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

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**Animal model used, if applicable:** N/A.

**Underlying hypothesis:** We tested the following hypothesis: 1) women would have a lower anaerobic capacity than men, even when normalized to the lean mass of the lower extremities (LLM), 2) following high-intensity exercise to exhaustion, women would have a lower functional reserve than men; 3) during repeated fatiguing high-intensity exercise, women would recover from fatigue faster than men; 4) during repeated fatiguing high-intensity exercise women would achieve greater O2 extraction than men; and 5) post-exercise ischaemia would reveal higher metaboreflex-induced heart rate and ventilatory responses in men than women.

**Definitions of ‘n’:**

‘n’ is defined as the number of participants at each time-point.

**Statistical summary table:**

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| **Experimental question number\*** | **Finding/ conclusion** | **Experimental location/ variable** | **Mean value** | **SD** | **n val.** | **P\*\*** | **Units** | **Data comparisons** | **Statistical test** | **Any other variable** | **Figure/ table** | **Comments** |
| 1. Anaerobic capacity | Women have lower anaerobic capacity than men | Accumulated oxygen deficit (AOD) during the best exercise bout at 120 % of VO2max | M: 166.6  W: 136.7 | M 36.6  W 27.9 | 18 M  18 W | **0.010** | mL·kg LLM-1 | Men vs Women | Students’ t test, unpaired,  two-tailed |  | Table 1 |  |
| 2.  Functional reserve | Men and women have a similar functional reserve | Work performed at 120 % of VO2max in the 2nd and 3rd exercise bouts preceded by ischaemic recovery | M: 0.31  W: 0.36 | M: 0.13  W: 0.24 | 18 M  18 W | 0.39 | KJ·kg LLM-1 | Men vs Women | Sex contrast repeated-measures ANOVA |  | Figure 2A | First bout excluded from the analysis, since the functional reserve manifests after exhaustion |
| 3. Components of the functional reserve | Men and women have similar accumulated VO2 after the first bout of exercise | Accumulated VO2 normalized to the LLM in the 2nd and 3rd exercise bouts after ischaemic recovery | M: 44.1  W: 54.2 | M: 23.8  W: 44.4 | 18 M  18 W | 0.47  0.68 | mL·kg LLM-1 | Men vs Women | Sex contrast repeated-measures ANOVA  Bout x sex interaction |  | Figure 2B | First bout excluded from the analysis since the functional reserve manifests after exhaustion. |
| 4. Components of the functional reserve | Men and women achieve similar oxygen deficits after the first bout of exercise preceded by ischaemic recovery. | Oxygen deficit in the 2nd and 3rd exercise bouts after ischaemic recovery | M: 24.2  W: 25.0 | M: 7.7  W: 11.2 | 18 M  18 W | 0.92  0.85 | mL·kg LLM-1 | Men vs Women | Sex contrast repeated-measures ANOVA  Bout x sex interaction | Lactate equivalent of the oxygen deficit | Figure 2C | First bout excluded from the analysis since the functional reserve manifests after exhaustion.  Analysis with logarithmically transformed variables |
| 5. Components of the functional reserve | Men and women have similar glycolytic rates after the first bout of exercise | Glycolytic rates in the 2nd and 3rd exercise bouts after ischaemic recovery | M: 0.176  W: 0.159 | M: 0.049  W: 0.050 | 18 M  18 W | 0.30  0.92 | mmol·kg LLM-1 · s-1 | Men vs Women | Sex contrast repeated-measures ANOVA  Bout x sex interaction |  | N/A  Results section | Analysis restricted to the 2nd and 3rd bouts after ischaemic recovery, to prevent PCr resynthesis and replenishment of O2 stores |
| 6. Components of the functional reserve | Men and women sustain a similar VO2 after the first bout of exercise | VO2 normalized to the LLM per min in the 2nd and 3rd exercise bouts after ischaemic recovery | M: 123.1  W: 124.2 | M: 20.6  W: 25.1 | 18 M  18 W | 0.88  0.92 | mL.kg LLM-1 · min-1 | Men vs Women | Sex contrast repeated-measures ANOVA  Bout x sex interaction |  | Figure 2D | First bout excluded from the analysis since the functional reserve manifests after exhaustion. |
| 7.  Influence of ischaemic recovery on the energy metabolism during subsequent exercise | The fractional contribution of the anaerobic metabolism to the total energy yield was larger in the bouts preceded by occlusions, with this effect being similar in men and women. | Percentage of the overall energy expenditure provided by substrate-level phosphorylation in all exercise bouts | M: 32.4  W: 30.5 | M: 5.2  W: 5.5 | 18 M  18 W | 0.29  < **0.001**  < **0.001**  0.92 | % | Men vs Women  Ischaemic vs free circulation recovery  1st vs 2nd vs 3rd bouts | Sex contrast repeated-measures ANOVA  Occlusion effect  Bout effect  Bout x sex interaction |  | Figure 3D |  |
| 8.  Influence of ischaemic recovery on the energy metabolism during subsequent exercise | The rate at which substrate-level phosphorylation provides energy is higher after ischaemic than free circulation recovery, with a similar response in men and women | Oxygen deficit in the 2nd and 3rd bouts and time to exhaustion | Occ: 1.28  Free: 0.72 | Occ: 0.38  Free: 0.23 | 18 M  18 W | < **0.001**  0.84 | mL.kg LLM-1 · s-1 | Ischaemic vs free circulation recovery | Main occlusion effect repeated-measures ANOVA  Bout x sex interaction |  | Table 2 |  |
| 9. Contribution of the aerobic metabolism to work performed | The O2 expended per unit of work during supramaximal exercise is similar in men and women and is similarly reduced in both sexes when the exercise is performed after occlusion | VO2 per kJ of work produced in each bout of exercise | M: 149.9  W: 151.0  Occ: 135.9  Free: 172.1 | M: 15.8  W: 18.8  Occ: 24.7  Free: 17 | 18 M  18 W | 0.84  < **0.001**  < **0.001**  0.88 | mL · kJ-1 | Men vs Women  Ischaemic vs free circulation recovery  1st vs 2nd vs 3rd bouts | Sex contrast repeated-measures ANOVA  Occlusion effect  Bout effect  Bout x sex interaction |  | Table 2 | The occlusion effect is only tested using the 2nd and 3rd bouts of exercise |
| 10.  Sex differences in fatigability during repeated supramaximal exercise to exhaustion | Men and women fatigue similarly during repeated supramaximal exercise | Time to exhaustion (endurance time) | M: 63.9  W: 62.2 | M: 13.4  W: 11.4 | 18 M  18 W | 0.80  0.34 | s | Men vs Women | Sex contrast repeated-measures ANOVA  Bout x sex interaction |  | Table 2 | Statistical analysis with logarithmically transformed values  All exercise bout included in the analysis |
| 11.  Sex differences in recovery during repeated supramaximal exercise to exhaustion | Men and women recover similarly from supramaximal exercise | Percentage of work accomplished respect the work performed in the first bout | M: 21.3  W: 25.3 | M: 5.4  W: 7.3 | 18 M  18 W | 0.06  0.54 | % | Men vs Women | Sex contrast repeated-measures ANOVA  Occlusion x sex x bout interaction |  | Figure 2C | First bout excluded from the analysis since the focus is on the percentage of recovery achieved in 2nd and 3rd bouts |
| 12.  Mechanisms of fatigue: sex differences in brain oxygenation | Brain oxygenation changes similarly with repeated supramaximal exercise in men and women, but women have lower levels of brain oxygenation during repeated supramaximal exercise | Frontal lobe tissue oxygenation index (TOI) during each bout | M: 66.9  W: 61.7 | M: 4.8  W: 3.9 | 18 M  17 W | **0.001**  < **0.001**  0.48  0.82 | TOI units  (A.U.) | Men vs Women | Sex contrast repeated-measures ANOVA  Bout effect  Bout x sex interaction  Occlusion x sex interaction |  | Figure 4A | TOI signal lost in one female. |
| 13.  Mechanisms of fatigue: sex differences in O2 extraction | Women exhibited greater relative O2 extraction capacity during the last two bouts of exercise | TOI muscle O2 extraction index computed as observed TOI - minimum TOI value during ischaemia  TOIOBV - TOIMIN | M: 9.2  W: 6.5  Occ: 6.1  Free: 9.6 | M: 4.1  W: 2.7  Occ: 3.6  Free: 5.9 | 18 M  17 W | **0.031**  **0.002**  **0.04**  0.50 | TOI units  (A.U.) | Men vs Women | Sex contrast repeated-measures ANOVA  Occlusion effect  Occlusion x sex interaction  Bout x sex interaction |  | Figure 4C | TOI signal lost in one female  First bout excluded from the analysis since the functional reserve manifests after exhaustion |
| 14.  Mechanisms of fatigue: sex differences in metaboreflex activation (heart rate) | No sign of sex differences in metaboreflex activation during post-exercise ischaemia | Heart rate during the ischaemic recovery periods | Occlusion  M: 171.8  W: 175.5  Free circulation  M: 179.9  W: 182.7 | Occlusion  M: 12.2  W: 10.0  Free circulation  M: 6.7  W: 8.2 | 16 M  16 W | 0.36  0.30  0.98 | Beats · min-1 | Men vs Women  Men vs Women | Unpaired t-test  Unpaired t-test  Occlusion x sex interaction |  | Table 3 | HR signal lost in two females and 2 males.  Only the recovery periods are analysed |
| 15.  Mechanisms of fatigue: sex differences in metaboreflex activation (pulmonary ventilation) | No sign of sex differences in metaboreflex activation during post-exercise ischaemia | Pulmonary ventilation during the ischaemic recovery periods | Occlusion  M: 120.1  W: 77.9  Free circulation  M: 126.3  W: 78.1 | Occlusion  M: 29.2  W: 11.4  Free circulation  M: 24.7  W: 13.8 | 18 M  18 W | < **0.001**  < **0.001**  0.26 | L · min-1 | Men vs Women  Men vs Women | Unpaired t-test  Unpaired t-test  Occlusion x sex interaction | Findings supported also by the similar PETCO2 and PETO2 responses to ischaemic recovery observed in men and women in comparison with the response observed during recovery with free circulation | Table 3 | Only the recovery periods are analysed  A greater metaboreflex response in men should have caused higher levels of relative hyperventilation during post-exercise ischaemia in men, compared to the response observed during recovery with free circulation |
| 16.  Sex differences in muscle metabolism during the early recovery after high-intensity exercise | No sex differences in O2 debt per LLM during the first 20 s of postexercise recovery | O2 debt of lower extremities normalized to the LLM | M: 12.6  W: 12.3 | M: 5.4  W: 4.5 | 18 M  18 W | 0.87 | mL · kg LLM-1 | Men vs Women | Sex contrast repeated-measures ANOVA |  | N/A  See results section | Can only be calculated for the experiments with ischaemic recovery  Only the recovery periods are analysed |

\*You may use multiple lines for the same question to indicate multiple comparisons

\*\* Authors may wish to make the text bold where p is considered significant against a stated confidence limit.