovery with free circulation ($P < 0.001$, ES = −0.68 (CI: −0.96, −0.25), occlusion main effect, ANOVA), with similar responses in males and females ($P = 0.56$, ES = −0.19 (CI: −0.85, 0.46), sex contrast) (Table 2).

Sex differences in fatigability and recovery during repeated supramaximal exercise to exhaustion

No sex differences were observed in times to exhaustion (and work), in the second and third bouts ($P = 34$, ES = −0.32 (CI: −0.98, 0.34), sex contrast; $P = 0.88$, occlusion by sex by bout interaction) (Table 2).

There were no sex differences in recovery of work capacity after the first bout of exercise ($P = 0.06$, ES = −0.64 (CI: −1.31, 0.06), sex contrast, ANOVA). After the first 120% CP bout with total occlusion of the circulation during the recovery period, males and females performed 18.5 ± 8.3 and 22.7 ± 12.7% of the initial work ($P = 0.24$, ES = −0.40 (CI: −1.06, 0.27), unpaired *t*-test, males *vs*. females) (Fig. 3*A*). After the second 120% CP bout with post-exercise occlusion, they performed 14.6 ± 7.0 and 17.6 ± 11.6% of the initial work, respectively ($P = 0.54$, ES = −0.20 (CI: −0.86, 0.45), unpaired *t*-test males *vs*. females) (Fig. 3*A*). Recovery with free circulation allowed for a greater restoration of performance in both sexes, but males had a poorer recovery than females (average of the last two bouts: 26.0 ± 5.7 and 30.5 ± 7.2% of initial work for males and females, respectively, $P = 0.045$, ES = −0.69 (CI: −1.38, 0.01), unpaired *t*-test males *vs*. females) (Fig. 3*A*).

However, due to their lower AOD attained in the first bout, females recovered a greater fraction of their AOD than males (average of the last two bouts with and without occlusion: 16.0 ± 4.0 and 19.9 ± 6.0%, males and females, respectively, $P = 0.027$, ES = −0.69 (CI: −1.46, −0.06), sex contrast, ANOVA) (Fig. 3*C*).

Brain oxygenation and relative muscle O_2 extraction

At rest, males had higher brain tissue oxygenation index (TOI) than females (67.1 ± 3.9 and 62.9 ± 3.7 TOI units, respectively, $P = 0.002$, ES = 1.11 (CI: 0.34, 1.86), sex contrast, ANOVA). Brain oxygenation was 4–6 units (TOI) lower in females than males during all exercise bouts ($P < 0.001$, ES = 1.25 (CI: 0.45, 2.02), sex contrast, ANOVA) (Fig. 4*A*) (Table 2). During the first exercise bout, brain oxygenation was increased by 1 TOI unit ($P = 0.001$, ES = −0.60 (CI: −0.96, −0.24), paired *t*-test), and then was reduced by 2 ($P = 0.009$, ES = 0.47 (CI: 0.11, 0.81), paired *t*-test), and 3 TOI units ($P < 0.001$, ES = 0.98 (CI: 0.57, 1.38), paired *t*-test), in the second and third bout, respectively (bout main effect $P < 0.001$, ANOVA), with similar responses in males and females (Fig. 4*A*) ($P = 0.45$, bout × sex interaction; $P = 0.82$, occlusion × sex interaction, ANOVA).

At rest, vastus lateralis TOI values were similar in males and females (75.7 ± 3.4 and 75.5 ± 2.7, respectively, $P = 0.82$, ES = 0.08 (CI: −0.59, 0.74), unpaired *t*-test). During exercise, vastus lateralis oxygenation was reduced to 63.2 ± 4.9 ($P < 0.001$, ES = 2.45 (CI: 1.78, 3.11)), 62.6 ± 5.1 ($P < 0.001$, ES = 2.48 (CI: 1.80, 3.14)), and 62.3 ± 5.1 A.U. ($P < 0.001$, ES = 1.83 (CI: 1.83, 3.19)), during the first, second and third bouts, respectively (bout main effect $P < 0.001$; all $P < 0.001$ compared to rest, paired *t*-test), well above the minimum value observed during ischaemia (52.1 ± 7.5 and 57.2 ± 6.6 A.U., in males and females, respectively, $P = 0.04$, ES = −0.72 (CI: −1.42, −0.01), sex contrast, ANOVA) (Fig. 4*B*). Females exhibited greater relative O_2 extraction capacity than males in the fatigued state, as reflected by the lower difference in TOI between the mean value observed during the last two bouts of exercise and the value corresponding to ischaemia, here called TOI O_2 extraction index (see Appendix) (bout × sex interaction $P = 0.01$; 9.2 ± 4.1 and 6.5 ± 2.7 A.U., for males and females, respectively $P = 0.031$, ES = 0.76 (CI: 0.04, 1.46), sex contrast, ANOVA; a lower value indicates more O_2 extraction) (Fig. 4*C*). In the whole group of subjects, relative muscle extraction capacity was enhanced in the exercise bouts performed after ischaemia (occlusion main effect $P = 0.011$; bout × occlusion interaction $P < 0.001$). The mean muscle extraction index during the last two bouts was 6.1 ± 3.6 and 9.6 ± 5.9 A.U., for

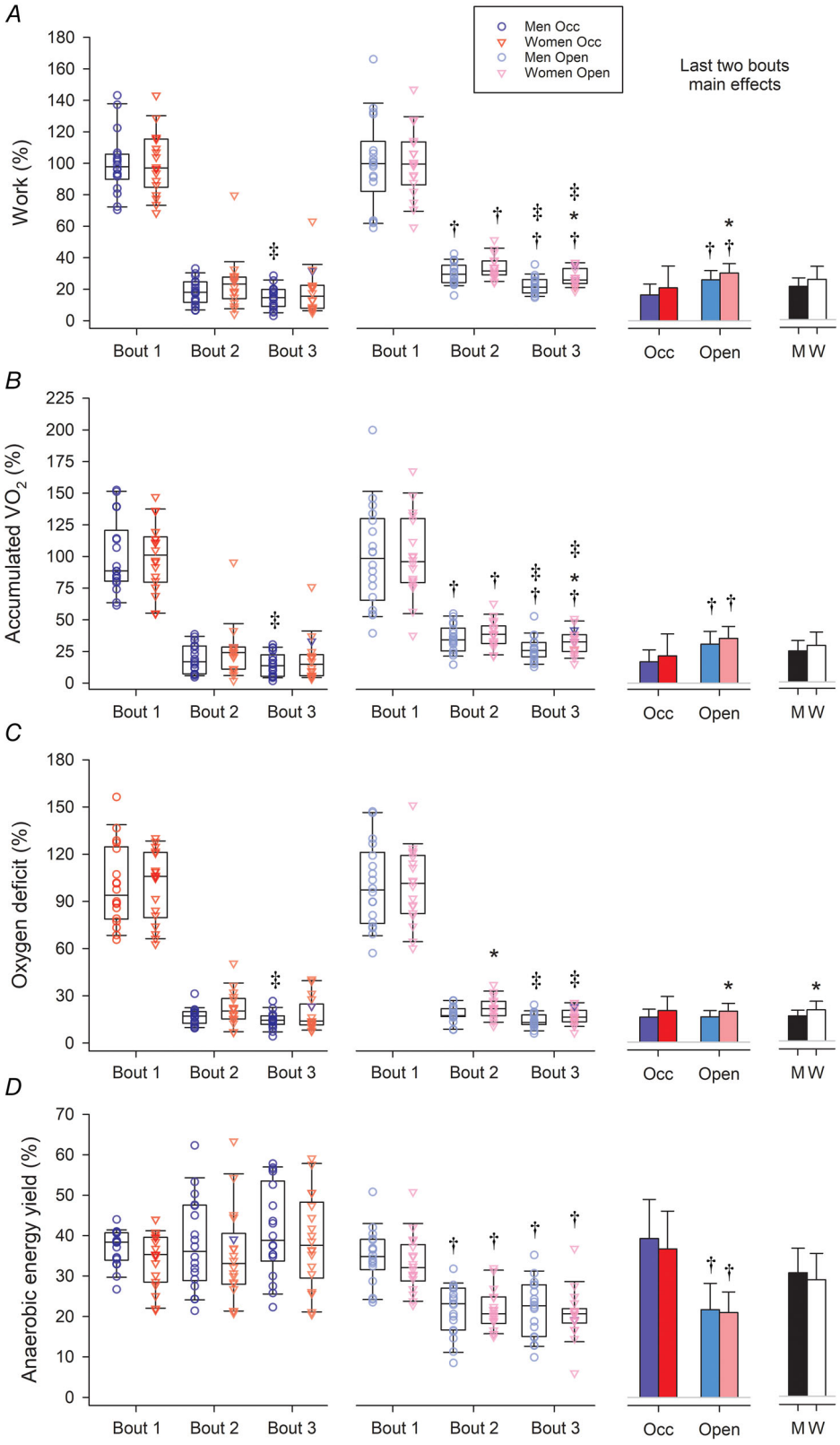

Figure 3. Performance and overall energy expenditure during three bouts of constant power exercise to exhaustion at 120% of $\dot{V}_{O_2max}$, interspaced by 20 s recovery periods with (dark colours) or without (light colours) ischaemia

A, work; *B*, accumulated oxygen; *C*, O_2 deficit; *D*, anaerobic energy yield as percentage of the whole energy expenditure. In *A*–*C* values are normalized to the first bout, of each experimental day, i.e. the first bout is taken as 100%. Black bars: mean responses for the last two bouts in females; white bars: mean responses for the last two bouts in males. Error bars: standard deviation. *$P < 0.05$ males compared to females; †$P < 0.05$ free circulation compared to occlusion; ‡$P < 0.05$ compared to the second bout. $n = 18$ for males and $n = 18$ for females. [Colour figure can be viewed at wileyonlinelibrary.com]

the occlusion and free circulation, respectively, $P = 0.002$, ES = −0.60 (CI: −0.96, −0.24), occlusion main effect, ANOVA) (Fig. 4*C*).

Cardiorespiratory responses, brain oxygenation and metaboreflex activation during the recovery periods

Cardiorespiratory responses during the recovery periods are summarized in Table 3. The occlusion of the circulation reduced the $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ by 24.1 ($P < 0.001$, ES = −1.81 (CI: −2.34, −1.27)), and 14.4% ($P < 0.001$, ES = −1.09 (CI: −1.49, −0.67)), respectively, with similar responses in males and females ($P = 0.63$, ES = 0.16 (CI: −0.49, 0.82) and 0.53 ES = 0.21 (CI: −0.45, 0.86), unpaired *t*-test), for the between-sex comparison, respectively, sex contrast, ANOVA). During recovery, $\dot{V}_E$ was not significantly altered by the occlusion ($P = 0.33$, ES = −0.16 (CI: −0.49, 0.17), occlusion main effect), with similar effects in males and females ($P = 0.26$, occlusion × sex interaction, ANOVA). In contrast, during the occlusion P_{ETCO_2} was reduced by 8.7% ($P < 0.001$, ES = −0.93 (CI: −1.32, 0.53)), and P_{ETO_2} increased by 4.4% ($P < 0.001$, ES = 1.51 (CI: 1.02, 1.98)), with similar responses in males and females ($P = 0.93$, ES = −0.03 (CI: −0.68, 0.62) and 0.97 ES = −0.01 (CI: −0.66, 0.64)), for the between-sex comparison, respectively, sex contrast, ANOVA). During the occlusions, $\dot{V}_E/\dot{V}_{O_2}$, $\dot{V}_E/\dot{V}_{CO_2}$ and RER were increased, while tidal volume (V_T) and HR were reduced by 28.8 ($P < 0.001$, ES = 1.56 (CI: 1.06, 2.04)), 12.7 ($P < 0.001$, ES = 0.94 (CI: 0.45, 1.33)), 13.6 ($P < 0.001$, ES = 1.73 (CI: 1.20, 2.24)), 5.9 ($P < 0.001$, ES = 0.70 (CI: −1.06, −0.33)) and 4.2% ($P < 0.001$, ES = −0.88 (CI: −1.29, −0.46), all occlusion main effects), respectively, with similar responses in males and females ($P = 0.42$, ES = −0.27 (CI: −0.93, 0.39); $P = 0.43$, ES = −0.27 (CI: −0.92, 0.40); $P = 0.85$, ES = −0.06 (CI: −0.72, 0.59); $P = 0.71$, ES = 0.12 (CI: −0.53, 0.78); and $P = 0.74$, ES = −0.12 (CI: −0.82, 0.59); for the between-sex comparisons, respectively, ANOVA).

The $\dot{V}_{O_2}$ per kg of LLM was similar in males and females, during recovery with occlusion ($P = 0.84$, ES = −0.06 (CI: −0.72, 0.59)) and free circulation ($P = 0.55$, ES = 0.20 (CI: −0.46, 0.85), both unpaired *t*-test for the mean of the two recoveries). The legs contributed to 24.6 ± 8.7% of the O_2 debt during the first 20 s of recovery, with this fraction being similar in males and females (i.e. 24.4 ± 9.6 and 24.9 ± 7.9%, respectively, $P = 0.86$, ES = −0.06 (CI: −0.72, 0.61), sex contrast, ANOVA). The O_2 debt of the leg muscles after the first bout of exercise was similar in males and females (11.6 ± 5.1 and 11.4 ± 4.1 ml kg^{-1}, $P = 0.89$, ES = 0.05 (CI: −0.62, 0.71), unpaired *t*-test), as well as after the second bout of exercise (13.7 ± 6.1 and 13.3 ± 5.4 ml kg^{-1}, $P = 0.84$, ES = 0.07 (CI: −0.60, 0.73), unpaired *t*-test).

Brain oxygenation was 4–6 units (TOI) lower in females than males during the recovery periods ($P = 0.003$, ES = 1.10 (CI: 0.33, 1.85), sex contrast) (Table 3). Brain oxygenation was ∼1.5 units lower in the second than in the first recovery period ($P < 0.001$, ES = 1.40 (CI: 0.92, 1.86), bout main effect, ANOVA), and this response was similar during the experiments with and without occlusion, and in men and females (Table 3).

The muscle pain reported during the occlusions was similar in males and females (7.1 ± 2.3 and 6.8 ± 1.8 A.U., $P = 0.70$, ES = 0.13 (CI: −0.54, 0.81), sex contrast, ANOVA).

Discussion

The main finding of the present investigation is the demonstration that males and females respond similarly to repeated supramaximal whole-body exercise to exhaustion and that at task failure a large functional reserve remains in both sexes. Although males and females performed the exercise at an intensity which should have exhausted the anaerobic capacity (Medbo & Tabata, 1993; Gastin *et al.* 1995; Calbet *et al.* 1997), a similar functional reserve in substrate-level phosphorylation energy supply per kg of lower extremities lean mass was observed at task failure in both sexes. Using indirect methods, we have shown that this functional reserve depends on the glycolytic component of substrate-level phosphorylation.

Accounting for their lower anaerobic capacity and blood haemoglobin concentration, females performed slightly less during the first bout of exercise and similarly to males during the subsequent bouts, likely due to their greater capacity to extract O_2 during high-intensity exercise. Compared to males, females achieved lower P_{ETCO_2} and brain oxygenation during supramaximal exercise to exhaustion, without an apparent negative repercussion for performance.

The anaerobic capacity does not determine task failure during supramaximal exercise to exhaustion

In agreement with previous studies, we have found that males incurred higher maximal O_2 deficits than

Table 3. Cardiorespiratory variables during the 20 s recovery periods after constant intensity exercise at 120% of $\dot{V}_{O_2max}$ until exhaustion in 18 males and 18 females (means ± SD)

		Occlusion 1	Occlusion 2	Recovery with free circulation 1	Recovery with free circulation 2	Oc	Oc × Sex	Bout	Bout × sex	Oc × Bout	Oc × Sex × Bout
Accumulated O_2 (ml)	M	752 ± 102	741 ± 113	980 ± 130	1010 ± 138	<0.001	0.68	0.99	0.41	0.002	0.93
	W	531 ± 104	518 ± 125	674 ± 88	687 ± 23						
Accumulated CO_2 (ml)	M	1160 ± 170	1052 ± 143	1338 ± 221	1274 ± 201	<0.001	0.051	<0.001	0.022	0.049	0.42
	W	763 ± 124	713 ± 136	862 ± 138	831 ± 28						
$\dot{V}_E$ (l min^{-1})	M	119.5 ± 30.4	120.6 ± 28.7	123.0 ± 26.3	129.6 ± 23.7	0.23	0.26	<0.001	0.19	0.019	0.57
	W	76.3 ± 12.6	79.5 ± 11.6	72.1 ± 19.5	84.0 ± 3.2						
$\dot{V}_E/\dot{V}_{O_2}$	M	53.1 ± 11.9	54.6 ± 11.8	41.7 ± 6.6	42.8 ± 5.5	<0.001	0.34	0.002	0.041	0.25	0.26
	W	48.5 ± 7.3	52.2 ± 7.7	36.3 ± 9.8	41.1 ± 1.4						
$\dot{V}_E/\dot{V}_{CO_2}$	M	34.1 ± 5.2	38.0 ± 6.0	30.7 ± 4.8	34.0 ± 4.5	<0.001	0.64	<0.001	0.15	0.51	0.14
	W	33.5 ± 3.5	37.6 ± 3.8	28.3 ± 7.5	33.9 ± 1.2						
P_{ETO_2} (mmHg)	M	119.1 ± 4.5	120.3 ± 4.3	113.9 ± 4.3	115.3 ± 3.3	<0.001	0.99	<0.001	0.31	0.69	0.25
	W	117.7 ± 4.0	119.9 ± 3.5	113.0 ± 3.9	114.6 ± 1.0						
P_{ETCO_2} (mmHg)	M	34.3 ± 4.5	31.0 ± 4.4	37.5 ± 4.7	33.9 ± 3.8	<0.001	0.93	<0.001	0.92	0.63	0.19
	W	34.2 ± 3.9	30.5 ± 3.2	37.0 ± 4.4	33.9 ± 1.1						
RR (breaths min^{-1})	M	44.7 ± 9.5	47.1 ± 10.8	44.4 ± 10.4	47.6 ± 9.8	0.65	0.58	<0.001	0.79	0.80	0.16
	W	40.2 ± 6.1	43.3 ± 5.5	39.9 ± 6.9	42.0 ± 1.5						
V_T (ml)	M	2690 ± 393	2595 ± 376	2814 ± 406	2769 ± 409	<0.001	0.85	0.009	0.28	0.033	0.59
	M	1933 ± 343	1862 ± 309	2028 ± 386	2039 ± 78						
RER	M	1.55 ± 0.15	1.43 ± 0.12	1.36 ± 0.09	1.26 ± 0.08	<0.001	0.94	<0.001	0.018	0.73	0.30
	W	1.44 ± 0.13	1.39 ± 0.13	1.28 ± 0.09	1.21 ± 0.01						
Accumulated O_2 LLM (ml kg^{-1})	M	38.1 ± 3.8	37.6 ± 4.3	49.7 ± 5.1	51.3 ± 5.7	<0.001	0.68	0.99	0.41	0.002	0.93
	W	38.8 ± 8.0	37.8 ± 9.4	49.0 ± 5.1	49.9 ± 1.3						
Accumulated CO_2 LLM (ml kg^{-1})	M	58.8 ± 6.9	53.3 ± 5.3	67.8 ± 8.8	64.6 ± 8.0	<0.001	0.38	<0.001	0.24	0.041	0.65
	W	55.6 ± 9.6	52.0 ± 9.8	62.6 ± 8.4	60.4 ± 1.6						
Brain oxygenation (TOI) (A.U.)	M	68.1 ± 6.7	66.7 ± 6.8	65.9 ± 6.0	64.5 ± 6.4	0.37	0.53	<0.001	0.58	0.57	0.74
	W	62.4 ± 5.6	61.0 ± 5.7	62.9 ± 5.9	61.2 ± 1.5						
HR (beats min^{-1})*	M	171.6 ± 12.2	172.1 ± 12.7	178.2 ± 7.1	181.5 ± 6.6	<0.001	0.78	<0.001	0.98	0.20	0.17
	W	174.5 ± 10.2	176.5 ± 10.4	181.7 ± 9.1	183.6 ± 1.8						

Accumulated O_2: total amount of O_2 consumed the recovery period; accumulated CO_2: total amount of CO_2 produced during the recovery period; Oc: recovery main effect (occlusion *vs.* free circulation); bout: bout main effect. *P*-values reported in the last six columns. *n = 16 M and 16 W; rest n = 18 M and 18 W. A.U., arbitrary units; HR, heart rate; LLM, lean mass of the lower extremities; P_{ETCO_2}, end tidal CO_2 pressure; P_{ETO_2}, end tidal O_2 pressure; RER, respiratory exchange ratio; RR, respiratory rate; TOI, tissue oxygenation index; $\dot{V}_E$, pulmonary ventilation; $\dot{V}_{O_2}$, oxygen uptake; V_T, tidal volume.

females (Medbo & Burgers, 1990; Weyand *et al.* 1993; Ramsbottom *et al.* 1997; Hill & Vingren, 2014), but as a novelty, here we show that this greater capacity of males persists after accounting for differences in muscle mass. Nevertheless, after the second and third bouts to exhaustion, the capacity to produce energy through substrate-level phosphorylation was similar in both sexes, after normalization to the LLM. Neither in males nor in females was task failure caused by exhaustion of anaerobic capacity. Otherwise, resumption of exercise would have

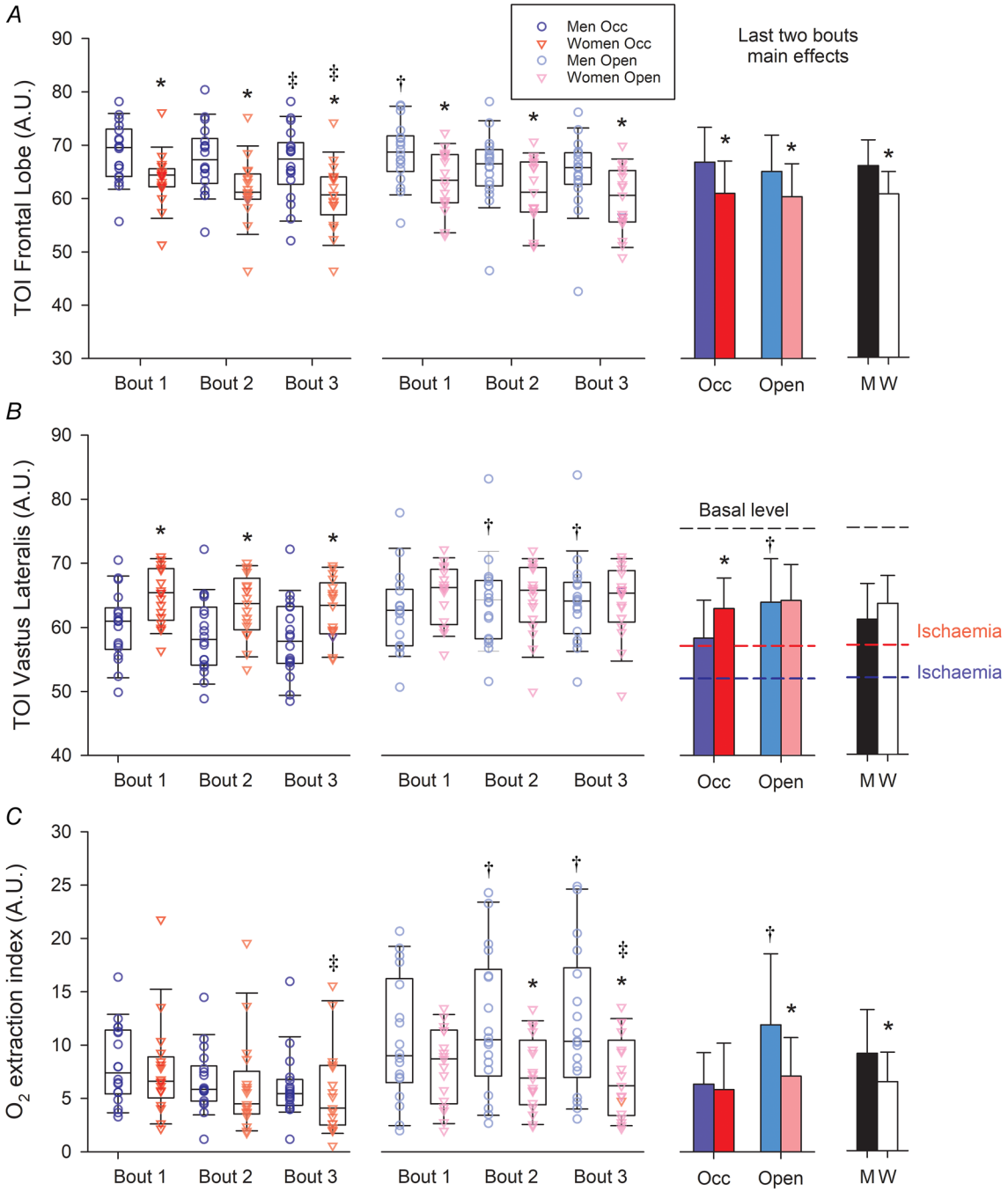

Figure 4. Tissue oxygenation index (TOI) and vastus lateralis O_2 extraction index
Brain oxygenation (*A*), vastus lateralis oxygenation (*B*), and vastus lateralis O_2 extraction (*C*) during three bouts of constant intensity exercise to exhaustion at 120% of $\dot{V}_{O_2max}$, interspaced by 20 s recovery periods with (dark colours) or without (light colours) ischaemia. Black bars: mean responses for the last two bouts in females; white bars: mean responses for the last two bouts in males. Error bars: standard deviation. *$P < 0.05$ males compared to females; †$P < 0.05$ free circulation compared to occlusion; ‡$P < 0.05$ compared to the second bout. $n = 18$ males and $n = 17$ for females. [Colour figure can be viewed at wileyonlinelibrary.com]

been impossible after the occlusion because the intensity was supramaximal. Since the occlusion of the circulation at exhaustion impedes the recovery of PCr and muscle pH (Harris *et al.* 1976; Blei *et al.* 1993; Quistorff *et al.* 1993; Morales-Alamo *et al.* 2015), and the exercise intensity was always above $\dot{V}_{O_2max}$, our subjects would not have been able to restart pedalling at the end of the first and second 20 s occlusions without such a functional reserve in substrate-level phosphorylation. Since previous work has demonstrated that at the moment of occlusion PCr levels are very low, and ADP and Cr concentration increased (Morales-Alamo *et al.* 2015), mitochondrial respiration should be maximally stimulated (Sahlin & Harris, 2011; Calbet *et al.* 2020). Consequently, in less than 3–5 s the small amount of O_2 remaining bound to myoglobin (Richardson *et al.* 1995) and the O_2 present in the capillary beds (Harris *et al.* 1975; Blei *et al.* 1993) is consumed, as previously explained (Morales-Alamo *et al.* 2015). During the occlusion, the glycolytic component of substrate-level phosphorylation remains active, increasing the accumulation of lactate and the level of intracellular acidification (Morales-Alamo *et al.* 2015). Although the circulation was open during the second bout of exercise, no reduction of muscle lactate or increase of PCr concentration is possible during supramaximal exercise (Parolin *et al.* 1999). If anything, the metabolic state should be even worse at the end of the second bout of exercise (Parolin *et al.* 1999) and further impaired if preceded by ischaemia (Morales-Alamo *et al.* 2015). Moreover, as the exercise should have started with very low levels of PCr (Harris *et al.* 1976; Morales-Alamo *et al.* 2015), the only metabolic pathway that can significantly supplement the aerobic energy production during post-occlusion supramaximal exercise (120% above $\dot{V}_{O_2max}$, in the present study) is the glycolytic component of substrate-level phosphorylation (Morales-Alamo *et al.* 2015). This allows for an estimate of the amount of lactate produced during the second and third bouts, which was 3.47 and 2.94 mmol kg LLM^{-1} (i.e. 0.16 and 0.17 mmol kg^{-1} s^{-1}, respectively). These values represent ∼17–20% of the maximal glycolytic rates achieved by six males during a Wingate test (Parolin *et al.* 1999).

Despite this unfavourable metabolic environment, all subjects were able to perform some exercise during the third bout (from 4 to 52 s), revealing that even after the second bout of exercise, and despite impeding muscle recovery with the occlusion, some functional reserve remains at exhaustion. Nevertheless, four males and five females exercised for less than 10 s during the third bout, indicating that some subjects used a large fraction of their functional reserve after the second occlusion.

Effects of post-exercise ischaemia on subsequent exercise

This research shows that muscle O_2 extraction capacity is increased similarly in males and females when the exercise is performed after ischaemia, as indicated by the lower TOI values observed during exercise after ischaemia. Following post-exercise ischaemia, time to exhaustion is reduced similarly in males and females due to a lower provision of energy by oxidative phosphorylation. The lower $\dot{V}_{O_2}$ during exercise after ischaemia is partly compensated for by increasing the rate of substrate-level phosphorylation to 1.8-fold above that observed when the exercise is performed after recovery with free circulation, with similar responses in males and females. The latter demonstrates that task failure is not caused by the achievement of a limiting glycolytic rate. Moreover, the fact that the O_2 deficit was the same during the bouts performed after ischaemia and free circulation implies that fatigue occurs after a defined amount of energy has been produced through substrate-level phosphorylation, and that the mechanism causing task failure is likely linked to a direct or indirect effect of the metabolites and ion disturbances associated with substrate-level phosphorylation in critical subcellular compartments (Fitts, 1994; Allen *et al.* 2008).

Post-exercise ischaemia may have contributed to either facilitate or impair recovery by several mechanisms. Post-exercise ischaemia may have facilitated recovery, first, through an increase of muscle temperature (Coupland *et al.* 2001; Pedersen *et al.* 2003) caused by the elevated metabolic rate during post-exercise ischaemia (Morales-Alamo *et al.* 2015), which combined with the occlusion of the circulation, would limit the transfer of heat (Westerblad *et al.* 1997; Pedersen *et al.* 2003); second, by the positive effects of lactate and H^+ in the recovery of sarcolemmal excitability (Pedersen *et al.* 2003; Allen & Westerblad, 2004; Copithorne *et al.* 2020); third, by reducing the rate of RONS production by mitochondrial respiration due to the lack of O_2 (Bruton *et al.* 2008); fourth, by facilitating the entry of P_i into the sarcoplasmic reticulum, where it might precipitate with Ca^{2+} (Fryer *et al.* 1995; Ferreira *et al.* 2021; Hinks *et al.* 2021); and fifth, by increasing the firing of group III/IV muscle afferents, facilitating motoneuron discharge, as recently suggested by neurophysiological experiments in humans (Brandner *et al.* 2015; Copithorne *et al.* 2020).

Alternatively, post-exercise ischaemia could have exerted a negative influence in the recovery of muscle contractile capacity by several mechanisms. First, it may have done so by increasing nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase (NOX) and xanthine oxidase (XO) RONS production at the start of the exercise by an ischaemia–reperfusion mechanism,

which may have elicited the production of OH· and H_2O_2 through the Fenton reaction (Morales-Alamo & Calbet, 2014; Larsen *et al*. 2016; Calbet *et al*. 2020). This is supported by the fact that administration of polyphenolic compounds with potent direct (by quenching RONS) and indirect (by inhibiting XO and NOX RONS production) antioxidant effects enhances O_2 consumption and performance when subjects are requested to sprint maximally with normal blood flow after a short period of post-exhaustive exercise ischaemia (Gelabert-Rebato *et al*. 2018; Gelabert-Rebato *et al*. 2019*b*). Second, it may have exerted a negative influence by reducing the levels of muscle glycogen (Gejl *et al*. 2014; Jensen *et al*. 2020) due to the reliance on glycolysis to maintain cellular viability in the absence of O_2 (Morales-Alamo *et al*. 2015). However, since the reduction in muscle excitability is rather small (Senefeld *et al*. 2018; Copithorne *et al*. 2020) and all subjects were able to perform the second and the third exercise bouts despite the occlusions, our results suggest that task failure is elicited by a muscle mechanism that can be recovered very quickly (within seconds) despite the progressive accumulation of metabolites during ischaemia, and/or by a mechanism residing outside the skeletal muscles, i.e. in the central nervous system (Kayser, 2003; Marcora & Staiano, 2010; Calbet *et al*. 2015; Morales-Alamo *et al*. 2015; Torres-Peralta *et al*. 2016; Angius *et al*. 2018).

During a single bout of supramaximal exercise, females produce less ATP through substrate-level phosphorylation, but both sexes recruit anaerobic pathways similarly in subsequent bouts of supramaximal exercise

In the present investigation, females incurred a lower O_2 deficit during the first exercise bout and had a lower increase in RER during the exercise, both compatible with less activation of glycolysis during the first exercise bout. This interpretation agrees with the low RER values observed at exhaustion in MacArdle's disease patients despite remarkable hyperventilation. MacArdle's disease patients cannot rely on the glycolytic component of substrate level phosphorylation during high-intensity exercise (Hagberg, 1982).

Our results concur with the reported lower muscle lactate accumulation after repeated sprint exercise in females than males (Jacobs *et al*. 1983), as well as lower activation of glycogenolysis in females than males in type I fibres after repeated Wingate tests (Esbjornsson-Liljedahl *et al*. 2002). Compared to males, females have lower activities of some enzymes regulating the glycolytic rate, such as lactate dehydrogenase (Green *et al*. 1984; Simoneau & Bouchard, 1989; Esbjornsson Liljedahl et al. 1996), hexokinase (Green *et al*. 1984; Simoneau & Bouchard, 1989), phosphofructokinase (Green *et al*. 1984; Simoneau & Bouchard, 1989; Jaworowski *et al*. 2002), pyruvate kinase (Green *et al*. 1984) and phosphorylase (Green *et al*. 1984). Although differences in enzyme activities could account for a lower maximal glycolytic rate, they cannot explain why during the second and third bouts the contribution of glycolysis was the same in both sexes, as demonstrated by the almost identical O_2 deficits and calculated glycolytic rates observed in the second and third bouts performed after ischaemia.

Interestingly, the O_2 deficits were similar in the bouts with free circulation compared to the bouts performed after ischaemia. It should be considered that in the bouts performed after ischaemia, the O_2 stores were likely exhausted at the start of the exercise, meaning that all the deficit corresponds to the glycolytic component of substrate-level phosphorylation. In contrast, during recovery with free circulation some PCr resynthesis and replenishment of muscle O_2 stores should have occurred (Harris *et al*. 1975; Bangsbo *et al*. 1990). Therefore, the actual anaerobic energy production must have been 10–20% lower in the bouts preceded by free circulation recovery than calculated from the assessment of the O_2 deficit. The latter would match even more closely the actual O_2 deficits of the bouts preceded and not preceded by ischaemia.

Since performance was 42% greater after recovery with free circulation and the rate of utilization of the O_2 deficit 1.8-fold higher after exercise preceded by ischaemia, these results strongly suggest that task failure occurs after the utilization of a fixed amount of the anaerobic capacity. This is likely related to the accumulation of muscle metabolites altering sarcolemmal excitability, which is rapidly restored upon cessation of contractile activity and by the application of ischaemia (Copithorne *et al*. 2020) or the sequestration of P_i into the sarcoendoplasmic reticulum (Ferreira *et al*. 2021). The recovery with free circulation should have allowed a higher recovery (yet incomplete) of ion perturbations, PCr and O_2 stores, permitting a slowing of the glycolytic substrate-level phosphorylation rate and, therefore, prolonging the exercise bout.

To recapitulate, our data and previous research support that females have a lower anaerobic capacity than men and this may be the main reason why they produce a smaller amount of energy through anaerobic pathways than men in the first exercise bout. In the subsequent exercise bout, the male advantage disappears likely due to the partial inhibition of glycolysis elicited by the accumulation of metabolites (Gaitanos *et al*. 1993), with a greater influence in a muscle with more type II fibres, which are more abundant in males than females. A lower capacity to utilize PCr in females than males is unlikely since no sex differences have been reported for PCr utilization during sprint exercise (Esbjornsson-Liljedahl *et al*. 2002).

Moderately active males and females have similar $\dot{V}_{O_2max}$ values when expressed per kg of active lean mass

Males and females had a similar $\dot{V}_{O_2max}$ when normalized to the lean mass of the lower extremities, in agreement with our previous observation (Perez-Gomez *et al.* 2008). Consequently, during the exercise at 120% of $\dot{V}_{O_2max}$ (first bout) the mean rate of $\dot{V}_{O_2}$ per kg of muscle mass was almost identical, as it was also during the two last bouts of exercise with or without occlusion during the recovery periods. This indicates that the relative intensity was well matched between sexes and the metabolic demand elicited by the supramaximal exercise and the recoveries (with or without occlusion) on the subsequent exercise bouts was similar in both sexes. In both cases, the occlusion limited the capacity to utilize O_2 in the subsequent bout, potentially through partial inhibition of oxidative phosphorylation (Jubrias *et al.* 2003) or due to reduced O_2 delivery to the contracting muscles. The fact that during the second and third bouts of exercise with free circulation both males and females were able to reach mean metabolic rates close to $\dot{V}_{O_2max}$ indicates that there was minimal if any inhibition of oxidative phosphorylation. Since during whole-body exercise $\dot{V}_{O_2max}$ is limited by O_2 delivery, the fact that during the second and third bout preceded by free circulation recovery $\dot{V}_{O_2}$ was close to maximal can only be explained by an almost maximal O_2 conductance and mitochondrial utilization of O_2 in both sexes. Since females have lower haemoglobin concentration, this can only be explained by a larger O_2 extraction. This agrees with the recent observation of higher complex I, complex II and oxidative phosphorylation capacity (complex I + complex II) respiratory capacity (normalized to the amount of mitochondrial protein content) in females than males (Cardinale *et al.* 2018). The latter finding has the effect of lowering mitochondrial activation rate, lowering P_{50} (higher mitochondrial O_2 affinity) in females compared to males (Cardinale *et al.* 2018) and enhancing O_2 extraction capacity (Cardinale *et al.* 2019), explaining the similar $\dot{V}_{O_2}$ observed in the present investigation. In agreement, our NIRS data also indicate greater O_2 extraction capacity in females.

Oxygen debt

Here we report the first assessment of post-exercise O_2 debt of the lower extremities after whole-body exercise to exhaustion using a novel methodological approach. We have demonstrated that the LLM accounts for just 24% of the total O_2 debt paid during the first 20 s of recovery, with the observed values being almost identical between sexes when normalized to the LLM. Our results agree well with the values obtained for quadriceps muscle after exhaustive single-leg knee-extension exercise (Bangsbo *et al.* 1990). The fact that there were no sex differences indicates a similar muscle metabolism after exhaustion in males and females. Only a minor fraction of the O_2 debt is expended in PCr, ATP and glycogen resynthesis (Bangsbo *et al.* 1990); the rest likely represents the energy expended by the ATPases involved in ion regulation, as previously discussed (Morales-Alamo *et al.* 2015). Interestingly, the value of the O_2 debt incurred in the first 20 s of the exercise represented 24% of the $\dot{V}_{O_2max}$, a metabolic rate slightly lower than the 32% of $\dot{V}_{O_2max}$ measured for the vastus lateralis during 60 s ischaemia applied at exhaustion (Morales-Alamo *et al.* 2015). The small difference may relate to differences in the metabolic rates during recovery between different muscles of the lower extremities (Heinonen *et al.* 2012).

Limitations

The main limitation of this study resides in the indirect measurement of substrate-level phosphorylation energy turnover. For this purpose, we have assumed constant muscle efficiency during supramaximal exercise regardless of the level of fatigue (Medbo & Tabata, 1993; Jubrias *et al.* 2008). Although *in vitro* experiments indicate that the muscle efficiency may deteriorate with fatigue (Barclay, 1996), conclusive evidence is lacking in humans (Woledge, 1998). Nonetheless, the effect of severe fatigue on muscle efficiency is likely small during dynamic contractions in humans (Myburgh, 2004; Jubrias *et al.* 2008; Morales-Alamo *et al.* 2015). In our previous study, we applied 10 and 60 s occlusions after incremental exercise to exhaustion (Morales-Alamo *et al.* 2015). Despite a greater level of metabolic disturbance after 60 than 10 s occlusions, the $\dot{V}_{O_2}$ per power output was lower after 60 s of ischemia than after 10 s, which is not compatible with a marked reduction of muscle efficiency during supramaximal exercise under severe fatigue conditions (Morales-Alamo *et al.* 2015). Nevertheless, if muscle efficiency should deteriorate with fatigue, we could have underestimated the actual glycolytic rates. However, the latter would imply that the metabolic functional reserve is even greater than we have estimated.

Although the menstrual cycle phase was not controlled, most studies have shown no impact of the menstrual cycle on high-intensity exercise performance (Botcazou *et al.* 2006; Bushman *et al.* 2006) nor on oxygen deficit (Shaharudin *et al.* 2011).

Despite the high number of subjects included in the study, we cannot rule out a type 2 error in some sex comparisons. For example, males achieved 10 and 12% higher glycolytic rates during the second and third exercise bouts preceded by ischaemic recovery, respectively. Since we report the size effects, these can be used to define the sample size required to determine whether this sex difference is real, and at least 404

Table 4. Relationship between leg O_2 extraction and the tissue oxygenation index (TOI) measured by near-infrared spectroscopy in the vastus lateralis of both legs during an incremental exercise test to exhaustion on the cycle ergometer

Subject	Sex	Intercept	95% CI intercept		Slope	95% CI slope		R^2	SEE
Left leg									
1	M	193.7	158.7	228.6	−1.88	−2.40	−1.37	0.95	2.07
2	M	224.6	139.1	310.2	−2.09	−3.25	−0.93	0.72	5.25
3	M	168.2	122.3	214.1	−1.58	−2.32	−0.83	0.82	5.10
4	F	261.0	233.5	288.5	−2.72	−3.13	−2.31	0.97	1.42
5	M	115.3	108.7	121.9	−0.68	−0.79	−0.57	0.97	1.20
6	M	173.5	147.2	199.7	−1.45	−1.86	−1.04	0.86	3.79
7	F	229.7	168.7	290.7	−2.32	−3.19	−1.45	0.90	2.96
8	F	151.9	119.7	184.1	−1.07	−1.53	−0.61	0.95	1.00
9	F	255.3	182.2	328.3	−2.39	−3.39	−1.38	0.79	2.85
10	M	144.6	124.8	164.3	−1.14	−1.44	−0.84	0.93	1.49
11	F	273.6	247.5	299.7	−2.85	−3.23	−2.47	0.98	1.06
12	M	189.1	169.9	208.3	−1.61	−1.88	−1.35	0.99	0.79
Right Leg									
1	M	168.9	148.0	189.9	−1.51	−1.81	−1.20	0.97	1.62
2	M	251.4	168.6	334.2	−2.54	−3.70	−1.38	0.79	4.06
3	M	167.0	135.5	198.5	−1.54	−2.04	−1.03	0.90	2.67
4	F	293.0	227.8	358.2	−3.29	−4.27	−2.30	0.90	3.66
5	M	139.9	123.7	156.1	−1.08	−1.34	−0.82	0.95	1.93
6	M	253.7	224.4	282.9	−2.59	−3.02	−2.16	0.95	2.68
7	F	159.8	129.5	190.1	−1.42	−1.88	−0.96	0.93	2.62
8	F	125.0	88.1	162.0	−0.73	−1.27	−0.19	0.86	1.44
9	F	266.1	194.9	337.3	−2.74	−3.78	−1.70	0.82	2.66
10	M	134.9	105.8	163.9	−1.04	−1.50	−0.59	0.84	3.10
11	F	305.1	278.8	331.5	−3.24	−3.62	−2.87	0.98	1.08
12	M	183.3	111.7	254.9	−1.57	−2.60	−0.54	0.82	2.15

Leg O_2 extraction reported as % and TOI in TOI units (arbitrary units). All regression analyses were statistically significant at $P < 0.01$. No significant differences were observed between males and females (unpaired *t*-test for intercepts and slopes). The left and right legs had similar intercepts and slopes (paired *t*-test). CI, confidence interval; F, female; M, male; SEE, standard error of estimate.

volunteers (202 males and 202 females) will be required. On the other hand, our conclusion regarding the lack of sex differences in most of the variables assessed is robust given the small magnitude of the differences observed.

In summary, our data indicate that both males and females fatigue to a similar extent and recruit the aerobic and anaerobic energy pathways during repeated supramaximal exercise in a remarkably similar fashion when differences in lean mass (a surrogate of muscle mass in the present study) are considered. Males have higher anaerobic capacity than females, even after normalization to the lower extremities lean mass, but this advantage is only manifested during the first bout of supramaximal exercise. Through the utilization of post-exercise ischaemia, we have shown that both sexes have a functional reserve which depends on the glycolytic component of substrate-level phosphorylation. In the fatigued state, the amount of exercise that can be performed at supramaximal exercise intensities depends on the rate of utilization of the remaining functional reserve, which supplements the aerobic energy production. We have shown that after ischaemia, muscle O_2 extraction capacity is increased, but muscle $\dot{V}_{O_2}$ is reduced similarly in males and females. Metaboreflex activation during post-exercise ischaemia and the O_2 debt per kg of active lean mass are also similar in males and females after supramaximal exercise. Finally, P_{ETCO_2} and brain oxygenation are lower in females than males, without apparent negative repercussion on performance. Females had no faster recovery of performance after accounting for sex differences in lean mass.

Appendix

Validation of the near-infrared spectroscopy assessment of muscle O_2 extraction

In the present investigation, muscle O_2 extraction was indirectly assessed using NIRS, as described in Methods.

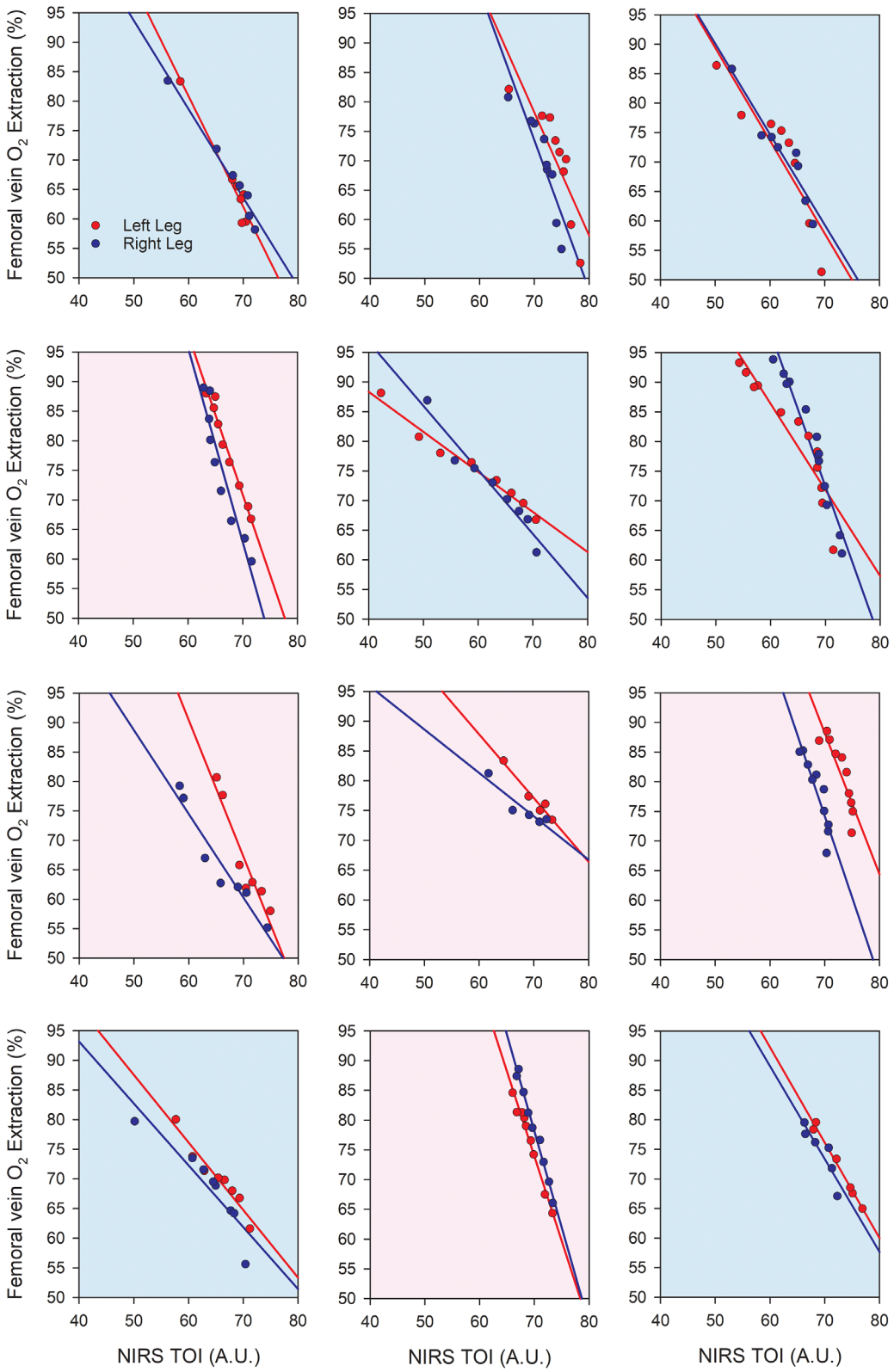

Figure 5. Relationship between the tissue oxygenation index (TOI) and leg O_2 extraction in 12 volunteers, seven males (blue backgrounds) and five females (pink backgrounds), during an incremental exercise test to exhaustion on the cycle ergometer

Dark-blue and red colours represent the right and left leg, respectively. The individual parameters defining the regression lines are reported in Table 4. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 5. Relationship between leg O_2 extraction and TOI (tissue oxygenation index) O_2 extraction index computed as the difference between TOI observed (TOI_{OBV}) and the minimum TOI registered (TOI_{MIN}) during a 60 s ischaemia applied instantaneously at task failure, at the end of an incremental exercise to exhaustion on the cycle ergometer

Subject	Sex	Intercept	95% CI intercept		Slope	95% CI slope		R^2	SEE
Left leg									
1	M	96.0	87.5	104.4	−1.88	−2.40	−1.37	0.95	2.07
2	M	104.3	85.0	123.6	−2.09	−3.25	−0.93	0.72	5.25
3	M	105.6	88.8	122.3	−1.58	−2.32	−0.83	0.82	5.10
4	F	106.3	102.6	110.0	−2.70	−3.05	−2.36	0.98	1.21
5	M	87.0	84.8	89.1	−0.68	−0.79	−0.57	0.97	1.20
6	M	99.3	93.6	105.1	−1.45	−1.86	−1.04	0.86	3.79
7	F	95.9	84.7	107.1	−2.32	−3.19	−1.45	0.90	2.96
8	F	91.6	85.2	98.0	−1.07	−1.53	−0.61	0.95	1.00
9	F	117.9	102.4	133.4	−2.39	−3.39	−1.38	0.79	2.85
10	M	88.7	83.6	93.7	−1.14	−1.44	−0.84	0.93	1.49
11	F	107.7	103.5	111.9	−2.85	−3.23	−2.47	0.98	1.06
12	M	105.1	99.6	110.5	−1.61	−1.88	−1.35	0.99	0.79
Right Leg									
1	M	90.8	85.7	95.8	−1.51	−1.81	−1.20	0.97	1.62
2	M	105.2	88.7	121.7	−2.54	−3.70	−1.38	0.79	4.06
3	M	105.9	94.3	117.4	−1.54	−2.04	−1.03	0.90	2.67
4	F	105.9	96.6	115.2	−3.29	−4.24	−2.34	0.91	3.56
5	M	94.5	89.0	100.1	−1.08	−1.34	−0.82	0.95	1.93
6	M	121.4	114.2	128.7	−2.59	−3.02	−2.16	0.95	2.68
7	F	77.8	73.3	82.3	−1.42	−1.88	−0.96	0.93	2.62
8	F	83.9	77.3	90.5	−0.73	−1.27	−0.19	0.86	1.44
9	F	108.3	96.7	119.9	−2.74	−3.78	−1.70	0.82	2.66
10	M	83.7	76.6	90.9	−1.04	−1.50	−0.59	0.84	3.10
11	F	116.5	112.0	121.0	−3.24	−3.62	−2.87	0.98	1.08
12	M	101.5	83.6	119.4	−1.57	−2.60	−0.54	0.82	2.15

TOI was measured by near-infrared spectroscopy. Leg O_2 extraction reported as % and TOI O_2 extraction index in TOI units (arbitrary units). All regression analyses were statistically significant at $P < 0.01$. No significant differences were observed between males and females (unpaired *t*-test for intercepts and slopes). The left and right legs had similar intercepts and slopes (paired *t*-test). CI, confidence interval; F, female; M, male; SEE, standard error of estimate.

Essentially, we define the new O_2 extraction index as $TOI_{OBV} - TOI_{MIN}$, where TOI_{OBV} is the mean TOI value registered during exercise and TOI_{MIN} is the minimal 1 s rolling average TOI value registered during ischaemia. To validate this index, we used data collected in ongoing research designed to study muscle signalling during ischaemia–reperfusion, approved by the Ethical Committee of the University of Las Palmas de Gran Canaria (Ref.: CEIH-2017-13). In this study, volunteers were catheterized in both femoral veins and equipped with NIRS optodes in both vastus lateralis before doing incremental exercise to exhaustion, followed immediately by ischaemia. Data obtained in the first 12 subjects (seven males and five females) were available to validate the O_2 extraction index here defined. The seven males (age = 22.0 ± 2.0 years, height = 180.1 ± 9.4 cm, body mass = 76.0 ± 6.6 kg, body fat = 19.6 ± 6.8%, and $\dot{V}_{O_2max} = 40.4 \pm 6.8$ ml kg^{-1} min^{-1}) and five females (age = 22.6 ± 2.2 years, height = 161.1 ± 2.7 cm, body mass = 52.8 ± 7.3 kg, body fat = 26.4 ± 1.1%, and $\dot{V}_{O_2max} = 36.3 \pm 5.3$ ml kg^{-1} min^{-1}) were healthy, physically active sports science students.

Subjects were catheterized in both femoral veins as previously described (Curtelin *et al.* 2018). Also, a hand vein was catheterized and heated to obtain arterialized blood. After catheterization, NIRS optodes were carefully placed over both vastus lateralis, as described in Methods by the same research personnel. The same device used in the main experiments was employed in the validation study (NIRO-200NX, Hamamatsu, Japan). Arterial saturation was estimated by haemoglobin oxygen saturation (S_{pO_2}) measured with a finger pulse oximeter (OEM III module, 4549-000, Plymouth, MN, USA). The exercise protocol

consisted of an incremental exercise test to exhaustion starting at 20 W, which was increased by 15 and 20 W every 3 min in females and males, respectively, until the RER was >1.00. During this phase, 1 ml blood samples were withdrawn with heparinized Radiometer syringes (PICO50, Radiometer Medical ApS, Brønshøj, Denmark) simultaneously from both femoral veins, 30 s before each increase of the load. After completing the highest workload with an RER $\geq$ 1.00, the ergometer was unloaded while the subject continued pedalling at a slow cadence (∼30–40 rpm) for 2 min. At the end of the 2 min, the subjects started an incremental exercise test at the load reached in the previous phase, increased by 10 and 15 W every minute until exhaustion. Close to exhaustion, a final blood sample was obtained from both femoral veins and the heated hand vein. At task failure, the circulation of one leg, randomly assigned, was occluded instantaneously with a cuff connected to the same rapid cuff inflator described in Methods (Hokanson E20 AG101, Bellevue, WA, USA), using the same pressure, i.e. 300 mmHg.

The blood samples were immediately analysed to determine blood gases, electrolytes and haemoglobin concentration (ABL90, Radiometer), as previously reported (Curtelin *et al*. 2018). The arterial O_2 content (C_{aO_2}) was calculated using S_{pO_2}. The concentration of O_2 in blood samples was calculated from the saturation and [Hb] (i.e. $(1.34 \times [Hb] \times S_{O_2}) + (0.03 \times P_{O_2})$). The femoral vein O_2 extraction was calculated for each leg as $(C_{aO_2} - C_{fvO_2}) \times 100/C_{aO_2}$, where C_{fvO_2} represents the O_2 concentration in the corresponding femoral vein. The TOI data were averaged every 20 s around the sampling point. During the 1 min post-exercise ischaemia, the TOIs were averaged every 5 s, and the lowest 5 s average was used as the minimum TOI to calculate the extraction index. The relationship between O_2 extraction, TOI, and NIRS-derived extraction index, was determined by linear regression (SPSS v.15.0 for Windows; SPSS Inc., Chicago, IL, USA).

As depicted in Fig. 5, a linear relationship was observed during exercise between leg O_2 extraction and TOI values for each leg in all subjects. In Tables 4 and 5, the equation parameters defining the relationship between leg O_2 extraction and TOI (Table 4 and Fig. 5) and between leg O_2 extraction and the TOI-derived O_2 extraction index (i.e. TOI-derived O_2 extraction index = $TOI_{OBV} - TOI_{MIN}$) are reported for all subjects and for each leg. The intercepts and slopes of the regression lines were compared between legs (two-tailed paired *t*-test) and between males and females (two-tailed unpaired *t*-test). No significant differences were observed either between legs or between sexes. Similar results were obtained when the O_2 content of the femoral vein was regressed against TOI values.

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Additional information

Data availability statement

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

Competing interests

No conflicts of interest, financial or otherwise, are declared by the authors.

Author contributions

Conceptualization: J.A.C. Methodology: D.M.-A., J.A.C. and M.M.-R. Formal analysis: J.A.C., M.M.-R., M.G.-R., M.P.-V. and V.G.A. Investigation: C.D., J.A.C., J.H., M.G.-R., M.M.-R., M.P.-V., R.B. and V.G.A. Resources: D.M.-A., J.A.C. and M.M.-R. Data curation: J.A.C., M.G.-R., M.P.-V. and V.G.A. Writing – original draft preparation: J.A.C. and M.M.-R. Writing – review and editing: C.D., J.A.C., J.H., M.G.-R., M.M.-R., M.P.-V., R.B. and V.G.A. Validation: J.A.C., M.G.-R. and V.G.A. Visualization: J.A.C. and M.M.-R. Supervision: C.D., D.M.A. and J.A.C. Project Administration: D.M.-A., M.G.-R. and M.M.-R. Funding acquisition: C.D., D.M.-A., J.A.C. and R.B. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

brain oxygenation, fatigue, ischaemia, oxygen debt, oxygen deficit, oxygen extraction, performance, sex dimorphism

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Statistical Summary Document
Peer Review History