

Appendix I: Adherence to pre-test protocol questionnaire

Appendix II: Health-history questionnaire

Appendix III: Additional tables and results

Table 1. Average percentage of maximal oxygen uptake attained from the five submaximal exercise bouts and trial specific body mass

| Trained | | | | | Untrained | | | |
|--------------------------------|---|----------------|---------------|---------------|---|----------------|----------------|----------------|
| | Speed of bouts km · t ⁻¹ | HIGH CHO | LOW CHO | FASTED | Speed of bouts km · t ⁻¹ | HIGH CHO | LOW CHO | FASTED |
| % VO _{2max} bout 1 | 7.4 (1.0) | 45.3 (4.2) | 46.0 (2.7) | 46.7 (4.6) | 4.6 (0.6) | 48.2 (7.4) | 52.1 (6.0) | 48.1 (5.9) |
| % VO _{2max} bout 2 | 8.9 (1.1) | 52.5 (4.8)* | 54.7 (4.4) | 55.1 (5.8) | 5.7 (0.6) | 59.8 (6.2)* | 62.3 (6.2) | 61.0 (6.0) |
| % VO _{2max} bout 3 | 10.7 (1.3) | 62.4 (5.7) | 63.5 (5.5) | 64.9 (5.8) | 6.5 (0.5) | 70.8 (6.8) | 73.2 (9.8) | 71.4 (8.1) |
| % VO _{2max} bout 4 | 12.4 (1.3) | 72.7 (7.7) | 75.1 (6.6) | 75.9 (8.3) | 6.9 (1.2) | 77.1 (8.8) | 79.5 (5.2) | 77.7 (8.4) |
| % VO _{2max} bout 5 | 14.0 (1.6) | 80.8 (7.8) | 85.2 (6.3) | 85.5 (7.8) | 7.7 (0.9) | 84.2 (7.1) | 85.2 (5.0) | 84.5 (7.3) |
| BM (kg) | | 77.5 (8.7) | 77.5 (9.2) | 77.5 (9.0) | | 92.1 (11.3) | 91.7 (12.1) | 92.1 (12.5) |

Data are presented as mean (SD). High CHO = pre-event meal with a high carbohydrate content, low CHO = pre-event meal with a low carbohydrate content, fasted = test in fasted state, BM = body mass measured each respective test, N = number of participants in each group for each bout of exercise, % VO_{2max} bout x = intensity of bouts of exercise expressed as a percentage of their individual VO_{2max} for each trial in the trained and untrained group respectively, * = significant effect of pre-test condition after a follow-up test with both groups included.

The effect of pre-test condition on the relative intensity was significant for the 2nd bout ($F(2, 38) = 3.543$, $p = 0.039$, partial $\eta^2 = 0.157$). There was not a statistically significant interaction effect of training status between the effect of pre-test condition on the relative intensity of the bouts of exercise ($F(2, 36) = 0.898$, $p = 0.416$, partial $\eta^2 = 0.048$). The intensity was 2.13 lower following the high CHO-trial compared to the fasted trial ($p = 0.098$, CI 95%: -4.556 to 0.301), and 2.26 lower compared to the low CHO-trial ($p = 0.076$, CI 95%: -4.71 to 0.182). Ignoring pre-test condition, the intensity was significantly lower in the trained group compared the untrained group in the 2nd ($F(2, 18) = 9.08$, $p = 0.007$, partial $\eta^2 = 0.335$), and 3rd bout ($F(2,$

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19) = 9.71, $p = 0.006$, partial $\eta^2 = 0.338$). There was not a significant effect of pre-test condition on BM ($F(2, 38) = 0.746$, $p = 0.481$, partial $\eta^2 = 0.038$).

Table 2. Bout specific work economy for the five submaximal exercise bouts

| Energy Cost | Trained | | | Untrained | | |
|--|-----------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|
| | HIGH | LOW | FASTED | HIGH | LOW | FASTED |
| 1 st bout (Kcal · km ⁻¹) | 104.44 (13.00) N = 11 | 105.27 (10.74) N = 11 | 106.66 (17.41) N = 11 | 136.31 (20.85) N = 9 | 145.32 (27.18) N = 10 | 134.62 (31.00) N = 10 |
| 2 nd bout (Kcal · km ⁻¹) | 100.91 (13.45) N = 10 | 104.59 (14.76) N = 10 | 103.95 (16.13) N = 10 | 143.06 (24.59) N = 9 | 145.30 (22.63) N = 10 | 141.60 (25.58) N = 10 |
| 3 rd bout (Kcal · km ⁻¹) | 101.87 (15.37) N = 10 | 101.18 (11.92) N = 11 | 103.37 (16.54) N = 11 | 156.50 (19.24) N = 4 | 146.88 (23.62) N = 8 | 143.80 (27.31) N = 10 |
| 4 th bout (Kcal · km ⁻¹) | 97.96 (14.86)*# N = 8 | 106.24 (14.54) N = 9 | 105.66 (18.00) N = 10 | 144.72 (4.65) N = 3 | 149.30 (22.39) N = 7 | 142.62 (28.69) N = 7 |
| 1 st bout (Kcal · kg ^{-0.75} · km ⁻¹) | 4.00 (0.28) N = 11 | 4.04 (0.27) N = 11 | 4.07 (0.40) N = 11 | 4.49 (0.60) N = 9 | 4.89 (0.74) N = 10 | 4.52 (0.73) N = 10 |
| 2 nd bout (Kcal · kg ^{-0.75} · km ⁻¹) | 3.86 (0.29) N = 10 | 3.95 (0.38) N = 10 | 3.97 (0.37) N = 10 | 4.70 (0.56) N = 9 | 4.89 (0.45) N = 10 | 4.77 (0.51) N = 10 |
| 3 rd bout (Kcal · kg ^{-0.75} · km ⁻¹) | 3.87 (0.33) N = 10 | 3.88 (0.34) N = 10 | 3.95 (0.36) N = 11 | 4.90 (0.32) N = 4 | 4.98 (0.75) N = 8 | 4.84 (0.58) N = 10 |
| 4 th bout (Kcal · kg ^{-0.75} · km ⁻¹) | 3.81 (0.47)*# N = 8 | 4.04 (0.38) N = 9 | 4.00 (0.44) N = 10 | 4.99 (0.48) N = 3 | 5.08 (0.94) N = 7 | 4.85 (0.91) N = 7 |
| 1 st bout (J · kg ⁻¹ · m ⁻¹) | 5.65 (0.38) N = 11 | 5.70 (0.44) N = 11 | 5.75 (0.50) N = 11 | 6.03 (0.80) N = 9 | 6.63 (1.06) N = 10 | 6.12 (0.92) N = 10 |
| 2 nd bout (J · kg ⁻¹ · m ⁻¹) | 5.42 (0.34) N = 10 | 5.56 (0.52) N = 10 | 5.64 (0.46) N = 10 | 6.29 (0.72) N = 9 | 6.59 (0.66) N = 10 | 6.46 (0.63) N = 10 |
| 3 rd bout (J · kg ⁻¹ · m ⁻¹) | 5.41 (0.36) N = 10 | 5.48 (0.52) N = 10 | 5.58 (0.42) N = 11 | 6.46 (0.26) N = 4 | 6.77 (1.10) N = 8 | 6.55 (0.73) N = 10 |
| 4 th bout (J · kg ⁻¹ · m ⁻¹) | 5.42 (0.66)*# N = 8 | 5.70 (0.55) N = 9 | 5.66 (0.56) N = 10 | 6.81 (0.96) N = 3 | 6.91 (1.52) N = 7 | 6.60 (1.38) N = 7 |

Data are presented as mean (SD). High CHO = pre-event meal with a high carbohydrate content, low CHO = pre-event meal with a low carbohydrate content, fasted = test in fasted state, N = number of participants with accepted values for each bout of exercise, Energy cost = kcal per km, kcal per kg BM per km and Joules per meter in the trained and untrained group, respectively, * = significant effect of pre-test condition after a follow-up test with both groups included, *# = significant main effect of pre-test condition after a follow-up test for each group separately.

WE could only be calculated with the method used if RER was < 1.00. In the 4th bout of exercise, RER was only < 1.00 in three untrained participants following the high CHO- trial,

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thus, only the trained group was included in the follow up test. In the 5th bout, RER was only < 1.00 following the high CHO-trial in one trained participant, thus, EC was not calculated for this bout.

The effect of pre-test condition was statistically significant on WE in the 4th bout in the trained group (Kcal per km $F(2, 14) = 3.918$, $p = 0.044$, partial $\eta^2 = 0.359$), (scaled $F(2, 14) = 4.313$, $p = 0.035$, partial $\eta^2 = 0.381$), (SI-units $F(2, 14) = 4.423$, $p = 0.032$, partial $\eta^2 = 0.387$). WE was significantly lower following the high CHO-trial compared to the low CHO-trial (Kcal per km $p = 0.026$, CI 95%: -10.363 to -0.738) (scaled $p = 0.037$, CI 95%: -0.424 to -0.014) (SI-units $p = 0.059$, CI 95%: -0.619 to 0.013) but not the fasted trial (Kcal per km $p = 0.325$, CI 95%: -9.88 to 2.561) (scaled $p = 0.256$, CI 95%: -0.362 to 0.079) (SI-units $p = 0.181$, CI 95%: -0.501 to 0.083). Ignoring pre-test condition, WE was significantly lower for the four first bouts of exercise in the trained group compared to the untrained group when stated as EC per km (1st: $F(2, 18) = 15.742$, $p < 0.001$, partial $\eta^2 = 0.467$), (2nd: $F(2, 17) = 21.447$, $p < 0.001$, partial $\eta^2 = 0.558$), (3rd: $F(2, 12) = 29.594$, $p < 0.001$, partial $\eta^2 = 0.711$), as scaled EC (1st: $F(2, 18) = 7.752$, $p = 0.012$, partial $\eta^2 = 0.301$), (2nd: $F(2, 17) = 20.673$, $p < 0.001$, partial $\eta^2 = 0.549$), (3rd: $F(2, 12) = 24.538$, $p < 0.001$, partial $\eta^2 = 0.672$) and as SI-units: (1st: $F(2, 18) = 7.752$, $p = 0.012$, partial $\eta^2 = 0.301$), (2nd: $F(2, 17) = 20.673$, $p < 0.001$, partial $\eta^2 = 0.549$), (3rd: $F(2, 12) = 24.538$, $p < 0.001$, partial $\eta^2 = 0.672$).

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Table 3. Bout specific oxygen cost for the five submaximal exercise bouts

| Oxygen cost | Trained | | | Untrained | | |
|---|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | HIGH | LOW | FASTED | HIGH | LOW | FASTED |
| 1 st bout relative (ml · kg ⁻¹ · min ⁻¹) | 32.54 (3.48) | 33.12 (3.24) | 33.57 (3.77) | 22.58 (3.55) | 24.36 (2.72) | 22.57 (3.20) |
| 2 nd bout relative (ml · kg ⁻¹ · min ⁻¹) | 37.78 (4.37)* | 38.98 (4.30) | 39.61 (4.73) | 28.07 (3.67) | 29.23 (3.33) | 28.61 (3.51) |
| 3 rd bout relative (ml · kg ⁻¹ · min ⁻¹) | 44.89 (5.48) | 45.72 (5.84) | 46.58 (4.93) | 33.17 (3.73) | 34.20 (4.15) | 33.45 (4.14) |
| 4 th bout relative (ml · kg ⁻¹ · min ⁻¹) | 52.17 (6.32) | 54.07 (6.50) | 54.50 (6.31) | 36.17 (5.03) | 37.22 (2.88) | 36.67 (4.86) |
| 5 th bout relative (ml · kg ⁻¹ · min ⁻¹) | 58.43 (7.85)*# | 62.22 (7.93) | 61.80 (7.00) | 39.30 (3.75) | 39.74 (1.97) | 39.42 (3.55) |
| 1 st bout scaled (ml · kg ^{-0.75} · kg ⁻¹) | 96.44 (10.61) | 98.07 (9.25) | 99.55 (12.16) | 69.79 (10.88) | 75.27 (8.04) | 69.81 (10.85) |
| 2 nd bout scaled (ml · kg ^{-0.75} · kg ⁻¹) | 111.91 (12.68)* | 115.95 (12.54) | 117.37 (14.37) | 86.90 (12.31) | 90.39 (10.88) | 88.46 (12.21) |
| 3 rd bout scaled (ml · kg ^{-0.75} · kg ⁻¹) | 132.98 (15.78) | 135.36 (15.92) | 138.08 (14.60) | 102.72 (13.10) | 105.69 (12.24) | 103.51 (14.97) |
| 4 th bout scaled (ml · kg ^{-0.75} · kg ⁻¹) | 154.56 (18.28) | 160.02 (17.29) | 161.43 (18.35) | 112.93 (17.9) | 115.15 (10.46) | 113.61 (17.65) |
| 5 th bout scaled (ml · kg ^{-0.75} · kg ⁻¹) | 172.53 (24.04)*# | 184.57 (21.98) | 183.48 (20.64) | 120.93 (12.85) | 122.20 (8.78) | 121.14 (12.89) |

Data are presented as mean (SD). High CHO = pre-event meal with a high carbohydrate content, low CHO = pre-event meal with a low carbohydrate content, fasted = test in fasted state, x bout relative = ml of oxygen per kg body mass per minute, x bout scaled = ml of oxygen per kg^{-0.75} body mass in the trained and untrained group, respectively, * = significant effect of pre-test condition after a follow-up test with both groups included, # = significant main effect of pre-test condition after a follow-up test for each group separately.

The effect of pre-test condition was statistically significant on the OC of the 2nd bout in both groups (relative $F(2, 36) = 4.099$, $p = 0.025$, partial $\eta^2 = 0.185$) (scaled $F(2, 36) = 3.960$, $p = 0.028$, partial $\eta^2 = 0.180$) and the OC of the 5th bout in the trained group (relative_Trained $F(2, 16) = 6.347$, $p = 0.009$, partial $\eta^2 = 0.442$), (relative_Untrained $F(2, 14) = 0.136$, $p = 0.874$, partial $\eta^2 = 0.019$), (scaled_Trained $F(2, 16) = 6.266$, $p = 0.010$, partial $\eta^2 = 0.439$), (scaled_Untrained $F(2, 14) = 0.126$, $p = 0.882$, partial $\eta^2 = 0.018$). There was a statistically significant interaction effect between training status and the effect of pre-test condition for the 5th bout (relative $F(2, 30) = 3.457$, $p = 0.045$, partial $\eta^2 = 0.187$), (scaled $F(2, 30) = 3.404$, $p = 0.047$, partial $\eta^2 = 0.185$). In the 2nd bout relative OC was 1.37 lower following the high CHO-trial compared to the fasted trial ($p = 0.04$, CI 95%: -2.69 to -0.052) and 1.31 lower compared to the low CHO-trial ($p = 0.074$, CI 95%: -2.71 to 0.099). In the trained group the OC was consistently lowest following the high CHO-

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trial for all bouts of exercise, significantly so in 5th bout where it was 3.63 lower compared to the fasted trial ($p = 0.025$, CI 95%: -6.773 to -0.493) and 4.08 lower compared to the low CHO-trial ($p = 0.041$, CI 95%: -7.983 to -0.170).

Table 4. Macronutrient composition, energy content and total weight of the pre- event meals

| | Trained | | Untrained | |
|-------------------------|---------------|----------------|----------------|----------------|
| | High | Low | High | Low |
| Carbohydrate (g) | 231.9 (16.2) | 36.8 (3.1) | 269.1 (37.9) | 45.6 (8.8) |
| Fat (g) | 8.6 (1.3) | 85.1 (9.4) | 11.52 (2.9) | 107.0 (23.11) |
| Protein (g) | 31.1 (4.4) | 149.6 (4.7) | 36.8 (8.7) | 43.0 (9.3) |
| Energy (kcal) | 1116.5 (78.8) | 1062.7 (105.1) | 1330.4 (193.0) | 1317.4 (271.6) |
| Total weight (g) | 449.4 (32.3) | 394.3 (36.4) | 522.6 (55.5) | 464.4 (160.5) |

Data are presented as mean (SD). High CHO = pre-event meal with a high carbohydrate content, low CHO = pre-event meal with a low carbohydrate content, fasted = test in fasted state, total weight not including liquids such as water and skimmed milk, in the trained and untrained group, respectively

Table 5. Average self-reported carbohydrate and energy intake

| | Trained | Untrained |
|-------------------------|----------------|----------------|
| Carbohydrate (g) | 236.3 (54.9) | 226.5 (47.3) |
| Energy (kcal) | 2414.3 (474.4) | 1718.1 (209.0) |

Data are presented as mean (SD) of the nine days the participants monitored their diets in the trained and untrained group, respectively.

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Table 6. Blood glucose concentrations at rest and following warm-up, all five submaximal exercise bouts and after the GXT

| Blood glucose mmol · L ⁻¹ | Trained | | | Untrained | | |
|---|---|---|---|---|--|--|
| | High vs Fasted | High vs Low | Low vs Fasted | High vs Fasted | High vs Low | Low vs Fasted |
| Rest | 0.37 (0.33) p = 0.855 CI 95%: -0.59 to 1.33 | 0.34 (0.36) p = 1.000 CI 95%: -0.70 to 1.38 | 0.03 (0.16) p = 1.000 CI 95%: -0.43 to 0.49 | 0.56 (0.19) p = 0.046 CI 95%: 0.01 to 1.11 | 0.65 (0.22) p = 0.051 CI 95%: -0.00 to 1.30 | -0.09 (0.19) p = 1.000 CI 95%: -0.65 to 0.47 |
| Warm-up | -0.41 (0.29) p = 0.582 CI 95%: -1.25 to 0.43 | -0.29 (0.32) p = 1.000 CI 95%: -1.20 to 0.62 | -0.12 (0.13) p = 1.000 CI 95%: -0.50 to 0.26 | 0.73 (0.21)*# p = 0.027 CI 95%: 0.09 to 1.38 | 0.33 (0.21) p = 0.437 CI 95%: -0.29 to 0.96 | 0.40 (0.14) p = 0.065 CI 95%: -0.02 to 0.824 |
| 1st bout | -0.80 (0.28) p = 0.051 CI 95%: -1.60 to 0.00 | -0.25 (0.33) p = 1.000 CI 95%: -1.19 to 0.70 | -0.56 (0.13)*# p = 0.004 CI 95%: -0.92 to -0.19 | 0.53 (0.25) p = 0.180 CI 95%: -0.19 to 1.25 | 0.45 (0.22) p = 0.209 CI 95%: -0.19 to 1.09 | 0.08 (0.12) p = 1.000 CI 95%: -0.28 to 0.44 |
| 2nd bout | -0.53 (0.26) p = 0.225 CI 95%: -1.30 to 0.24 | -0.39 (0.28) p = 0.606 CI 95%: -1.22 to 0.44 | -0.14 (0.14) p = 1.000 CI 95%: -0.56 to 0.28 | 0.25 (0.24) p = 0.950 CI 95%: -0.44 to 0.94 | 0.22 (0.17) p = 0.209 CI 95%: -0.29 to 0.73 | 0.03 (0.13) p = 1.000 CI 95%: -0.36 to 0.42 |
| 3rd bout | -0.88 (0.28)*# p = 0.038 CI 95%: -1.71 to -0.05 | -0.42 (0.29) p = 0.554 CI 95%: -1.28 to 0.44 | -0.46 (0.18) p = 0.180 CI 95%: -1.09 to 0.17 | 0.01 (0.19) p = 1.000 CI 95%: -0.54 to 0.56 | -0.06 (0.25) p = 1.000 CI 95%: -0.80 to 0.68 | 0.07 (0.15) p = 1.000 CI 95%: -0.37 to 0.51 |
| 4th bout | -0.95 (0.21)*# p = 0.003 CI 95%: -1.55 to -0.34 | -0.66 (0.18)*# p = 0.012 CI 95%: -1.16 to -0.15 | -0.29 (0.24) p = 0.761 CI 95%: -0.98 to 0.40 | -0.06 (0.23) p = 1.000 CI 95%: -0.74 to 0.62 | -0.40 (0.33) p = 0.751 CI 95%: -1.36 to 0.56 | 0.34 (0.19) p = 0.307 CI 95%: -0.21 to 0.89 |
| 5th bout | -1.48 (0.41)* p = 0.014 CI 95%: -2.65 to -0.31 | -1.00 (0.39) p = 0.082 CI 95%: -2.11 to 0.11 | -0.48 (0.44) p = 0.906 CI 95%: -1.75 to 0.79 | -0.19 (0.26)* p = 1.000 CI 95%: -1.00 to 0.62 | 0.00 (0.30) p = 1.000 CI 95%: -0.92 to 0.92 | -0.19 (0.19) p = 1.000 CI 95%: -0.79 to 0.42 |
| Post GXT | -0.81 (0.65) p = 0.747 CI 95%: -2.78 to 1.16 | -0.19 (0.38) p = 1.000 CI 95%: -1.34 to 0.97 | -0.62 (0.44) p = 1.000 CI 95%: -2.45 to 1.21 | -0.09 (0.39) p = 1.000 CI 95%: -1.27 to 1.09 | -0.03 (0.49) p = 1.000 CI 95%: -1.50 to 1.43 | -0.6 (0.23) p = 1.000 CI 95%: -0.75 to 0.64 |

Data are presented as mean difference (Std. error), p-value and confidence interval 95%. High = pre-event meal with a high relative CHO-content, Low = pre-event meal with a low relative CHO-content, fasted = fasted trial in the trained and untrained group, respectively, * = significant effect of pre-test condition after a follow-up test with both groups included, *# = significant main effect of pre-test condition after a follow-up test for each group separately.

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The effect of pre-test condition on BG was statistically significant for BG measured at rest, ($F(2, 38) = 5.064, p = 0.019, \text{partial } \eta^2 = 0.21$), the measurements after the warm-up (Trained: $F(2, 20) = 1.298, p = 0.288, \text{partial } \eta^2 = 0.115$), (Untrained: $F(2, 16) = 7.467, p = 0.005, \text{partial } \eta^2 = 0.483$), after the 1st bout (Trained: $F(2, 20) = 4.972, p = 0.04, \text{partial } \eta^2 = 0.332$), (Untrained: $F(2, 18) = 3.963, p = 0.037, \text{partial } \eta^2 = 0.306$), after the 3rd bout (Trained: $F(2, 18) = 5.49, p = 0.014, \text{partial } \eta^2 = 0.379$), (Untrained: $F(2, 18) = 0.071, p = 0.932, \text{partial } \eta^2 = 0.008$), after 4th (Trained: $F(2, 20) = 10.554, p = 0.001, \text{partial } \eta^2 = 0.513$), (Untrained: $F(2, 18) = 1.435, p = 0.264, \text{partial } \eta^2 = 0.138$), and after the 5th exercise bout ($F(2, 36) = 5.737, p = 0.007, \text{partial } \eta^2 = 0.242$). There was a statistically significant interaction effect of training status between the effect of pre-test condition on BG after the warm-up ($F(2, 36) = 5.767, p = 0.016, \text{partial } \eta^2 = 0.243$), after the 1st bout ($F(2, 38) = 7.908, p = 0.006, \text{partial } \eta^2 = 0.294$), after the 3rd ($F(2, 36) = 3.616, p = 0.037, \text{partial } \eta^2 = 0.167$), and after the 4th bout of exercise ($F(2, 38) = 3.857, p = 0.03, \text{partial } \eta^2 = 0.169$). Ignoring pre-test condition, BG was significantly higher in the trained group compared to the untrained group from the 2nd bout to the last measurement following the GXT; (2nd: $F(2, 18) = 6.272, p = 0.022, \text{partial } \eta^2 = 0.258$), (3rd: $F(2, 18) = 17.55, p = 0.001, \text{partial } \eta^2 = 0.494$), (4th: $F(2, 18) = 15.013, p = 0.001, \text{partial } \eta^2 = 0.441$), (5th: $F(2, 17) = 20.403, p < 0.001, \text{partial } \eta^2 = 0.546$), (post- GXT: $F(2, 16) = 30.856, p < 0.001, \text{partial } \eta^2 = 0.658$).

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Table 7. Blood lactate concentrations at rest and following warm-up, all five submaximal exercise bouts and after the GXT

| | Trained | | | Untrained | | |
|---|--|--|--|--|--|--|
| Blood lactate mmol · L ⁻¹ | High vs Fasted | High vs Low | Low vs Fasted | High vs Fasted | High vs Low | Low vs Fasted |
| Rest | 0.52 (0.08)* p = 0.001 CI 95%: 0.27 to 0.77 | 0.56 (0.19)* p = 0.050 CI 95%: -0.01 to 1.12 | -0.04 (0.20) p = 1.000 CI 95%: -0.61 to 0.54 | 0.28 (0.09)* p = 0.051 CI 95%: -0.00 to 0.57 | 0.348 (0.07)* p = 0.003 CI 95%: 0.14 to 0.55 | -0.06 (0.08) p = 1.000 CI 95%: -0.29 to 0.16 |
| Warm-up | 0.32 (0.10) p = 0.028 CI 95%: 0.03 to 0.61 | 0.29 (0.09) p = 0.028 CI 95%: 0.03 to 0.55 | 0.03 (0.10) p = 1.000 CI 95%: -0.26 to 0.32 | 0.17 (0.19) p = 1.000 CI 95%: -0.39 to 0.74 | 0.22 (0.20) p = 0.956 CI 95%: -0.39 to 0.74 | -0.04 (0.13) p = 1.000 CI 95%: -0.43 to 0.34 |
| 1st bout | 0.34 (0.18) p = 0.244 CI 95%: -1.64 to 0.85 | 0.30 (0.20) p = 0.479 CI 95%: -0.27 to 0.87 | 0.04 (0.07) p = 1.000 CI 95%: -0.16 to 0.24 | -0.15 (0.22) p = 1.000 CI 95%: -0.80 to 0.49 | 0.24 (0.11) p = 0.184 CI 95%: -0.09 to 0.57 | -0.39 (0.23) p = 0.382 CI 95%: -1.08 to 0.29 |
| 2nd bout | 0.26 (0.15) p = 0.358 CI 95%: -0.19 to 0.72 | 0.27 (0.21) p = 0.688 CI 95%: -0.35 to 0.90 | -0.01 (0.11) p = 1.000 CI 95%: -0.33 to 0.32 | 0.24 (0.22) p = 0.900 CI 95%: -0.40 to 0.89 | 0.45 (0.21) p = 0.162 CI 95%: -0.15 to 1.05 | -0.21 (0.21) p = 0.992 CI 95%: -0.81 to 0.39 |
| 3rd bout | -0.10 (0.14) p = 1.000 CI 95%: -0.51 to 0.32 | 0.04 (0.19) p = 1.000 CI 95%: -0.50 to 0.59 | -0.14 (0.18) p = 1.000 CI 95%: -0.65 to 0.37 | -0.18 (0.26) p = 1.000 CI 95%: -0.93 to 0.57 | 0.41 (0.25) p = 0.405 CI 95%: -0.32 to 1.13 | -0.59 (0.24) p = 0.105 CI 95%: -1.28 to 0.11 |
| 4th bout | -0.34 (0.32) p = 0.971 CI 95%: -1.26 to 0.59 | -0.17 (0.22) p = 1.000 CI 95%: -0.79 to 0.45 | -0.17 (0.28) p = 1.000 CI 95%: -0.97 to 0.63 | -0.38 (0.34) p = 0.896 CI 95%: -1.40 to 0.65 | -0.07 (0.30) p = 1.000 CI 95%: -0.99 to 0.84 | -0.30 (0.27) p = 0.888 CI 95%: -1.12 to 0.52 |
| 5th bout | -0.12 (0.36) p = 1.000 CI 95%: -1.16 to 0.92 | -0.40 (0.21) p = 0.256 CI 95%: -1.00 to 0.20 | 0.28 (0.34) p = 1.000 CI 95%: -0.69 to 1.25 | -0.44 (0.49) p = 1.000 CI 95%: -1.97 to 1.09 | -0.62 (0.47) p = 0.672 CI 95%: -2.08 to 0.84 | 0.18 (0.35) p = 1.000 CI 95%: -0.92 to 1.28 |
| Post GXT | 0.42 (0.34) p = 0.728 CI 95%: -0.55 to 1.39 | 0.29 (0.28) p = 0.965 CI 95%: -0.50 to 1.08 | 0.13 (0.34) p = 1.000 CI 95%: -0.85 to 1.11 | 0.64 (0.83) p = 1.000 CI 95%: -2.09 to 3.38 | 0.07 (0.74) p = 1.000 CI 95%: -2.49 to 2.35 | 0.71 (0.39) p = 0.366 CI 95%: -0.59 to 2.00 |

Data are presented as mean difference (Std. error), p-value and confidence interval 95%. High = pre-event meal with a high relative CHO-content, Low = pre-event meal with a low relative CHO-content, fasted = fasted trial in both the trained and untrained group, * = significant effect of pre-test condition after a follow-up test with both groups included.

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The effect of pre-test condition on [La]b was statistically significant for [La]b measured at rest ($F(2, 38) = 14.051$, $p < 0.001$, partial $\eta^2 = 0.44$), the [La]b measurements after the warm-up ($F(2, 38) = 4.251$, $p = 0.022$, partial $\eta^2 = 0.183$), and after the 2nd exercise bout ($F(2, 36) = 4.034$, $p = 0.026$, partial $\eta^2 = 0.183$). Ignoring pre-test condition, [La]b was significantly higher for the first three bouts of exercise in the untrained group compared to the trained group (1st: $F(1, 19) = 12.427$, $p = 0.002$, partial $\eta^2 = 0.395$), (2nd: $F(1, 17) = 18.164$, $p < 0.001$, partial $\eta^2 = 0.517$), (3rd: $F(1, 19) = 15.738$, $p < 0.001$, partial $\eta^2 = 0.453$).

Table 8. Respiratory exchange ratio (RER) during the warm-up and all five submaximal exercise bouts

| | Trained | | | Untrained | | |
|---|-----------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|----------------------------|
| RER ($\text{VCO}_2 \cdot \text{VO}_2^{-1}$) | High vs Fasted | High vs Low | Low vs Fasted | High vs Fasted | High vs Low | Low vs Fasted |
| 1st bout | 0.07 (0.01)* $p < 0.001$ | 0.05 (0.01)* $p = 0.001$ | 0.02 (0.01) $p = 0.180$ | 0.06 (0.01)* $p = 0.004$ | 0.05 (0.01)* $p < 0.001$ | 0.01 (0.01) $p = 1.000$ |
| | CI 95%: 0.04 to 0.11 | CI 95%: 0.02 to 0.08 | CI 95%: -0.01 to 0.06 | CI 95%: 0.02 to 0.10 | CI 95%: 0.03 to 0.07 | CI 95%: -0.02 to 0.04 |
| 2nd bout | 0.07 (0.01)* $p = 0.001$ | 0.05 (0.01)* $p < 0.001$ | 0.02 (0.01) $p = 0.350$ | 0.05 (0.01)* $p = 0.003$ | 0.04 (0.01)* $p = 0.001$ | 0.02 (0.01) $p = 0.640$ |
| | CI 95%: 0.03 to 0.10 | CI 95%: -0.03 to 0.07 | CI 95%: -0.01 to 0.045 | CI 95%: 0.02 to 0.09 | CI 95%: 0.02 to 0.06 | CI 95%: -0.02 to 0.05 |
| 3rd bout | 0.06 (0.01)* $p = 0.001$ | 0.04 (0.01)* $p = 0.001$ | 0.02 (0.01) $p = 0.216$ | 0.05 (0.01) $p = 0.005$ | 0.03 (0.01)* $p = 0.062$ | 0.02 (0.01) $p = 0.343$ |
| | CI 95%: 0.03 to 0.09 | CI 95%: 0.02 to 0.06 | CI 95%: -0.01 to 0.05 | CI 95%: 0.02 to 0.08 | CI 95%: -0.01 to 0.06 | CI 95%: -0.02 to 0.06 |
| 4th bout | 0.06 (0.01)* $p = 0.006$ | 0.04 (0.01)* $p = 0.002$ | 0.02 (0.01) $p = 0.550$ | 0.03 (0.01)* $p = 0.044$ | 0.02 (0.01)* $p = 0.171$ | 0.02 (0.01) $p = 0.540$ |
| | CI 95%: 0.02 to 0.10 | CI 95%: 0.02 to 0.07 | CI 95%: -0.02 to 0.06 | CI 95%: 0.01 to 0.07 | CI 95%: -0.01 to 0.04 | CI 95%: -0.02 to 0.05 |
| 5th bout | 0.05 (0.02)* $p = 0.057$ | 0.02 (0.01)* $p = 0.017$ | 0.23 (0.02) $p = 0.609$ | 0.04 (0.02)* $p = 0.070$ | 0.01 (0.01)* $p = 1.000$ | 0.03 (0.02) $p = 0.354$ |
| | CI 95%: -0.01 to 0.09 | CI 95%: 0.01 to 0.04 | CI 95%: -0.03 to 0.07 | CI 95%: -0.01 to 0.09 | CI 95%: -0.03 to 0.05 | CI 95%: -0.02 to 0.09 |
| Post GXT | 0.06 (0.02)* $p = 0.045$ | 0.03 (0.02)* $p = 0.400$ | 0.04 (0.02) $p = 0.359$ | 0.08 (0.03)* $p = 0.076$ | 0.05 (0.02)* $p = 0.086$ | 0.03 (0.03) $p = 0.883$ |
| | CI 95%: 0.00 to 0.13 | CI 95%: -0.02 to 0.07 | CI 95%: -0.03 to 0.10 | CI 95%: -0.01 to 0.18 | CI 95%: -0.01 to 0.11 | CI 95%: -0.06 to 0.12 |

Data are presented as mean difference (Std. error), p-value and confidence interval 95%. High = pre-event meal with a high relative CHO-content, Low = pre-event meal with a low relative CHO-content, fasted = fasted trial in both the trained and untrained group, * = significant effect of pre-test condition after a follow-up test with both groups included.

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RER was consistently higher in the high CHO-trial compared to both the low CHO-trial and the fasted trial (figure 7 and 8). The effect of pre-test condition on RER was statistically significant for all points of measurement (1st: $F(2, 40) = 43.238$, $p < 0.001$, partial $\eta^2 = 0.684$), (2nd: $F(2, 38) = 41.852$, $p < 0.001$, partial $\eta^2 = 0.688$), (3rd: $F(2, 40) = 29.61$, $p < 0.001$, partial $\eta^2 = 0.597$), (4th: $F(2, 40) = 17.224$, $p < 0.001$, partial $\eta^2 = 0.463$), (5th: $F(2, 34) = 10.105$, $p = 0.002$, partial $\eta^2 = 0.373$), (Last minute GXT: $F(2, 36) = 10.269$, $p < 0.001$, partial $\eta^2 = 0.363$). Ignoring pre-test condition, RER was observed to be significantly higher in the untrained group for the first three exercise bouts (1st: $F(1, 18) = 16.484$, $p < 0.001$, partial $\eta^2 = 0.478$), (2nd: $F(2, 17) = 18.164$, $p < 0.001$, partial $\eta^2 = 0.517$), (3rd: $F(2, 19) = 15.264$, $p < 0.001$, partial $\eta^2 = 0.445$).

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Table 9. Rate of perceived exertion (RPE) after warm-up, all five submaximal exercise bouts and after the GXT

| Trained | | | | Untrained | | |
|----------------------------|---|---|--|--|---|--|
| RPE | High vs Fasted | High vs Low | Low vs Fasted | High vs Fasted | High vs Low | Low vs Fasted |
| Warm-up | -0.82 (0.63) p = 0.630 CI 95%: -2.63 to 0.989 | -0.73 (0.47)* p = 0.456 CI 95%: -2.07 to 0.62 | -0.09 (0.46) p = 1.000 CI 95%: -1.40 to 1.22 | -0.67 (0.44) p = 0.507 CI 95%: -2.00 to 0.66 | -1.89 (0.51)* p = 0.018 CI 95%: -3.43 to 0.66 | 1.22 (0.70) p = 0.361 CI 95%: -0.90 to 3.34 |
| 1st bout | -0.73 (0.47) p = 0.456 CI 95%: -2.07 to 0.62 | -0.27 (0.54) p = 1.000 CI 95%: -1.83 to 1.28 | -0.45 (0.41) p = 0.889 CI 95%: -1.64 to 0.73 | -0.50 (0.58) p = 1.000 CI 95%: -2.21 to 1.21 | -1.00 (0.54) p = 0.287 CI 95%: -2.58 to 0.57 | 0.50 (0.65) p = 1.000 CI 95%: -1.42 to 2.42 |
| 2nd bout | -0.73 (0.41) p = 0.312 CI 95%: -1.89 to 0.44 | -0.55 (0.39) p = 0.576 CI 95%: -1.67 to 0.57 | -0.18 (0.35) p = 1.000 CI 95%: -1.19 to 0.83 | -0.60 (0.76) p = 1.000 CI 95%: -2.84 to 1.64 | -1.10 (0.50) p = 0.171 CI 95%: -2.58 to 0.38 | 0.50 (0.64) p = 1.000 CI 95%: -1.37 to 2.37 |
| 3rd bout | -0.73 (0.24) p = 0.036 CI 95%: -1.41 to -0.05 | -0.64 (0.28)* p = 0.137 CI 95%: -1.44 to 0.16 | -0.09 (0.21) p = 1.000 CI 95%: -0.70 to 0.52 | -0.67 (0.73) p = 1.000 CI 95%: -2.86 to 1.52 | -1.33 (0.53)* p = 0.106 CI 95%: -2.92 to 0.26 | 0.67 (0.47) p = 0.585 CI 95%: -0.76 to 2.01 |
| 4th bout | -0.46 (0.25) p = 0.288 CI 95%: -1.16 to 0.26 | -0.27 (0.24) p = 0.830 CI 95%: -0.95 to 0.41 | -0.18 (0.23) p = 1.000 CI 95%: -0.83 to 0.47 | -0.78 (0.80) p = 1.000 CI 95%: -3.18 to 1.62 | -1.00 (0.53) p = 0.283 CI 95%: -2.59 to 0.589 | 0.22 (0.36) p = 1.000 CI 95%: -0.88 to 1.32 |
| 5th bout | -0.60 (0.40) p = 0.504 CI 95%: -1.77 to 0.57 | -0.40 (0.31) p = 0.669 CI 95%: -1.30 to 0.50 | -0.20 (0.33) p = 1.000 CI 95%: -1.16 to 0.76 | -0.78 (0.70) p = 0.902 CI 95%: -2.90 to 1.34 | -0.67 (0.41) p = 0.423 CI 95%: -1.90 to 0.57 | -0.11 (0.48) p = 1.000 CI 95%: -1.57 to 1.35 |
| Post GXT | -0.09 (0.34) p = 1.000 CI 95%: -1.07 to 0.89 | -0.27 (0.33) p = 1.000 CI 95%: -1.23 to 0.68 | 0.18 (0.12) p = 0.501 CI 95%: -0.17 to 0.53 | -0.33 (0.24) p = 0.585 CI 95%: -1.04 to 0.38 | -0.11 (0.31) p = 1.000 CI 95%: -1.04 to 0.82 | -0.22 (0.36) p = 1.000 CI 95%: -1.32 to 0.88 |

Data are presented as mean difference (Std. error), p-value and confidence interval 95%. High = pre-event meal with a high relative CHO-content, Low = pre-event meal with a low relative CHO-content, fasted = fasted trial in both the trained and untrained group, * = significant effect of pre-test condition after a follow-up test with both groups included, *# = significant main effect of pre-test condition after a follow-up test for each group separately.

The effect of pre-test condition on RPE was statistically significant after warm- up ($F(2, 36) = 5.782$, $p = 0.007$, partial $\eta^2 = 0.243$), and after 3rd bout ($F(2, 36) = 5.856$, $p = 0.006$, partial $\eta^2 = 0.245$). There was not observed a statistically significant interaction effect of training status on the effect of pre-test condition. RPE after warm- up was 1.25 lower following the high CHO-

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trial compared to the fasted- trial ($p = 0.008$, CI 95%: -2.2 to -0.3) and 0.75 lower compared to the low CHO-trial ($p = 0.208$, CI 95%: -1.773 to 0.273). Ignoring pre-test condition, RPE was not observed to be significantly different in the trained group compared to the untrained.