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Friederike Scharhag-Rosenberger^{ab}, Anja Carlsohn^a, Carsten Lundby^c, Stefan Schüler^a, Frank Mayer^a & Jürgen Scharhag^{ad}

^a Sports Medicine & Sports Orthopaedics, University Outpatient Clinic, University of Potsdam, Potsdam, Germany

^b National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg University Hospital, Heidelberg, Germany

^c Institute of Physiology, University of Zurich, Zurich, Switzerland

^d Department of Cardiology, Internal Medicine III, Heidelberg University Hospital, Heidelberg, Germany

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ORIGINAL ARTICLE

Can more than one incremental cycling test be performed within one day?

FRIEDERIKE SCHARHAG-ROSENBERGER^{1,2*}, ANJA CARLSOHN¹, CARSTEN LUNDBY³, STEFAN SCHÜLER¹, FRANK MAYER¹, & JÜRGEN SCHARHAG^{1,4}

¹Sports Medicine & Sports Orthopaedics, University Outpatient Clinic, University of Potsdam, Potsdam, Germany,

²National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg University Hospital, Heidelberg, Germany,

³Institute of Physiology, University of Zurich, Zurich, Switzerland, ⁴Department of Cardiology, Internal Medicine III, Heidelberg University Hospital, Heidelberg, Germany

Abstract

Changes in performance parameters over four consecutive maximal incremental cycling tests were investigated to determine how many tests can be performed within one single day without negatively affecting performance. Sixteen male and female subjects (eight trained (T): 25 ± 3 yr, BMI 22.6 ± 2.5 kg·m⁻², maximal power output (P_{max}) 4.6 ± 0.5 W·kg⁻¹; eight untrained (UT): 27 ± 3 yr, BMI 22.3 ± 1.2 kg·m⁻², P_{max} 2.9 ± 0.3 W·kg⁻¹) performed four successive maximal incremental cycling tests separated by 1.5 h of passive rest. Individual energy requirements were covered by standardised meals between trials. Maximal oxygen uptake ($\dot{V}O_{2max}$) remained unchanged over the four tests in both groups ($P = 0.20$ and $P = 0.33$, respectively). P_{max} did not change in the T group ($P = 0.32$), but decreased from the third test in the UT group ($P < 0.01$). Heart rate responses to submaximal exercise were elevated from the third test in the T group and from the second test in the UT group ($P < 0.05$). The increase in blood lactate shifted rightward over the four tests in both groups ($P < 0.001$ and $P < 0.01$, respectively). Exercise-induced net increases in epinephrine and norepinephrine were not different between the tests in either group ($P \geq 0.15$). If $\dot{V}O_{2max}$ is the main parameter of interest, trained and untrained individuals can perform at least four maximal incremental cycling tests per day. However, because other parameters changed after the first and second test, respectively, no more than one test per day should be performed if parameters other than $\dot{V}O_{2max}$ are the prime focus.

Keywords: Maximal oxygen uptake, cardiopulmonary exercise testing, consecutive tests, study design, exhaustion, fatigue

Introduction

Maximal incremental cycling tests are a well-established testing procedure (Bentley, Newell, & Bishop, 2007) and commonly used in physiological studies. Some study designs include more than one maximal incremental cycling test for each subject, for example to determine intervention effects under different conditions. In this case, tests are often performed on separate days because it remains unclear whether several maximal incremental cycling tests can be performed within one day without negatively affecting performance. However, it would be advantageous if several tests could be scheduled within one day, as day-to-day variability in performance would be

eliminated and intervention effects could be tested on one defined date. Furthermore, in certain experimental settings, tests must be performed on one day. For example, Robach et al. (2008) tested their subjects with arterial catheterisation for invasive cardiac output and blood pressure measurements under normoxia and hypoxia. Other studies had similar designs with arterial catheterisation and multiple tests under different conditions (Lundby et al., 2008; Rasmussen et al., 2010). From an ethical point of view, invasive techniques should not be repeated on separate days if testing on the same day was possible.

Two previous studies with a different focus (exercise-induced arterial hypoxemia) described the

Correspondence: Friederike Scharhag-Rosenberger, National Center for Tumor Diseases (NCT) Heidelberg, Medical Oncology, Im Neuenheimer Feld 460, 69120 Heidelberg, Germany. Email: fsr@exercise-science.de
Current address: Anja Carlsohn, Institute of Health Sciences, Faculty I, University of Education, Schwäbisch Gmünd, Germany

responses to two subsequent maximal incremental cycling tests within one day (Caillaud, Anselme, & Prefaut, 1996; McKenzie, Lama, Potts, Sheel, & Coutts, 1999). In both studies, participants were highly endurance-trained athletes. The recovery period between the tests was set to 30 and 60 min, and the subjects were not provided with food between the tests. Both studies revealed no influence on $\dot{V}O_{2\max}$ while P_{\max} was observed to decrease in one study but not in the other (Caillaud et al., 1996; McKenzie et al., 1999). Based on these findings, several investigators have performed two exercise tests within one day, separated by 1–2 h of rest (Helgerud, Storen, & Hoff, 2010; Lundby et al., 2008; Rasmussen et al., 2010; Robach et al., 2008). However, it remains unclear whether untrained subjects can also perform two maximal incremental cycling tests within one day without changes in $\dot{V}O_{2\max}$ and how many consecutive tests can at most be performed in trained and untrained individuals without significant changes in performance.

The impact of preceding maximal cycling tests on subsequent tests is likely to depend on the duration of the recovery period. After a resting period of 1–2 h, oxygen uptake ($\dot{V}O_2$), heart rate (HR) and blood lactate concentration (bLa) have returned to baseline levels and this duration therefore appears sufficient (Oyono-Enguelle et al., 1990; Short & Sedlock, 1997). Another important factor is the nutritional status of the subjects because carbohydrate (CHO) availability affects endurance performance (Jeukendrup, 2011) and even short strenuous exercise bouts decrease muscle glycogen content considerably (Fairchild et al., 2003). Therefore, adequate CHO intake between consecutive tests appears crucial. Finally, the impact of preceding tests on subsequent tests might differ between trained and untrained individuals because endurance training elicits numerous adaptations which accelerate recovery, e.g. altered postexercise hormonal responses of the pituitary adrenal axis (Duclos, Corcuff, Rashedi, Fougere, & Manier, 1997) and faster intracellular pH recovery after exhausting exercise (Hug, Bendahan, Le Fur, Cozzone, & Grelot, 2005).

It was therefore investigated in the present study how many maximal incremental cycling tests can be performed within one day without negatively affecting performance. Changes in performance parameters were observed over four consecutive maximal incremental cycling tests with 1.5 h of passive rest and adequate CHO supply between tests in trained and untrained individuals. From the results, recommendations were derived whether multiple maximal incremental cycling tests can be performed in a single day.

Methods

Participants

Sixteen healthy male and female subjects between 20 and 35 years of age participated in the study. Eight of them were endurance trained (T; 4 males and 4 females; inclusion criteria: ≥ 3 endurance training sessions/week, maximal power output [P_{\max}] $> 4.5 \text{ W}\cdot\text{kg}^{-1}$ for males and $>4.0 \text{ W}\cdot\text{kg}^{-1}$ for females) and eight were untrained (UT; 4 males and 4 females; inclusion criteria: no regular endurance training, $P_{\max} < 3.5 \text{ W}\cdot\text{kg}^{-1}$ for males and $< 3.0 \text{ W}\cdot\text{kg}^{-1}$ for females). Anthropometric data and performance characteristics of the subjects were: age: T: 25 ± 3 yr, UT: 27 ± 3 yr ($P = 0.29$); height: T: 1.72 ± 0.03 m, UT: 1.75 ± 0.08 m ($P = 0.31$); weight: T: 67 ± 8 kg, UT: 68 ± 7 kg ($P = 0.66$); P_{\max} : T: $4.6 \pm 0.5 \text{ W}\cdot\text{kg}^{-1}$, UT: $2.9 \pm 0.3 \text{ W}\cdot\text{kg}^{-1}$ ($P < 0.001$). All participants gave written informed consent to take part in the study which had been approved by the University's ethics committee (approval number 11/29).

General design

Each subject performed four maximal stepwise incremental cycling tests, separated by 1.5 h of passive rest. CHO and energy requirements were covered by means of standardised meals of adapted portion size before and between the tests. Ad libitum water intake was recorded and analyses of urine-specific gravity and weighing before each test served to ensure appropriate hydration.

Prestudy day protocol

The subjects followed a predefined diet consisting of $\geq 5 \text{ g}\cdot\text{kg}^{-1}$ CHO and adequate individually calculated total energy intake (Burke, Cox, Culmings, & Desbrow, 2001). The food intake on the prestudy-day was recorded in a protocol and analysed based on the German Food Database BLS II using computer software (PRODI Compact Plus, Nutri-Science, Hausach, Germany).

Study day protocol

The subjects entered the laboratory at 08:00 after an overnight fast. They consumed a standardised breakfast of $1.5 \text{ g}\cdot\text{kg}^{-1}$ CHO, composed of cornflakes with milk and apple juice (Burke et al., 2001). Then, a medical check-up was completed between 08:30 and 09:30, including medical history, physical examination, resting electrocardiogram and blood analysis. An indwelling venous catheter (B. Braun, Melsungen, Germany) was inserted into a forearm vein. Anthropometric data were assessed and weight

measurements were repeated before each test. One and a half hours after breakfast (at about 10:00), the subjects started the first maximal incremental cycling test. This was followed by 1.5 h of passive rest. Fifteen minutes after cessation of exercise, the subjects received a snack individually dosed to cover the CHO and energy requirements of the preceding cycling test plus the resting period, which was composed of cereal bars and apple juice (American College of Sports Medicine, 2007). The second, third and fourth maximal incremental cycling tests followed analogously after 1.5 h of passive rest (approximately at 12:00, 14:00 and 16:00). The snacks were recalculated and given once again 15 min after the second and third test.

Cycling tests

All testings were performed on the same electronically braked cycling ergometer (Lode Excalibur, Groningen, The Netherlands) with identical adjustments of saddle and handle bars within each subject. After a 3 min resting period, the protocol started at 50 W. Work rate was increased every 3 min by 50 W for the T and 25 W for the UT group until voluntary exhaustion, indicated by cadence < 40 rpm. Exercise time, power output and HR were blinded to the subjects who were encouraged to spend maximal effort in each test. Cadence was recorded in the first test and repeated in the following tests. The subjects rated their perceived exertion at maximum on a 6–20 rating of perceived exertion (RPE) scale shortly after cessation of exercise (Borg, 1982).

Blood lactate and HR measurements

Capillary blood samples were taken from the earlobe at rest, after each exercise stage and in the postexercise period. Blood lactate concentrations were determined via an enzymatic-amperometric method (Biosen S-line, EKF Diagnostic Sales, Magdeburg, Germany). HR was recorded continuously by means of a telemetric system (Polar RS400, Polar Electro, Kempele, Finland) and analysed at rest, in the last 10 s of the first five exercise stages and at maximum.

Gas exchange measurements

Gas exchange measurements were conducted continuously in all tests using a ZAN 600 metabolic device (nSpire Health, Oberthulba, Germany; stationary breath-by-breath system). Maximal oxygen uptake ($\dot{V}O_{2\max}$) was determined as the highest 30 s average. To evaluate oxygen consumption ($\dot{V}O_2$) at submaximal work rates, the last 30 s of the first four exercise stages was considered (before subjects demonstrated a plateau in oxygen uptake).

Venous blood sampling and analyses

Venous blood samples were drawn from the indwelling venous catheter before and within the first 3 min after cessation of each exercise test in a standardised sitting position on the cycle ergometer. Blood was drawn into serum gel plastic tubes and tubes pretreated with ethylenediaminetetraacetic acid (EDTA) (Sarstedt, Nümbrecht, Germany). Aliquots of the whole blood were analysed for hemoglobin (Hb) and hematocrit (Hct) (Horiba ABX Micros 60, Axonlab, Stuttgart, Germany) and plasma volume shifts were calculated by means of Hb and Hct (Dill & Costill, 1974). The tubes were then centrifuged and glucose and triglycerides were assessed from serum (Horiba ABX Pentra 400, Axonlab, Stuttgart, Germany). For the determination of epinephrine, norepinephrine, cortisol and insulin, serum and EDTA-plasma aliquots were frozen at $-18\text{ }^{\circ}\text{C}$ until analysis was performed. Epinephrine and norepinephrine were assessed via an enzyme-linked immunosorbent assay (ELISA, IBL international, Hamburg Germany). Insulin and cortisol were analysed via electrochemiluminescence immunoassays (Cobas, Roche Diagnostics, Mannheim, Germany). Concentrations are reported as measured values not corrected for plasma volume shifts to reflect the actual exposure of the tissue to hormones and substrates. Exercise-induced net changes in venous blood parameters (differences between the post- and pre-test values) were calculated and compared between the four tests to allow for circadian variation in baseline levels.

Statistical analyses

The findings are reported separately for T and UT subjects. All outcome measures were tested for normality by using Shapiro–Wilk W tests. The data were normally distributed, except for RPE and venous blood parameters which were partly skewed. Differences in subjects' characteristics between groups were tested with Student's *t*-tests for independent samples. Within each group, changes over the four tests in maximal performance parameters were examined by using one-way repeated-measures analyses of variance (ANOVAs) with Scheffé tests post hoc. To evaluate the potential associations between the individual test duration and the changes in $\dot{V}O_{2\max}$ and P_{\max} , Pearson correlations were performed. To compare the pre-exercise values and the exercise-induced net changes in venous blood parameters as well as RPE over time, Friedman ANOVAs and Wilcoxon signed-rank tests for post hoc comparisons were used. Differences between the groups in the development of the outcome measures over the course of the four exercise tests were analysed using two-way repeated-measures ANOVAs (factor 1:

test and factor 2: group). The interaction effect (test*group) was evaluated. HR, bLa and $\dot{V}O_2$ at submaximal exercise stages were compared between the tests by means of two-way repeated-measures ANOVAs (factor 1: test and factor 2: exercise stage of the cycling test). The test or interaction effect was evaluated, respectively, and Scheffé tests were used for post hoc comparisons. The data are given as means \pm standard deviations (SD) unless otherwise stated. $P < 0.05$ was considered significant.

Results

Maximal performance

In the T group, neither $\dot{V}O_{2max}$ nor P_{max} changed significantly over the four exercise tests (Figure 1 and Table I). In the UT group, $\dot{V}O_{2max}$ did not change, but P_{max} was reduced from the third test by a total of $-7 \pm 2\%$ ($P < 0.01$). The development of $\dot{V}O_{2max}$ over the course of the four tests was not different between the T and the UT subjects ($P = 0.97$), whereas the development of P_{max} was ($P < 0.001$). Regarding maximal exhaustion (Table I), HR_{max} , bLa_{max} and RPE did not change over the four tests in the T group. In the UT group, HR_{max}

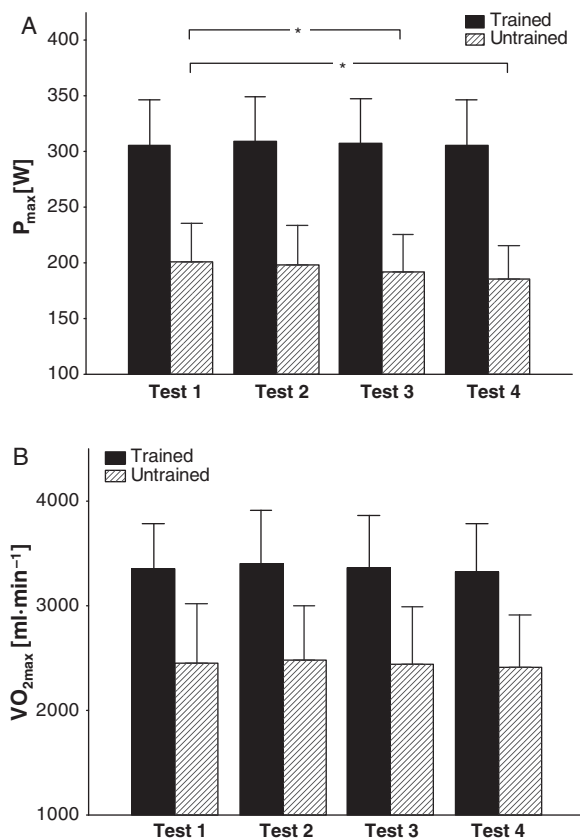


Figure 1. Maximal power output (P_{max} , A) and maximal oxygen uptake ($\dot{V}O_{2max}$, B) in the four exercise tests. Asterisks indicate significant differences within a group ($N = 8$ in each group, means \pm SD).

did not change either, but bLa_{max} was reduced from the third test ($P \leq 0.001$) and RPE increased from the first to the fourth test ($P < 0.05$). The development of HR_{max} and RPE over the tests did not differ between T and UT, but the development of bLa_{max} did ($P = 0.39$, $P = 0.13$ and $P < 0.01$, respectively). The duration of the first exercise test ranged between 15:36 and 26:24 min. This duration showed a trend towards an association with the decrease in $\dot{V}O_{2max}$ over the four tests ($r = -0.51$, $P = 0.05$, $N = 16$) and was significantly associated with the decrease in P_{max} over the four tests ($r = -0.57$, $P = 0.02$, $N = 16$).

Submaximal parameters

In the T group, resting HR and the HR curve (Figure 2) were elevated from the third test ($P < 0.01$ and $P < 0.05$, respectively; total shift in the HR curve: $+6 \pm 6$ beats·min $^{-1}$). In the UT group, resting HR did not change, but the HR curve was elevated from the second test ($P = 0.07$ and $P < 0.05$, respectively; total shift in the HR curve: $+9 \pm 8$ beats·min $^{-1}$). Resting bLa concentration was higher in the third test compared to the first test in the T group ($P < 0.05$) and did not change in the UT group ($P = 0.26$). The bLa curve (Figure 2) showed a rightward shift over the four exercise tests in both groups ($P < 0.001$ and $P < 0.01$, respectively). On the fourth and fifth exercise stage, bLa was significantly lower in the fourth compared to the first exercise test in both groups ($P < 0.01$).

Resting $\dot{V}O_2$ did not differ between the tests in either group (T: 359 ± 58 vs. 376 ± 61 vs. 392 ± 49 vs. 388 ± 68 $ml \cdot min^{-1}$, $P = 0.60$; UT: 402 ± 111 vs. 391 ± 74 vs. 393 ± 71 vs. 375 ± 70 $ml \cdot min^{-1}$, $P = 0.76$). In the T group, submaximal $\dot{V}O_2$ did not change over the four tests (average of the first four exercise stages: 1880 ± 129 vs. 1884 ± 107 vs. 1895 ± 168 vs. 1876 ± 152 $ml \cdot min^{-1}$, $P = 0.94$). In the UT group, however, submaximal $\dot{V}O_2$ changed over time ($P < 0.05$) with a trend ($P = 0.06$) towards higher values in the fourth compared to the second exercise test (average of the first four exercise stages: 1497 ± 175 vs. 1477 ± 168 vs. 1514 ± 167 vs. 1544 ± 168 $ml \cdot min^{-1}$).

Blood parameters

Considering the pre-exercise sample of the first cycling test as reference, plasma volume shifts to pre-exercise values of the second, third and fourth test were $+0.7 \pm 4.6$, $+2.1 \pm 4.5$ and $+2.5 \pm 6.3\%$ in the T group ($P = 0.52$, $N = 8$) and $+3.1 \pm 6.2$, $+5.5 \pm 6.7$ and $+3.4 \pm 8.5\%$ in the UT group ($P = 0.41$, $N = 7$). Within each test, exercise-induced plasma volume shifts amounted to -14.3 ± 5.8 , -14.3 ± 6.5 , -14.8 ± 4.5 and $-11.8 \pm 6.4\%$ in the T group

Table I. Maximal parameters and indicators of maximal effort in the four exercise tests ($N = 8$ in each group; means \pm SD)

	Test 1	Test 2	Test 3	Test 4	P
Trained					
$\dot{V}O_{2\max}$ [$\text{ml}\cdot\text{min}^{-1}$]	3350 \pm 439	3399 \pm 514	3363 \pm 497	3319 \pm 462	0.32
P_{\max} [W]	306 \pm 41	309 \pm 40	307 \pm 41	306 \pm 41	0.20
HR_{\max} [$\text{beats}\cdot\text{min}^{-1}$]	188 \pm 8	190 \pm 7	190 \pm 7	191 \pm 7	0.09
bLa_{\max} [$\text{mmol}\cdot\text{l}^{-1}$]	11.5 \pm 3.0	11.9 \pm 2.0	11.1 \pm 2.3	10.7 \pm 1.7	0.05
RPE []	18.1 \pm 0.9	18.6 \pm 0.8	18.4 \pm 0.8	18.8 \pm 1.1	0.09
Untrained					
$\dot{V}O_{2\max}$ [$\text{ml}\cdot\text{min}^{-1}$]	2456 \pm 567	2481 \pm 517	2444 \pm 511	2412 \pm 501	0.33
P_{\max} [W]	201 \pm 34	198 \pm 36	192 \pm 33	186 \pm 30	< 0.01
HR_{\max} [$\text{beats}\cdot\text{min}^{-1}$]	184 \pm 9	185 \pm 10	184 \pm 9	184 \pm 10	0.62
bLa_{\max} [$\text{mmol}\cdot\text{l}^{-1}$]	9.5 \pm 2.3	9.2 \pm 2.1	8.1 \pm 1.9	6.9 \pm 1.7	< 0.001
RPE []	17.8 \pm 1.6	17.5 \pm 2.1	18.1 \pm 2.1	19.0 \pm 1.6	< 0.01

($P = 0.57$, $N = 8$). In the UT group, changes of -13.7 ± 3.5 , -11.2 ± 3.1 , -12.0 ± 3.2 and $-7.6 \pm 6.7\%$ were observed within the four tests ($P = 0.08$, $N = 7$). All venous blood parameters are given in Figure 3. The exercise-induced net increases in

glucose decreased over the four exercise tests in both groups (T and UT: $P < 0.05$). The other exercise-induced net changes were not different between the four tests in either group ($P \geq 0.15$). Furthermore, the development of the exercise-induced net changes over time was not different between groups for any blood parameter ($P \geq 0.10$).

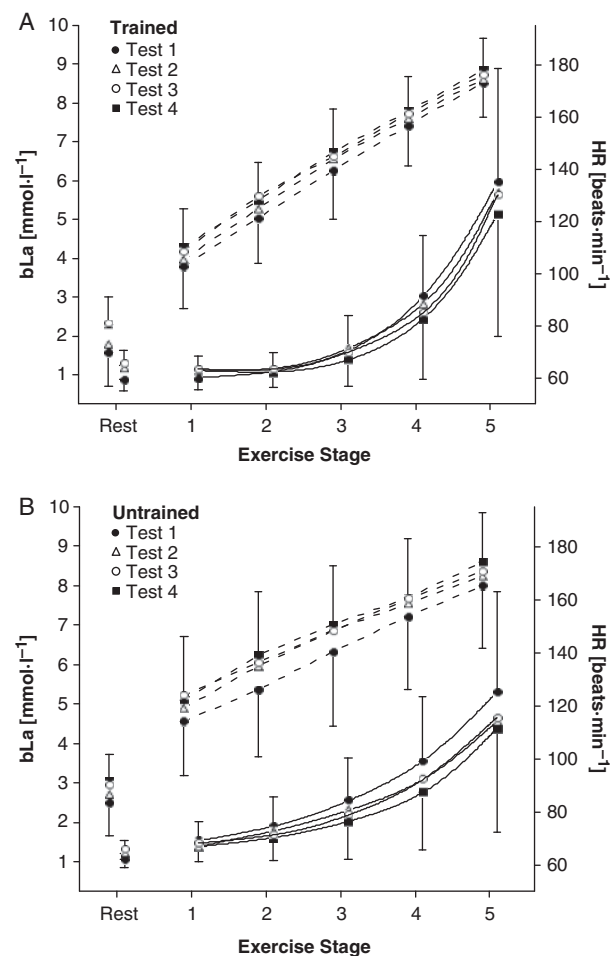


Figure 2. Heart rate (HR, dashed lines) and blood lactate curves (bLa, solid lines) in the four exercise tests in the trained group ($N = 8$, A) and the untrained group ($N = 8$, B, means \pm SD). Error bars are omitted for the second and third test to add clarity. Statistically significant differences: see text.

Energy intake and hydration

The subjects' CHO intake on the pre-study day was $6.5 \pm 2.6 \text{ g}\cdot\text{kg}^{-1}$ in the T and $5.8 \pm 1.1 \text{ g}\cdot\text{kg}^{-1}$ in the UT group. On the study day up to the fourth exercise test, the T group consumed $4.6 \pm 0.6 \text{ g}\cdot\text{kg}^{-1}$ of CHO, $1255 \pm 179 \text{ kcal}$ of energy and $3131 \pm 537 \text{ ml}$ of water. The UT group consumed $4.3 \pm 0.4 \text{ g}\cdot\text{kg}^{-1}$ of CHO, $1201 \pm 212 \text{ kcal}$ of energy and $3413 \pm 599 \text{ ml}$ of water. In the course of the study day, body weight remained unchanged (T: $+0.16 \pm 0.36 \text{ kg}$, $P = 0.09$; UT: $+0.24 \pm 0.59 \text{ kg}$, $P = 0.12$). Urine-specific gravity ranged from 1.000 to 1.010 $\text{g}\cdot\text{ml}^{-1}$ and did not indicate dehydration in any case.

Discussion

The present study demonstrates that in endurance-trained and endurance-untrained individuals, $\dot{V}O_{2\max}$ does not change over four repeated maximal incremental cycling tests separated by 1.5 h of passive rest. However, other parameters changed over the tests in both groups. These changes appear to depend on the parameter of interest, the training status of the subjects and the duration of the exercise test.

The finding that $\dot{V}O_{2\max}$ did not change over the four consecutive tests is in agreement with two previous studies investigating two repeated maximal incremental cycling tests within one day in highly trained athletes (Caillaud et al., 1996; McKenzie et al., 1999). It appears that $\dot{V}O_2$ approaches $\dot{V}O_{2\max}$

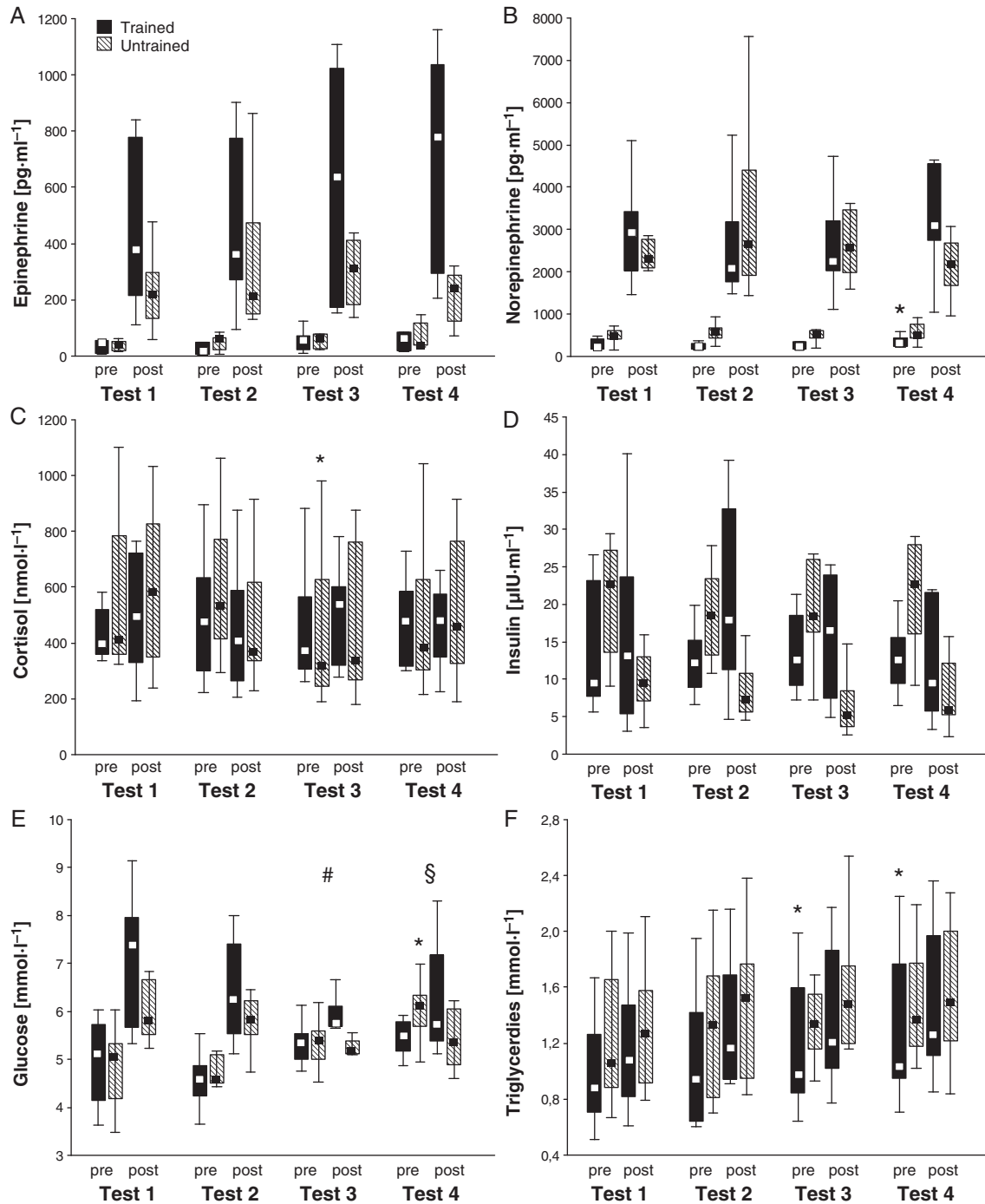


Figure 3. Blood parameters before and after the four exercise tests in the trained (black bars) and the untrained group (shaded bars). Asterisks indicate significant differences from the pre-test value of the first exercise test within a group. Hashes indicate significant differences in the exercise-induced net change from the first test within the trained group. Paragraph signs indicate significant differences in the exercise-induced net change from the first test within the untrained group (epinephrine: T: $N = 6$, UT: $N = 7$; norepinephrine: T: $N = 7$, UT: $N = 8$; cortisol and insulin: T: $N = 8$, UT: $N = 7$; glucose and triglycerides: T: $N = 8$, UT: $N = 6$; medians, quartiles and ranges without outliers).

in all maximal incremental cycling tests, even if voluntary exhaustion occurred earlier and earlier in the untrained group. This might be explained by oxygen uptake kinetics: following the first rise in $\dot{V}O_2$ in response to an increase in work rate, the $\dot{V}O_2$ slow component elevates $\dot{V}O_2$ to maximal values by

the point of exhaustion at all work rates within the severe intensity domain (Jones et al., 2011). The stepwise incremental exercise protocol used in the present study might have enabled this increase in $\dot{V}O_2$ up to $\dot{V}O_{2max}$ at work rates slightly below P_{max} . However, prior high-intensity exercise ('priming')

has been demonstrated to elicit faster $\dot{V}O_2$ kinetics in subsequent exercise bouts, reducing the amplitude of the slow component (Poole, Barstow, McDonough, & Jones, 2008). Although studies on priming used much shorter recovery periods of, e.g., 10 min (Burnley, Davison, & Baker, 2011), $\dot{V}O_2$ kinetics might have been affected by the previous exercise tests in the present study. Another possible reason for the unchanged $\dot{V}O_{2max}$ is that cycling economy decreased and $\dot{V}O_{2max}$ was therefore reached at lower work rates in the third and fourth test of the untrained group. Cycling economy is reflected by $\dot{V}O_2$ for a given work rate (Moseley & Jeukendrup, 2001). The untrained subjects demonstrated a trend towards an elevated submaximal $\dot{V}O_2$ from the fourth test, which supports this assumption. Altogether, the present data suggest that if $\dot{V}O_{2max}$ is the only parameter of interest, trained and untrained individuals can perform at least four maximal incremental cycling tests within one day.

In the trained group, P_{max} did not change over the four tests. However, McKenzie et al. (1999) reported a decrease in P_{max} over two consecutive maximal incremental cycling tests in highly trained athletes. These divergent findings might be attributable to a shorter resting period of 60 min in McKenzie's study (McKenzie et al., 1999). The present data and the study by Caillaud et al. (1996) suggest that P_{max} may remain unaffected by repeated exercise tests in trained individuals.

In the UT group, P_{max} decreased over the test which indicates that fatigue accumulated over the tests. Fatigue during cycling may be attributable to cardiovascular, muscular and nutritional processes (Abbiss & Laursen, 2005). Cardiovascular models of fatigue refer to the limited ability of the cardiovascular system to deliver oxygen to the muscles (Abbiss & Laursen, 2005). Because $\dot{V}O_{2max}$ remained unchanged, it is unlikely that oxygen delivery decreased and cardiovascular fatigue accumulated in the present study. On the muscular level, peripheral neuromuscular or biomechanical fatigue can occur (Abbiss & Laursen, 2005). Peripheral neuromuscular fatigue is related to processes involved in muscle excitation, recruitment and contraction, and usually is associated with increases in perception of effort (Abbiss & Laursen, 2005; St Clair Gibson, Lambert, & Noakes, 2001), which was observed in the untrained group of the present study. Furthermore, the decrease in P_{max} correlated with test duration which was 2:47 min longer in the untrained group on average and might have resulted in more pronounced peripheral neuromuscular fatigue. Accumulated peripheral neuromuscular fatigue therefore apparently contributed to the reduced maximal power output from the third test in the untrained group. Biomechanical fatigue refers

to the efficiency of movement. It is characterised by a reduction in a maximum voluntary force production and decreased cycling economy (Abbiss & Laursen, 2005). As mentioned above, the untrained subjects demonstrated a trend towards decreased cycling economy in the fourth compared to the second exercise test. Biomechanical fatigue might therefore also have contributed to the reduction in P_{max} over the four exercise tests in the untrained group.

In terms of nutritional reasons for fatigue, dehydration can be excluded because of stable weight, similar plasma volume levels and normal urine-specific gravity (Jeukendrup, 2011). Another potential nutritional factor for fatigue is glycogen depletion, despite adequate CHO intake (Abbiss & Laursen, 2005). In the present study, there was a rightward shift of the bLa curve, which indicates glycogen depletion (Hughes, Turner, & Brooks, 1982; Maassen & Busse, 1989). A reduced bLa concentration during submaximal exercise in a glycogen-depleted state is often accompanied by a reduced bLa_{max} and P_{max} as well as an increased submaximal exercise HR (Hughes et al., 1982; Mikulski, Ziemba, & Nazar, 2008). In the present study, bLa_{max} decreased over the tests in the untrained group and the HR curves increased in both groups. Altogether, the data indicate that glycogen stores may have become depleted over the four tests in the trained and untrained subjects despite adequate CHO feedings, and this may have led P_{max} to decrease in the untrained group. The rate of glycogen refill rather than the amount of CHO supply might have been the limiting factor.

In the present study, test duration ranged between 16 and 26 min which is within the range of test durations proposed for valid determination of $\dot{V}O_{2max}$ of 7–26 min (Midgley, Bentley, Luttikholt, McNaughton, & Millet, 2008). However, test duration was significantly associated with the decrease in P_{max} over the four tests and showed a trend towards an association with the decrease in $\dot{V}O_{2max}$. This suggests that exercise protocols of short duration are preferable when repeated tests within one day are performed.

Because the maximal incremental cycling tests in the present study were conducted at different times of a single day, circadian variation should be taken into account when interpreting the data. Although sports performance in general peaks in the late afternoon, different variables underlie diurnal variation to a different extent (Atkinson & Reilly, 1996; Drust, Waterhouse, Atkinson, Edwards, & Reilly, 2005). No circadian variation is reported for $\dot{V}O_{2max}$ and HR_{max} as well as for submaximal respiratory exchange ratio in most studies (Atkinson & Reilly, 1996; Drust et al., 2005). In contrast, bLa_{max} has been reported to be higher in the afternoon than in

the morning (Bessot et al., 2006) and resting HR and submaximal exercise HR demonstrate significant diurnal variations with a minimum at night or in the morning and a maximum in the afternoon (Drust et al., 2005). However, it should be recognised that the meals and cycling tests were likely to distribute the circadian rhythm in the present study (Drust et al., 2005). If at all, circadian variation might have played a role in the observed increases in resting HR and HR response to exercise.

The number of subjects within each group was relatively small in the present study. The statistical findings should therefore be interpreted cautiously. This particularly refers to the exact test in which each single parameter started to change. Both groups demonstrated changes in the HR, bLa and blood glucose responses to exercise. In addition, the untrained group demonstrated changes in P_{max} , HR_{max} , perceived exertion and cycling economy over time. Differences between groups might also be affected by the duration of the test protocol which was 2:47 min longer on average in the untrained group. Altogether, parameters other than $\dot{V}O_{2max}$ seem to change over repeated maximal incremental cycling tests and, therefore, trained and untrained individuals should not perform more than one maximal incremental cycling test within one single day if these parameters are of interest.

Conclusions

The present data demonstrate that if $\dot{V}O_{2max}$ is the main parameter of interest, at least four maximal incremental cycling tests can be performed within one single day in studies with trained and untrained participants, if 1.5 h of passive recovery and standardised meals are provided between the tests. From an ethical point of view, this finding reduces stress on subjects in studies with invasive measurements which do not have to be repeated on separate days. However, significant changes over the four tests were observed in parameters other than $\dot{V}O_{2max}$, indicating that neuromuscular and biomechanical fatigue may have occurred in the untrained group and glycogen stores depleted in both groups. The magnitude of these changes seems to depend on the parameter of interest, the training status of the subjects and the duration of the exercise test protocol. Therefore, if parameters other than $\dot{V}O_{2max}$ are the prime focus, no more than one test should be performed per day.

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