**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 36.6W 27.9 | 18 M18 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: 0.31W: 0.36 | M: 0.13W: 0.24 | 18 M18 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: 44.1W: 54.2 | M: 23.8W: 44.4 | 18 M18 W | 0.470.68 | mL·kg LLM-1 | Men vs Women | Sex contrast repeated-measures ANOVABout 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: 24.2W: 25.0 | M: 7.7W: 11.2 | 18 M18 W | 0.920.85 | mL·kg LLM-1 | Men vs Women | Sex contrast repeated-measures ANOVABout 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: 0.176W: 0.159 | M: 0.049W: 0.050 | 18 M18 W | 0.300.92 | mmol·kg LLM-1 · s-1 | Men vs Women | Sex contrast repeated-measures ANOVABout x sex interaction |  | N/AResults 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: 123.1W: 124.2 | M: 20.6W: 25.1 | 18 M18 W | 0.880.92 | mL.kg LLM-1 · min-1 | Men vs Women | Sex contrast repeated-measures ANOVABout 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: 32.4W: 30.5 | M: 5.2W: 5.5 | 18 M18 W | 0.29< **0.001**< **0.001**0.92 | % | Men vs WomenIschaemic vs free circulation recovery1st vs 2nd vs 3rd bouts | Sex contrast repeated-measures ANOVAOcclusion effectBout effectBout 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: 1.28Free: 0.72 | Occ: 0.38Free: 0.23 | 18 M18 W | < **0.001**0.84 | mL.kg LLM-1 · s-1 | Ischaemic vs free circulation recovery | Main occlusion effect repeated-measures ANOVABout 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: 149.9W: 151.0Occ: 135.9Free: 172.1 | M: 15.8W: 18.8Occ: 24.7Free: 17 | 18 M18 W | 0.84< **0.001**< **0.001**0.88 | mL · kJ-1 | Men vs WomenIschaemic vs free circulation recovery1st vs 2nd vs 3rd bouts | Sex contrast repeated-measures ANOVAOcclusion effectBout effectBout 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: 63.9W: 62.2 | M: 13.4W: 11.4 | 18 M18 W | 0.800.34 | s | Men vs Women | Sex contrast repeated-measures ANOVABout x sex interaction |  | Table 2 | Statistical analysis with logarithmically transformed valuesAll 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: 21.3W: 25.3 | M: 5.4W: 7.3 | 18 M18 W | 0.060.54 | % | Men vs Women | Sex contrast repeated-measures ANOVAOcclusion 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: 66.9W: 61.7 | M: 4.8W: 3.9 | 18 M17 W | **0.001**< **0.001**0.480.82 | TOI units(A.U.) | Men vs Women | Sex contrast repeated-measures ANOVABout effectBout x sex interactionOcclusion 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 ischaemiaTOIOBV - TOIMIN | M: 9.2W: 6.5Occ: 6.1Free: 9.6 | M: 4.1W: 2.7Occ: 3.6Free: 5.9 | 18 M17 W | **0.031****0.002****0.04**0.50 | TOI units(A.U.) | Men vs Women | Sex contrast repeated-measures ANOVAOcclusion effectOcclusion x sex interactionBout x sex interaction |  | Figure 4C | TOI signal lost in one femaleFirst 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 | OcclusionM: 171.8W: 175.5Free circulationM: 179.9W: 182.7 | OcclusionM: 12.2W: 10.0Free circulationM: 6.7W: 8.2 | 16 M16 W | 0.360.300.98 | Beats · min-1 | Men vs WomenMen vs Women | Unpaired t-testUnpaired t-testOcclusion 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 | OcclusionM: 120.1W: 77.9Free circulationM: 126.3W: 78.1 | OcclusionM: 29.2W: 11.4Free circulationM: 24.7W: 13.8 | 18 M18 W | < **0.001**< **0.001**0.26 | L · min-1 | Men vs WomenMen vs Women | Unpaired t-testUnpaired t-testOcclusion 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 analysedA 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: 12.6W: 12.3 | M: 5.4W: 4.5 | 18 M18 W | 0.87 | mL · kg LLM-1 | Men vs Women | Sex contrast repeated-measures ANOVA |  | N/ASee results section | Can only be calculated for the experiments with ischaemic recoveryOnly 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.