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Original Article

Oxygen uptake during the last bouts of exercise incorporated into high-intensity intermittent cross-exercise exceeds the $\dot{V}O_2$max of the same exercise mode

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ABSTRACT

Oxygen uptake ($\dot{V}O_2$) was measured during a non-exhaustive high-intensity intermittent cross-exercise (HIICE) protocol consisting of four alternating bouts of 20 s running (R) and three bouts of bicycle exercise (BE) at ~160% and ~170% maximal oxygen uptake ($\dot{V}O_2$max), respectively, with 10 s between-bout rests (sequence R-BE-R-BE-R-BE-R). The $\dot{V}O_2$ during the last BE ([52.2 ± 5.0] $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was significantly higher than the $\dot{V}O_2$max of the BE ([48.0 ± 5.4] $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $n = 30$) and similar to that of running. For clarifying the underlying mechanisms, a corresponding HIICE-protocol with BE and arm cranking ergometer exercise (AC) was used (sequence AC-AC-BE-AC-BE-AC-AC-BE). In some experiments, thigh blood flow was occluded by a cuff around the upper thigh. Without occlusion, the $\dot{V}O_2$ during the AC ([39.2 ± 7.1] $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ [6th bout]) was significantly higher than the $\dot{V}O_2$max of AC ([30.2 ± 4.4] $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $n = 7$). With occlusion, the corresponding $\dot{V}O_2$ ([29.8 ± 3.9] $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was reduced to that of the $\dot{V}O_2$max of AC and significantly less than the $\dot{V}O_2$ without occlusion. These findings suggest that during the last bouts of HIICE may exceed the of the specific exercise, probably because it is a summation of the $\dot{V}O_2$ for the ongoing exercise plus excess post-oxygen consumption (EPOC) produced by the previous exercise with a higher $\dot{V}O_2$max.

1. Introduction

A high-intensity intermittent/interval training (HIIT) technique known as "Tabata training"[1–3] is an intense form of physical training that has been shown to improve an individual's maximal oxygen uptake ($\dot{V}O_2$max) [1] and maximal accumulated oxygen deficit (MAOD[4]) [1] and health-related function,[5,6] resembling the benefits of traditional endurance training. Since the high-intensity intermittent exercises (HIIE) used with the HIIT technique are extremely demanding and exhaust the subjects,[7] even elite athletes may not dare to execute the authentic Tabata training during tapering period prior to main competitions. Furthermore, such HIIE may not be safe for some individuals as it may result in physical accidents and/or increased blood pressure. We therefore designed a non-exhaustive high-intensity intermittent cross-exercise (HIICE) protocol for HIIT that may have same effects on aerobic as the exhaustive HIIE and have wider applications to different populations, including health-oriented people and athletes. It consists of four and three bouts of 20 s high-intensity running and cycle ergometer exercises with a 10 s rest between exercise bouts. This HIICE adopts running (dominantly recruited muscle: calf muscles with the assistance of hamstring and thigh muscles[8–10] on a treadmill for the 1st, 3rd, 5th, and 7th bouts and cycle ergometer exercise (dominantly recruited muscle: thigh muscles[11,12] for the 2nd, 4th, and 6th bouts. The exercise intensity for the HIICE is the intensity that exhausts the subjects after completing the 6th or during 7th set of the exercise with 10 s rests between exercise sets.

This HIICE is not exhaustive, because 6–7 consecutive bouts of the bicycle exercise at the same intensity were shown to exhaust the subjects by consuming the MAOD, [7]whereas the HIICE involves only three bouts of bicycle exercise with 40 s rests between the bicycle exercise bouts and does not consume the MAOD, which is composed of the individual's highest lactate concentration and depletion of creatine phosphate in the dominantly recruited muscles for a specific exercise.[13]

Previously, we reported that oxygen uptake during the last bout of the high-intensity intermittent bicycle ergometer exercise was as high as the $\dot{V}O_2$max measured for bicycle ergometer exercise, suggesting that the aerobic energy-releasing system is maximally recruited.[7] It is well recognized that for most physical properties, the more demanding the

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Abbreviation

AC	arm cranking ergometer exercise
BE	bicycle ergometer exercise
CI	confidence interval
°	degree
EPOC	excess post-exercise oxygen consumption
HIIE	high intensity intermittent exercise
HIICE	high intensity intermittent cross exercise
HIIT	high intensity intermittent/interval training
HIICE-RB	high intensity intermittent cross exercise consisting of alternative bouts of four running and three bicycling ergometer exercises
HIIE-R:	exhaustive high intensity intermittent exercise consisting of 6–7 bouts of 20 s running with 10 s between-bout rests
HIIE-B:	exhaustive high intensity intermittent exercise consisting of 6–7 bouts of 20 s bicycle ergometer exercise with 10 s between-bout rests
kPa	k pascal
L:	litter
m	meter
min	minute
MAOD	maximal accumulated oxygen deficit
rpm	revolutions per minute
R	running
RPE	rating of perceived exertion
s	second
SD	standard deviation
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_2$max	maximal oxygen uptake
yr	year

training, the greater the improvement of that property, and it is thus necessary to maximally tax the aerobic energy-releasing systems during exercise training. This may explain why the $\dot{V}O_2$max increased so much after subjects trained using the HIIT for 6 weeks.[1]

As we described in our previous study,[7] we measured the oxygen uptake during each bout of the newly developed HIICE. We were surprised to observe that the subjects' oxygen uptakes during the last 10 s rest periods of the HIICE were quite high, suggesting that anaerobic energy release during the 20 s of high-intensity exercise may be partly recovered by aerobic processes during the 10 s rest period, for example by resynthesizing phosphocreatine, which again is broken down during the next 20 s exercise period. For this process, a large oxygen (blood) supply during the rest period of the HIICE is required.

More importantly, in the present study, we observed that the oxygen uptakes during the last bouts of the bicycle ergometer exercise of the HIICE were higher than the $\dot{V}O_2$max measured for the same exercise, which might challenge the general concept of $\dot{V}O_2$max.[14–22] Therefore, in the present study we performed a series of experiments to investigate possible mechanisms underlying this phenomenon.

2. Methods

2.1. Ethical approval

The protocols for the experiments and procedures involved in the present investigation were approved by the Ethics Committee of Ritsumeikan University (BKC-IRB-2011-05, BKC-IRB-2011-013, BKC-IRB-2013-007). After receiving a detailed explanation of the purpose, potential benefits, and risks of participating in the study, each subject gave written informed consent.

2.2. Experimental design

The study consisted of two experiments. The purposes of Experiment 1 were (*i*) to measure the subjects' $\dot{V}O_2$max for running and the bicycle ergometer exercise, (*ii*) to determine their oxygen uptake during exercise bouts of a HIICE protocol consisting of alternating running and a bicycle ergometer exercise, and (*iii*) to compare metabolic profiles with the exhaustive HIIE using running and bicycle ergometer only exercise. Experiment 2 was conducted to investigate possible mechanisms that may explain that, during HIICE, the $\dot{V}O_2$ during the last bouts of an exercise with a relatively low $\dot{V}O_2$max may be higher that the $\dot{V}O_2$max of that kind of exercise.

2.3. Pretest

Since the exercise intensity used in this study is expressed relative to the $\dot{V}O_2$max, that value was established based on pretests as follows.[1,4,7] First, to determine a linear relationship between the submaximal intensity of running (m·min^{-1}) and bicycling (watts) and the steady-state oxygen uptake (L·min^{-1}) for each individual, we measured the subjects' oxygen uptake during the last 1 or 2 min of six to nine different 10 min bouts of running and bicycling at a constant power between 35% and 90% of the $\dot{V}O_2$max. For the arm-cranking exercise, we measured the subjects' oxygen uptake during the last 1 min of six to nine different 5 min bouts of the exercise at a constant power between 35% and 90% of the $\dot{V}O_2$max.

Next, to determine the $\dot{V}O_2$max, we measured the subjects' oxygen uptake during the last two or three 30 s intervals during several bouts of supramaximal-intensity exercises that exhausted the subjects within 2–4 min.[1,4,7] For the bicycle exercise, the criterion for exhaustion was that the subject was unable to maintain the pedaling frequency at $\geq$ 85 rpm near the end of the bout. The criterion for exhaustion on the treadmill run exercise was determined by the subject when he was unable to follow the given speed. For arm-cranking exercise, the criterion for exhaustion was that the subject was unable to maintain the pedaling frequency at $\geq$ 55 rpm near the end of the bout. After we confirmed a leveling-off in the oxygen uptake by increasing the intensity, the highest oxygen uptake measured was taken as the subject's $\dot{V}O_2$max for each exercise.[18]

As a pretest to determine the exercise intensity for main test, the subjects performed 20 s intermittent exercises at an oxygen demand of 170% of the $\dot{V}O_2$max for the bicycling and arm cranking exercise, with 10 s rest intervals until exhaustion. For the running exercise, the oxygen demand was set to 160% of the $\dot{V}O_2$max. To determine the oxygen demand at such supramaximal intensity and the corresponding running, bicycling and arm-cranking exercise intensity, we carried out a linear extrapolation to higher powers using the established relationship between the exercise intensity (running: m·min^{-1}, bicycling: watts, arm cranking: watts) and the steady-state oxygen uptake described above (see Fig. 1 in Tabata et al.[7]).

If a subject was not able to complete the 6th bout or did complete the 7th bout of exercise during this pretest at the preset intensity, for the next pre-test, the subject again performed the same high-intensity intermittent exercise at a lower or higher intensity than the first test, respectively. The purpose of these pre-tests was to determine the exercise intensity for the main tests, *i.e.*, the intensity that exhausts the subject after the completion of the 6th set or during the 7th set of the 20 s exercise during the high-intensity intermittent exercise.

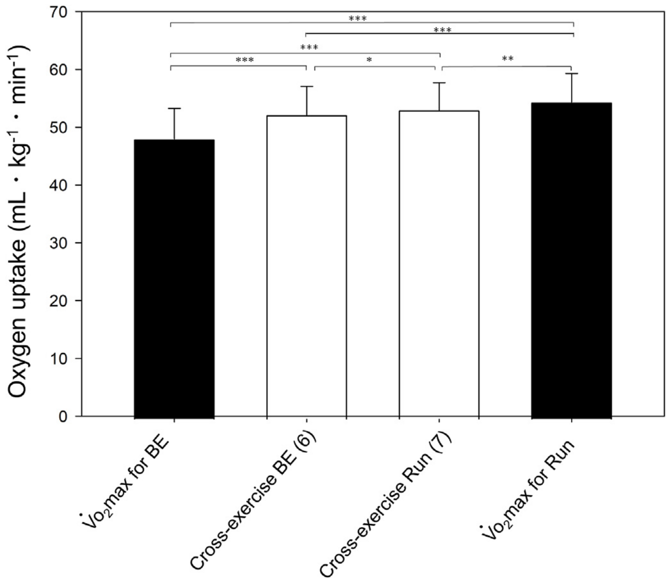

Fig. 1. The oxygen uptake during the last 20 s of bicycle ergometer exercise (BE) (the 6th bout) and running (Run) (the 7th bout) of the high-intensity intermittent cross-exercises versus the maximal oxygen uptake ($\dot{V}\,O_2$max) for BE and Run. *, **, and *** indicate a significant difference between the two values at $p < 0.05$, 0.01, and 0.001, respectively.

These pretests were carried out on 3–5 separate days for each exercise.

2.4. Main experiment

2.4.1. Experiment 1: the running and bicycle ergometer (BE) experiment

Thirty healthy male university students volunteered for the study: mean ± standard deviation (*SD*) age, (23 ± 1) yr; height, (1.74 ± 0.07) m; weight, (67.7 ± 5.2) kg. First, for the warm-up, the subject ran at an intensity of 50% of the $\dot{V}\,O_2$max for 10 min. The subject then performed the cross-exercise protocol, which was an intermittent 20 s running exercise on a treadmill for the 1st, 3rd, 5th, and 7th bouts, and a bicycle ergometer exercise (BE) for the 2nd, 4th, and 6th bouts (HIICE-RB). Each bout was separated by 10 s rest. The exercise intensity for each subject for the running and bicycling was taken from the pretest that exhausted the subject after 6th or during the 7th bout of each exercise as described above.

For the comparison of metabolic profiles of the HIICE-RB with exhaustive high-intensity intermittent exercise known as “Tabata training exercise”,[2,3] eight of the formerly mentioned 30 subjects (age, [23 ± 2] years; height, [1.73 ± 0.07] m; weight, [67.3 ± 5.2] kg) executed another two exhaustive high-intensity intermittent exercises of running on a treadmill (HIIE-R) and bicycling on an ergometer (HIIE-B), respectively. First, for a warm-up, the subject ran for 10 min or biked for 10 min, each at 50% of the $\dot{V}\,O_2$max measured for the same exercise. In the main experiment the subject exercised repeatedly for 20 s with 10 s rests between each bout. The exercise intensity used during each 20 s bout was the one that exhausted the subject after the 6th bout or during the 7th bout of each exercise in the former study.[7]

Expired gas was collected by a Douglas bag during the exercise to measure the subject's oxygen uptake. Blood from the subject's fingertip was obtained immediately after and at 1, 3, 6, and 9 min after the completion of the HIICE-RB and two HIIEs (HIIE-R, HIIE-B). After the subject's lactate concentration was measured, the highest value of the lactate concentration was used as the subject's peak lactate concentration. The rating of perceived exertion (RPE) was reported immediately after the end of the HIIEs and HIICE-RB using Borg's 6–20 RPE scale.[23,24]

2.4.2. Experiment 2: the bicycle-ergometer (BE) and arm-cranking ergometer (AC) experiment

First, eight healthy male students (age [22 ± 4] yr; height [1.67 ± 0.04] m; weight [62.8 ± 5.6] kg) volunteered for experiments to establish an appropriate high-intensity intermittent cross-exercise protocol for the blood-flow restriction experiment. This test used an intermittent cross-exercise with the bicycle ergometer and arm-cranking ergometer. The exercise time was 20 s, and the resting interval between the bouts was 10 s. The total number of exercise bouts was eight. The exercise intensity for bicycling and arm cranking was determined at pretests that exhausted the subject after completing 6th or during the 7th bouts of each exercise as described above.

Since the differences in the subjects' $\dot{V}\,O_2$max between the AC and the BE were large, the oxygen uptake during the bicycle ergometer exercise may not reach the $\dot{V}\,O_2$max measured for the bicycle ergometer exercise using the protocol for the Run-Bicycle experiment described above (Experiment 1). Therefore, to maximize the subjects' oxygen uptake during the bicycle ergometer exercise, we examined several cross-exercise protocols using the arm-cranking ergometer and bicycle ergometer. The protocols examined were as follows.

Cross exercise A: AC (1) – BE (2)– AC (3) – BE (4) – AC (5) – BE (6) – AC (7) – BE (8) (eight bouts, alternating AC and BE).

Cross exercise B: BE (1)– BE (2) – AC (3) – BE (4) – AC (5) – BE (6) – BE (7) – AC (8) (eight bouts, most emphasize on BE at the start).

Cross exercise C: AC (1)– BE (2) – BE (3) – AC (4) – BE (5) – AC (6) – BE (7) – BE (8) (eight bouts, most emphasize on BE at the end).

Since we planned to use the selected cross-exercise for future training exercise that is assumed to fully activate aerobic energy-releasing system for both AC and BE, we selected a protocol that (*i*) increased the subjects' oxygen uptake during the AC to a level higher than the subjects' $\dot{V}\,O_2$max for AC, and (*ii*) elevated the oxygen uptake during the last bouts of BE to the level of the subjects' $\dot{V}\,O_2$max for BE.

Second, seven male subjects (age [21 ± 0] yr; height [1.73 ± 0.07] m; weight [65.2 ± 6.9] kg) performed two high-intensity intermittent cross-exercises with and without thigh blood-flow restriction to thigh muscles by the thigh occlusion described below. For this experiment, we used Cross-exercise C, which in the above-described experiments elevated the subjects' oxygen uptake during the last bout of the bicycle ergometer exercises to the $\dot{V}\,O_2$max measured for the bicycle ergometer exercise.

Before the subject began the cross-exercise with blood-flow restriction, two cuffs were placed around the most proximal part of each thigh of the subject. The cuffs were connected to the bulb of a cylinder containing high-pressure nitrogen gas through a pressure gauge. Just after the end of the bicycle exercise (5th bout), pressure ≥ 53 kPa was applied to stop the blood flow to the thigh. This pressure was maintained continued until the end of the last arm-cranking ergometer exercise (6th bout), which was the end of this experiment (no performance of a 7th or 8th bouts of the bicycle ergometer exercise).

Expired gas was collected in a Douglas bag during the AC (6th bout) to measure the oxygen uptake for the cross-exercise both with and without the blood-flow restriction to the thigh muscles.

2.5. Instruments

2.5.1. Ergometers

All bicycle and arm-cranking ergometer tests were conducted on a mechanically braked cycle ergometer (Ergomedic 828E, Monark, Stockholm, Sweden) at 90 revolutions per minute (rpm) and with an arm-cranking ergometer (Ergomedic 891E, Monark, Stockholm, Sweden) at 60 rpm. All running experiments were done on a motor-driven treadmill (95T Achieve Treadmill, Life Fitness, Rosemont, Illinoi, USA) at 6° (10.5% inclination).

2.5.2. Methods of gas collection and analysis

During the HIIE and the HIICE, each subject's expired gas was collected in a single Douglas bag during the whole 20 s period of high-intensity exercise for the measurement of his oxygen uptake. Fractions of oxygen and carbon dioxide in the expired air were measured by a mass spectrometer (Arco 2000; Arcosystems, Kashiwa, Chiba, Japan). The gas volume was measured by a gasometer (Shinagawa Seisakusho, Shinagawa, Tokyo). Technical properties and limitations of measurements by the Douglas bag method and its advantages compared with commercial metabolic carts have been addressed in several studies.[25–28] In short, the Douglas bag method allows measurement with a technical error of around 0.5%, while the technical error in most commercial instruments is around 3%. Thus, our approach allows precise measurements that may detect even small systematic differences.

The lactate concentration was measured using an instantaneous lactate analyzer (Lactate Pro LT-1710, Arkray, Kyoto, Japan).

2.6. Statistics

Values are shown as mean ± *SD*. The data were analyzed by paired *t*-tests to detect possible differences between different exercise conditions. The significance level for all comparisons was set at $p < 0.05$. We have, in addition, calculated 95% confidence intervals (*CI*) for essential differences addressed. In a number of paired comparisons data for as many as 30 subjects are included. This, together with our high-quality system for measuring the oxygen uptake has allowed us to detect even small differences between experimental conditions.

3. Results

3.1. Experiment 1: the running and bicycle ergometer (BE) experiment

The $\dot{V}\,O_2$max for bicycling was ~12% less than that for running ($p < 0.001$) (Fig. 1). The $\dot{V}\,O_2$ during the last (7th) bout of the running exercise ([53.0 ± 4.8] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) was on the other hand only 2.5% less than the $\dot{V}\,O_2$max measured for running ([54.4 ± 5.0] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; 95% confidence interval [*CI*] of the difference −2.3 to −0.4 $mL{\cdot}\ kg^{-1}\ min^{-1}$, $p = 0.007$). The $\dot{V}\,O_2$ during the 6th bout of exercise (bicycling) ([52.2 ± 5.0] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) was 9% larger than the $\dot{V}\,O_2$max measured for bicycling ([48.0 ± 5.4] $mL{\cdot}kg^{-1}\ min^{-1}$; 95% *CI* of the difference 2.6–5.7 $mL\ kg^{-1}{\cdot}min^{-1}$, $p < 0.001$). In addition, the $\dot{V}\,O_2$ values during the 6th bout (bicycling) and the 7th bout (running) of exercise differed by only 1.5% (95% *CI* of the difference 0.1–1.5 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; $p = 0.02$). The $\dot{V}\,O_2$ during the 6th bout of exercise (bicycling) was only 4% less than the $\dot{V}\,O_2$max measured for running (95% *CI* of difference −3.0 to −1.4 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, $p < 0.001$). Thus, the $\dot{V}\,O_2$ during bicycling in the intermittent protocol was much closer to the values of running than to bicycling only.

The $\dot{V}\,O_2$ during the last bout of the exhaustive HIIE-R ([54.8 ± 5.8] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) and the HIIE-B ([51.1 ± 6.7] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) did not differ significantly from the $\dot{V}\,O_2$max measured for running ([55.7 ± 6.5] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; 95% *CI* of the difference −2.7 to + 0.7 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, $p = 0.25$) and bicycle ergometer exercise ([52.2 ± 7.4] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; 95% *CI* of the difference −3.2 to + 1.2 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, $p = 0.30$), respectively. The rating of perceived exertion (RPE) after HIICE-RB (15 ± 2) was less than that reported after the HIIE-R (20 ± 0) and the HIIE-B (20 ± 0; $p < 0.001$). The peak blood lactate concentration after HIICE-RB ([12.8 ± 1.0] $mmol{\cdot}L^{-1}$) was significantly lower than that measured after the HIIE-R ([15.8 ± 1.4] $mmol{\cdot}L^{-1}$; 95% *CI* of the difference −4.7 to – 1.5 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, $p = 0.003$) and the HIIE-B ([15.6 ± 1.5] $mmol{\cdot}L^{-1}$; 95% *CI* of the difference −4.5 to – 1.2 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, $p = 0.005$).

3.2. Experiment 2: the bicycle-ergometer (BE) and arm-cranking ergometer (AC) experiment

The $\dot{V}\,O_2$max for arm-cranking ergometer exercise ([30.4 ± 2.4] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) was only 57% of that of bicycling ([52.9 ± 6.3] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, $p < 0.001$).

The $\dot{V}\,O_2$ during the bicycle exercise of the high-intensity cross-exercise C increased gradually as the number of exercise bouts increased (Fig. 2). Finally, the $\dot{V}\,O_2$ during the bicycle exercise (8th bout) of cross-exercise C ([53.6 ± 4.5] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) was not different from the $\dot{V}\,O_2$max for bicycling (95 % *CI* of difference −1.2 to + 2.7 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; $p = 0.39$). This value was on the other hand considerably higher than both the highest $\dot{V}\,O_2$ during the bicycle ergometer exercise (8th bout) of cross-exercise A ([33.4 ± 2.9] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ and of cross-exercise B (7th bout, [44.8 ± 4.0] $mL{\cdot}kg^{-1}\ {\cdot}min^{-1}$, $p < 0.001$). These latter highest $\dot{V}\,O_2$ values during the bicycle exercise of cross-exercises A and B were on the other hand 38% and 16% less than the $\dot{V}\,O_2$max for bicycling ($p < 0.001$). Consequently cross-exercise protocol C was used for further studies.

The $\dot{V}\,O_2$ during the first bout of the arm-cranking exercise of cross-exercise C was only 56% of the $\dot{V}\,O_2$max for the arm-cranking ergometer exercise of (30.4 ± 2.4) $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ (Fig. 3). The $\dot{V}\,O_2$ of arm cranking rose by the number of bouts. Consequently, the $\dot{V}\,O_2$ during the 4th and 6th bout of arm-cranking ergometer exercise of cross-exercise C were (41.4 ± 5.1) $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ (+36%, 95% *CI* of the difference 6.2–15.8 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, $p < 0.01$) and (44.2 ± 2.7) $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ (+45%, 95% *CI* of the difference 12.3–15.1 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, $p < 0.001$), respectively higher than the $\dot{V}\,O_2$max for arm-cranking ergometer exercise. These two values were 22% (4th bout of arm cranking) and 16% (6th bout) less than the $\dot{V}\,O_2$max for the bicycle ergometer exercise ($p < 0.001$). Thus, during intermittent arm cranking and bicycling, the $\dot{V}\,O_2$ during arm cranking rose towards that of bicycling and was much higher than during only arm cranking.

The RPE values for the protocols were as follows: Protocol A, 15 ± 0; Protocol B, 16 ± 1, and Protocol C, 16 ± 1. No significant differences in the RPE among these three protocols were noted.

The $\dot{V}\,O_2$ during the last arm-cranking ergometer exercise (6th) of the cross-exercise without thigh occlusion ([39.2 ± 7.1] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) was significantly higher than $\dot{V}\,O_2$max for arm cranking ergometer exercise ([30.2 ± 4.4] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; 95% *CI* of the difference 8.1–15.3 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; $p = 0.002$), but this value was significantly lower than $\dot{V}$

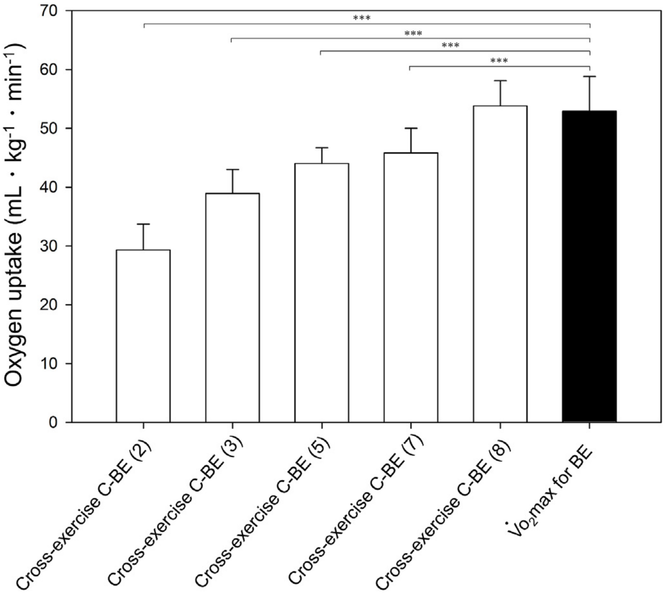

Fig. 2. The oxygen uptake during the bicycle ergometer exercise (BE) (the 2nd, 3rd, 5th, 7th, and 8th bouts) of the high-intensity intermittent cross-exercise C versus the maximal oxygen uptake ($\dot{V}\,O_2$max) for bicycle ergometer exercise (BE). *** indicates a significant difference from the $\dot{V}\,O_2$max measured during BE at $p < 0.001$.

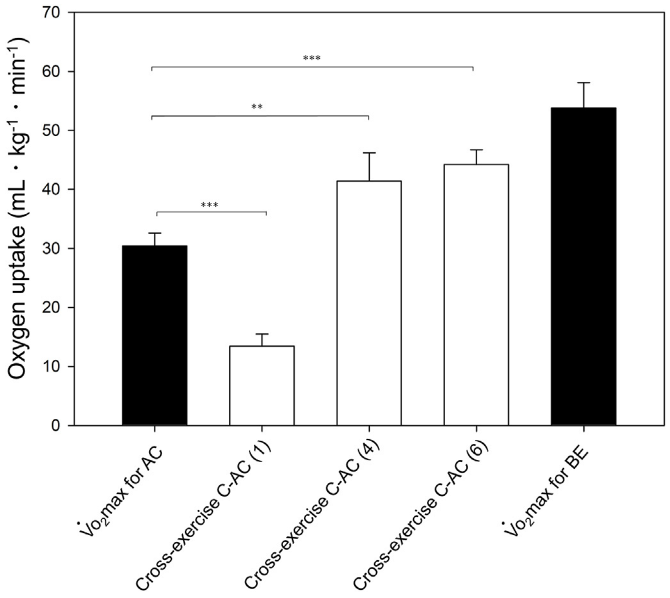

Fig. 3. The oxygen uptake during the arm-cranking ergometer exercise (AC) (the 1st, 4th, and 6th bouts) of the high-intensity intermittent cross-exercise C versus the maximal oxygen uptake ($\dot{V}$ O$_2$max) for AC and for bicycle ergometer exercise (BE). **, and *** indicate a significant difference from the $\dot{V}$ O$_2$max measured during AC at $p < 0.01$, and 0.001, respectively.

O$_2$max for the bicycle ergometer exercise ([50.9 ± 6.8] mL·kg^{-1}·min^{-1}; 95% *CI* of the difference −13.8 to −4.4 mL·kg^{-1}·min^{-1}; $p = 0.003$). The $\dot{V}$ O$_2$ during the last arm-cranking ergometer exercise (6th) of the cross-exercise with thigh occlusion ([29.8 ± 3.9] mL·kg^{-1}·min^{-1}) was 24% less than that observed during the same exercise without occlusion (95% *CI* of the difference −15.4 to −3.5 mL·kg^{-1}·min^{-1}; $p = 0.008$, Fig. 4). The value with occlusion did not differ from the $\dot{V}$ O$_2$max for the arm-cranking ergometer exercise (95% *CI* of the difference −4.7 to +4.0 mL·kg^{-1}·min^{-1}; $p = 0.84$), but that value was 41% less than $\dot{V}$ O$_2$max for the bicycle ergometer exercise ($p < 0.001$).

4. Discussion

The present investigation demonstrated that first the oxygen uptake during the last bout of bicycle exercise of the HIICE, which consists of alternative four 20 s running and three 20 s bicycle-ergometer exercise with 10 s rest between the exercise bout, increased to a level much higher than the $\dot{V}$ O$_2$max measured for bicycling and almost reached to the same values as the $\dot{V}$ O$_2$max for running exercise. Second, the oxygen uptake during the last bout of arm-cranking ergometer exercise (AC) of the HIICE, which consists of three 20 s AC and five 20 s bicycle-ergometer exercise (BE) (order of exercise: AC-BE-BE-AC-BE-AC-BE-BE) with 10 s rest between the exercise bouts, reached to the level much higher than for arm cranking only. Blood flow restriction to thigh muscles during the last bout of AC of the HIICE reduced oxygen uptake during the exercise bout to the level of $\dot{V}$ O$_2$max for AC.

Our previous study[7] showed that during the last bout of exhaustive high-intensity intermittent bicycle exercise, the oxygen uptake reached the $\dot{V}$ O$_2$max measured for bicycle exercise, suggesting that the aerobic energy-releasing system is fully activated by the exercise. The present investigation also demonstrated that the oxygen uptake during the last running bout (7th bout) of Experiment 1 nearly reached the $\dot{V}$ O$_2$max for running. We were able to detect small differences since our results are based on as many as 30 paired measurements with a high-quality method (Douglas bag method, addressed further in the Method section), thus allowing us to detect a difference of only ≈ 2%. In short, our results suggest that the cross-exercise activated the aerobic energy-releasing system almost maximally.

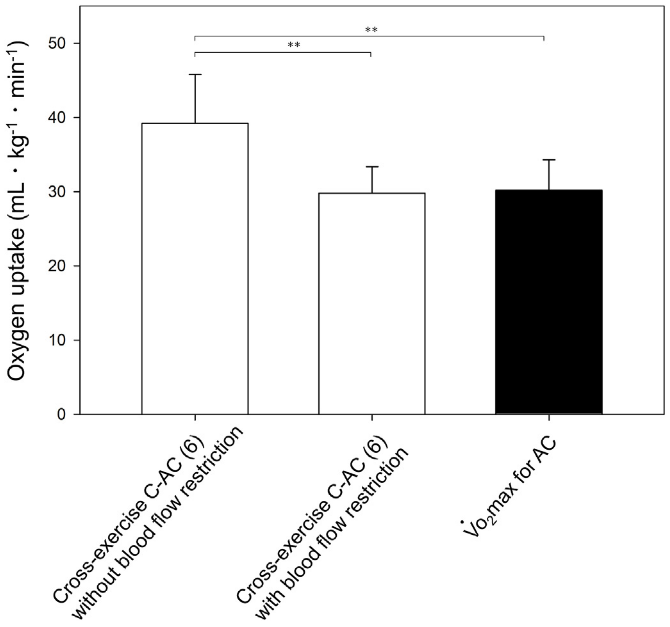

Fig. 4. The oxygen uptake during the last arm-cranking ergometer exercise (AC) (the 6th bout) of the cross-exercise C with or without blood-flow restriction to thigh muscles, and maximal oxygen uptake ($\dot{V}$ O$_2$max) for AC. ** indicates a significant difference from the oxygen uptake measured during AC[6] without blood flow restriction to thigh muscles at $p < 0.01$.

In the present investigation, we observed that the oxygen uptake during the last cycling bout (6th bout) of the cross-exercise was significantly greater than the $\dot{V}$ O$_2$max of the bicycling exercise and nearly as high as the $\dot{V}$ O$_2$max of running. To explain this phenomenon, we hypothesized that the oxygen uptake during the 6th bout of bicycling of the cross-exercise is a summation of the oxygen uptake for the ongoing bicycling exercise and the excess post-exercise oxygen consumption (EPOC), which is calculated as the difference between the oxygen uptake at rest after exercise and the resting oxygen uptake,[29–34] induced by the preceding running exercise (5th bout). Although the exact biochemical bases explaining EPOC at specific time points after exercise have not yet been elucidated,[29] in this condition, most of the EPOC is thought to be used for the aerobic re-synthesis of phosphocreatine broken during the previous 20 s of high-intensity exercises.

To test the EPOC-hypothesis addressed above, we might have used a classical occlusion technique[35] which shuts off the blood flow and return to and from the recruited muscle by the previous running exercise of the cross-exercise, and which may abolish the EPOC of a previous running exercise. However, since the muscles recruited for a running exercise are both upper and lower leg muscles, also the most proximal line of the upper legs should be occluded. However, this occlusion may also limit the blood flow to thigh muscles, which are recruited during the bicycling exercise and affect the oxygen uptake for bicycling. To avoid these problems, we used a different high-intensity intermittent cross-exercise involving bicycling and arm-cranking ergometer exercises.

Arm-cranking ergometer exercise has some advantages. First, the difference in $\dot{V}$ O$_2$max between the arm-cranking and bicycling is much larger than the corresponding difference between the running and bicycling exercises.[36] The second advantage is that occluding muscles of the lower legs only limits the gas exchange of the leg muscles, which are recruited for the bicycling but not for arm cranking. The occlusion applied would expectedly not affect the oxygen uptake for the arm-cranking.

A high-intensity intermittent cross-exercise using bicycle and arm-cranking exercises in an alternating fashion, which has been adopted for running and bicycle ergometer exercise in previous studies, did not elevate the subjects' oxygen uptake during the last bicycling exercise to the $\dot{V}$ O_2max of cycling. We therefore designed several different high-intensity intermittent cross-exercises consisting of bicycle and arm-cranking ergometer exercises with the same intensity and duration but with different numbers and orders of the bouts, and we measured the oxygen uptake during the cross exercises. As shown in Fig. 2, the oxygen uptake during the last (8th) bicycling exercise of the cross-exercise C reached the $\dot{V}$ O_2max for bicycle ergometer exercise, while that of the cross-exercise A and B did not. We thus used cross exercise C protocol as our preferred tool to clarify possible effects of preceding high-intensity exercise with a relatively high $\dot{V}$ O_2max on the oxygen uptake during later exercise with a relatively low $\dot{V}$ O_2max during high-intensity intermittent cross-exercise.

The results demonstrated that the oxygen uptake during the arm-cranking ergometer exercise (6th bout) of the cross-exercise C with occlusion was reduced to the level of the $\dot{V}$ O_2max measured for the arm-cranking ergometer exercise only. This result may indicate that the measured oxygen uptake during the high-intensity arm-cranking exercise of the present cross-exercise without occlusion was affected by the oxygen uptake in the lower extremities, probably due to the EPOC of the muscles recruited by the preceding bicycling exercise. This result suggests, further, that aerobic energy releasing system of the arm may be fully functioning during the arm-cranking exercise of the cross-exercise.

Notably, our observation that the oxygen uptake during the last bout of the high-intensity intermittent cross-exercise (bicycle ergometer exercise) was higher than the $\dot{V}$ O_2max measured for the bicycle exercise (Fig. 1) appears to challenge the golden concept of $\dot{V}$ O_2max,[14–22] that is the oxygen uptake during a specific exercise cannot exceed the $\dot{V}$ O_2max for the specific exercise. If this is so, this oxygen uptake should be the $\dot{V}$ O_2max for the specific exercise.

Bergh et al.[36] reported that the oxygen uptake during maximal exercise combining bicycle and arm-cranking ergometer exercise (4.40 $L \cdot min^{-1}$) exceeded the $\dot{V}$ O_2max measured for arm cranking ([3.01 ± 0.40] $L \cdot min^{-1}$) and bicycle ergometer exercise ([4.12 ± 0.56] $L \cdot min^{-1}$), and reached the $\dot{V}$ O_2max measured for running exercise (4.44 $L \cdot min^{-1}$). Secher et al.[37] also reported that the $\dot{V}$ O_2max attained during an arm-plus-leg exercise was higher than that recorded during a leg exercise only, and they noted that the $\dot{V}$ O_2max reached the level of that measured during treadmill running. These previous studies suggested that, if there is more than one source that consumes oxygen maximally, the oxygen taken up by the multiple sources may exceed the $\dot{V}$ O_2max measured for a specific single exercise.[38] Since the EPOC of a preceding high-intensity intermittent cross-exercise-incorporated exercise at supramaximal intensity with a relatively high $\dot{V}$ O_2max is presumed to be quite large, such a large EPOC is also regarded as another source of oxygen consumption. Consequently, the oxygen uptake during subsequent exercise may exceed the $\dot{V}$ O_2max of that exercise.

Our method of measuring the oxygen uptake reports only the value for the whole body. It does not allow us to discriminate between consumption in different parts of the body. We do not suggest that the high oxygen uptake measured during arm cranking without occlusion means that the muscles in the upper body consumed more oxygen that that indicated by the $\dot{V}$ O_2max of arm cranking. We rather suggest that the high measured oxygen uptake during the last bout of arm cranking during the cross-exercise without occlusion was a combined effect of oxygen consumption by working muscles in the upper body and an EPOC-effect of the previous exercise in lower body muscles. This again suggests that the results obtained in the present investigation are consistent with the general principle of the $\dot{V}$ O_2max.

The RPE after the present study's high-intensity intermittent cross-exercise consisting of alternative bouts of running and bicycling bouts was significantly less than that reported after two high-intensity intermittent exercises adopting only one of the two exercises. The mean RPE was 15 (hard), while the previous two high-intensity intermittent exercises were exhausting (RPE: 20). In addition, the subjects' blood lactate concentrations after the high-intensity intermittent cross-exercise were significantly lower than those measured after the two high-intensity intermittent exercises (only running or the bicycle ergometer exercise).

This study may also provide practical suggestions for circuit training or intermittent strength-type training. Such training exercising different major muscle groups and consisting of 20 s of exercise with a 10 s rest between the exercise bouts at either maximal or non-maximal efforts. If properly designed, it may tax the aerobic system significantly and might improve maximal aerobic power. However, since the oxygen uptake (~30% of the $\dot{V}$ O_2max for running) during the last 20 s of a push-up exercise implemented in the protocol was far less than the $\dot{V}$ O_2max for running,[2] exercises recruiting minor muscle groups are not suitable for high-intensity circuit training in terms of improving the whole-body $\dot{V}$ O_2max. On the other hand, circuit training may allow recovery of one muscle group while another muscle group is exercised. Our study provides new basis for that model.

5. Conclusion

In conclusion, the present investigation demonstrated that, in non-exhaustive high-intensity intermittent cross-exercise, the oxygen uptake during the last bout of an exercise with a relatively low $\dot{V}$ O_2max increased to a level well above the exercise-specific $\dot{V}$ O_2max. This may be caused by a combined high oxygen consumption in the working muscles plus an oxygen-consumption in recovering muscles (an EPOC-effect).

Submission statement

All authors have read and agree with manuscript content. While this manuscript is being reviewed for this journal, the manuscript will not be submitted elsewhere for review and publication.

Ethical approval statement

The protocols for the experiments and procedures involved in the present investigation were approved by the Ethics Committee of Ritsumeikan University (BKC-IRB-2011-05, BKC-IRB-2011-013, BKC-IRB-2013-007). After receiving a detailed explanation of the purpose, potential benefits, and risks of participating in the study, each subject gave written informed consent.

Authors' contributions

YX, XL, TH, and IT conceived and designed the research. YX, XL and KT conducted experiments. YX, XL, and IT analyzed data. YX, TH, and IT prepared the manuscript.

Conflict of interest

The authors have no direct or indirect interests that are in direct conflict with the conduction of the study.

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