Effects of moderate-intensity exercise on diet-induced increase in resting oxygen uptake

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Abstract We measured and compared the diet-induced increase in resting oxygen uptake (DIIROU) after moderate-intensity exercise (MIE) with the DIIROU after high-intensity intermittent exercise (HIIE). Eight healthy adult males participated in six testing sessions, including the measurement of resting oxygen uptake with and without lunch after MIE, HIIE, and as a non-exercise control. The MIE was 30 min of exercise at an intensity of 70% V\textsubscript{O2max}, and the HIIE consisted of seven to eight 20 second bouts of exhaustive exercise at 170% V\textsubscript{O2max} with 10-sec rests between the bouts. The exercise time of the HIIE for the no-lunch (fasting) experiment (144.1 ± 10.0 sec) was not significantly different from that for the lunch experiment (142.8 ± 10.3 sec). Lunch (713 kcal) was served for the lunch experiment at 12:00, which corresponds to ~1.5 hr after each exercise. Compared to the non-exercise control results, the accumulated oxygen uptake (AOU) of the MIE and HIIE were significantly higher from the end of the exercise until 11:30 (p < 0.001). However, no difference in AOU was noted from 11:30 to 12:00 between the control and MIE or HIIE results, suggesting that excess post-exercise oxygen consumption wore off before 12:00. The values of DIIROU (quantified as the difference in AOU between the lunch and fasting experiment from 12:00 to 16:00) after HIIE, MIE, and the non-exercise control were 132.7 ± 37.2, 102.8 ± 48.0, and 77.8 ± 40.7 ml/kg, respectively. The ΔDIIROU for the MIE (25.0 ± 17.8 ml/kg) calculated as the difference in DIIROU from the non-exercise control was significantly less than that of the HIIE (55.0 ± 25.4 ml/kg) (p < 0.01). These results may indicate that MIE potentiates a diet-induced increase in resting oxygen uptake, even though this effect was less than that of HIIE and was quantitatively small.

Keywords: metabolic chamber, high-intensity intermittent exercise, diet-induced thermogenesis, oxygen uptake, moderate-intensity exercise, Tabata training

Introduction

Obesity is a major health problem in many industrialized countries due to its association with cardiovascular diseases, hypertension, and diabetes mellitus. A negative energy balance is essential for reducing body fat stores\textsuperscript{1}), and therefore physical activity/exercise is recommended as a major strategy to induce a negative energy balance by increasing the body’s energy consumption. Physical activity/exercise enhances energy consumption both during exercise and after the exercise (known as ‘excess post-exercise oxygen consumption’ [EPOC])\textsuperscript{2}). Since EPOC amounts to approximately 15% of energy consumption during exercise\textsuperscript{3}), EPOC has been speculated to play an important role in increasing the body’s energy consumption; and it has been suggested that EPOC may reduce body weight. In addition to these two effects of exercise on energy metabolism, it is possible that prior exercise elevates a diet-induced increase in the body’s resting oxygen uptake (DIIROU)\textsuperscript{4}).

Several studies indicated that prior moderate-intensity exercise (MIE) enhances the resting oxygen uptake after a meal\textsuperscript{4-6}). However, other investigators failed to confirm such a post-feeding response of the resting oxygen uptake after MIE\textsuperscript{7,8}). In their review, LaForgia et al.\textsuperscript{9)} concluded that the resting oxygen uptake after a meal was unlikely to be significantly influenced by MIE.

Regarding the effects of prior supramaximal intensity exercise on the DIIROU, the results of a study by Hazel et al.\textsuperscript{10)} suggest that supramaximal-intensity exercise may elevate the DIIROU. Another study showed that the EPOC from 3 to 9 hrs (including lunch) after supramaximal running (20 × 1-min) at 105% V\textsubscript{O2max} was higher than that observed on a non-exercise control day\textsuperscript{11}). In contrast, the resting oxygen uptake was measured after sprint-interval exercise during a 22-hr period (including three meals) with the use of a metabolic chamber\textsuperscript{12)}, and there were no observable differences between the exercise and non-exercise control days in the total resting oxygen uptake during the late recovery phase (3-22 hrs after the
exercise period, at which point the EPOC had worn off). The conflicting results from these studies regarding post-meal oxygen consumption after supramaximal intensity exercise remain to be resolved.

By using a metabolic chamber, we recently demonstrated that subjects’ energy consumption, induced by a diet after high-intensity intermittent exercise (HIIE) at supramaximal intensity (the oxygen demand of which was equivalent to 170% VO\textsubscript{2max}), was higher than that induced solely by the same diet, suggesting that such HIIE enhances the DIIROU\textsuperscript{13).} Since the best approach to measure long-term energy expenditure — especially to detect small changes in oxygen uptake from a baseline — is indirect calorimetry in a respiratory chamber (which has demonstrated a relatively small increase in a diet-induced increase in oxygen uptake), we expected that this method and the protocol that we used in our previous study\textsuperscript{13} could be used to detect potential differences in the DIIROU (ΔDIIROU) that may exist in long-term oxygen uptake between MIE and a no-exercise condition. However, no research has reported the effects of MIE on oxygen uptake between MIE and HIIE. Since the best approach to identify the quantitative difference in the ΔDIIROU between MIE and HIIE, which has demonstrated a relatively small increase in a diet-induced increase in oxygen uptake, we expected that this method and the protocol that we used in our previous study\textsuperscript{13} could be used to detect potential differences in the DIIROU (ΔDIIROU) that may exist in long-term oxygen uptake between MIE and a no-exercise condition. However, no research has reported the effects of MIE on oxygen uptake between MIE and HIIE. Since the best approach to identify the quantitative difference in the ΔDIIROU between MIE and HIIE, which has demonstrated a relatively small increase in a diet-induced increase in oxygen uptake, we expected that this method and the protocol that we used in our previous study\textsuperscript{13} could be used to detect potential differences in the DIIROU (ΔDIIROU) that may exist in long-term oxygen uptake between MIE and HIIE. Since the authors. After receiving a detailed explanation of the purpose, potential benefits, and risks of participating in the study, each subject gave written informed consent.

Subjects were excluded from this study if they had evidence of cardiovascular disease, anemia, diabetes, renal or hepatic disease, hypo- or hyperthyroidism, or a musculoskeletal problem. Smokers and individuals taking medications were also excluded. Subjects were told not to alter their dietary and exercise habits during the experimental period.

Pre-test. All exercises were conducted on the mechanically braked cycle ergometer at 90 repetitions per minute (rpm). Since the exercise intensity used in this investigation is expressed relative to participants’ VO\textsubscript{2max}, that value was established in pretests as follows. First, to determine a linear relationship between cycling power (watts) and steady-state oxygen uptake (L·min\textsuperscript{-1}) for each subject, we measured the oxygen uptake during the last 2 min of 6–9 different 10-min bouts of cycling at a constant power ranging between 35% and 90% VO\textsubscript{2max}. Next, to determine the VO\textsubscript{2max}, we measured the oxygen uptake during the last two or three 30-sec intervals during several bouts of supramaximal-intensity exercise that exhausted the subjects within 2–4 min. After confirming a leveling-off of oxygen uptake by increasing the intensity, we took the highest oxygen uptake measured as subject VO\textsubscript{2max}\textsuperscript{4,15}. To determine the power of the bicycle exercise (watts) that corresponds to the oxygen demand (L·min\textsuperscript{-1}) of 170% VO\textsubscript{2max}, we used the following procedures. First, the oxygen demand (L·min\textsuperscript{-1}) of 170% VO\textsubscript{2max} was calculated as 1.70 times the VO\textsubscript{2max} (L·min\textsuperscript{-1}). Then, cycling power (watts) was determined by extrapolation using the linear relationship between cycling power (watts) and the steady-state oxygen uptake (L·min\textsuperscript{-1}) established at the submaximal exercise intensity described above (see Fig. 1 in Tabata et al.\textsuperscript{16}).
**Lunch experiments.** Subjects refrained from any structured exercise on the day before each lunch or fasting experiment. They were also instructed to eat their usual meals on the day prior to the experiment. They were asked to record these meals, but were not required to report in detail (i.e., grams of all food items) of the content of meals ingested on the day before the first chamber experiment. An experienced registered dietitian ensured that the meals consumed by the subjects were adequate for Japanese individuals in terms of a food intake pattern that reflects calorie, carbohydrate, fat, and protein intake. No alcohol or caffeine was allowed for 24 hrs before either experiment. These instructions were consistent for all experiments (i.e., the lunch and fasting sets of experiments).

Several days before the first chamber experiment, subjects were invited to come to the chamber and stay for a couple of hours so that they could become familiar with it. At 08:00 on the experiment days, subjects ate breakfast (energy content, 554 kcal; energy ratio: protein 16%, fat 22%, carbohydrate 62%; Table 1, upper panel).

On the HIIE day, just before a subject entered the metabolic chamber at 10:00, he put on a mask for expired gas collection via the Douglas bag method. The subject continued to wear the mask after he entered the chamber. The door to the chamber was closed, and the subject himself connected his mask to a hose; the end of the hose was connected to a three-way cock located outside the chamber^13^.

Once the subject was inside the metabolic chamber, the operation for measuring oxygen consumption was initiated. All expired gas was collected by a mask worn by the subject and sent outside the chamber, so that there was no trace of oxygen consumption in the metabolic chamber after the first few minutes. Outside of the chamber, the tester manipulated the three-way cock as is done for an ordinary expired-gas measurement in a laboratory.

Twenty minutes before the subject started the HIIE at 10:30, he biked for 10 min at 50% \( \text{VO}_2\text{max} \) intensity as a warm-up exercise. The HIIE that the subjects performed in the metabolic chamber was an exhaustive exercise consisting of 7 to 8 bouts of 20-sec high-intensity intermittent bicycle exercise with 10-sec rests between bouts. The exercise intensity was 170% \( \text{VO}_2\text{max} \); 7 to 8 bouts at this level have been shown to exhaust a subject^14^.

This HIIE protocol has been shown to stimulate both aerobic and anaerobic energy-releasing systems maximally, and to improve both aerobic and anaerobic energy-releasing systems simultaneously^16,17^.

The subject continued to wear the mask and sit on the bicycle ergometer for 15 min after the end of the HIIE. The subject then removed the mask himself. Through a glass window, a tester outside the metabolic chamber monitored the workload continuously and gave verbal instructions through a microphone with a speaker inside the chamber. This was to ensure that the work output was precisely the same as that observed in experiments conducted in ordinary experimental rooms.

The subject then alternated between two activities - 20 min of lying on a bed awake, and 10 min of sitting on a chair doing deskwork until 16:00, when the subject was allowed to leave the chamber. During this time, the subject read books or studied. The subjects were not allowed to watch DVDs or videos.

Lunch was served at 12:00. The meals were prepared based on the estimated energy requirement for Japanese subjects^18^ assuming a physical activity level of 1.5. The energy content of lunch was 713 kcal. The meals contained 15% protein, 25% fat and 60% carbohydrate in energy equivalents, which is the mean value for the Japanese population^19^.

On the MIE experimental day, the subject entered the chamber at 09:00 after he ate breakfast at 08:00. At 10:04, he started the MIE (duration, 30 min; intensity, 70% \( \text{VO}_2\text{max} \)). His oxygen uptake was measured in the metabolic chamber during the entire experimental period. After the exercise, the subject followed the same procedure as for the HIIE experiment.

For the control days, the subjects followed the same protocol as on the HIIE day until 16:00, except that they did not perform the HIIE.

**Fasting experiments.** On the fasting HIIE and fasting MIE experiment days, subjects entered the metabolic chamber at 10:00 and 09:00, respectively after eating breakfast at 8:00 (Table 1, lower panel). They started the HIIE and MIE at 10:30 and 10:04, respectively, following the same protocol as in the lunch experiments. The only difference was that the subjects were not served lunch. Otherwise the subjects followed the same protocol as on the exercise day of the lunch experiments, until 16:00, when they left the chamber.

For the control days, the subjects followed the same protocol as on the HIIE day until 16:00, except that they did not perform the HIIE.

**Oxygen uptake measurement using a Douglas bag.** The 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 air were measured by a mass spectrometer (Arco 2000; Arcosystems, Kashiwa, Chiba, Japan). We used the Douglas bag method for this metabolic chamber study in order to avoid the assumed artifact effects of HIIE on the calculated oxygen uptake during and after the HIIE, which induces sudden and rapid increases in oxygen uptake and carbon dioxide production that may interfere with the calculation of oxygen consumption using the modified Henning equation^19,20^.

Therefore, the Douglas bag method for measuring the subjects’ oxygen uptake was used only from the start of the HIIE to 14 min after the cessation of the HIIE. Our previous study showed that the calculated oxygen uptake...
### Table 1. Timetables of lunch and fasting experiments

#### Lunch Experiments

| Time   | Activity                        |
|--------|---------------------------------|
| 7:00   | Getting up                      |
| **8:00-8:20** Breakfast          | Sitting quietly                  |
| 8:20-10:00 |                     |
| 10:00-10:10 | Rest on the bicycle ergometer  |
| 10:10-10:20 | Warming up                      |
| 10:20-10:30 | Rest on the bicycle ergometer  |
| 10:30-10:34 | HIIE                           |
| 10:34-10:48 | Rest on the bicycle ergometer  |
| 10:48-11:00 | Lie awake in supine position    |
| 11:00-12:00 | Repeat                          |
|        | Desk work                       |
|        | Lie awake in supine position    |
| **12:00-12:20** Lunch            | **Lie awake in supine position**  |
| 12:20-12:30 | Lie awake in supine position    |
| 12:30-16:00 | Repeat                          |
|        | Desk work                       |
|        | Lie awake in supine position    |

#### Fasting Experiments

| Time   | Activity                        |
|--------|---------------------------------|
| 7:00   | Getting up                      |
| **8:00-8:20** Breakfast          | Sitting quietly                  |
| 8:20-10:00 |                     |
| 10:00-10:10 | Rest on the bicycle ergometer  |
| 10:10-10:20 | Warming up                      |
| 10:20-10:30 | Rest on the bicycle ergometer  |
| 10:30-10:34 | HIIE                           |
| 10:34-10:48 | Rest on the bicycle ergometer  |
| 10:48-11:00 | Lie awake in supine position    |
| 11:00-16:00 | Repeat                          |
|        | Desk work                       |
|        | Lie awake in supine position    |
uptake during the first minute (i.e., 15–16 min after the HIIE) using the chamber \((0.425 \pm 0.060 \text{ L} \cdot \text{min}^{-1})\) was comparable to the measured oxygen consumption during the last minute (i.e., 14–15 min after cessation of the HIIE) using the Douglas bag method \((0.433 \pm 0.061 \text{ L} \cdot \text{min}^{-1})\), demonstrating the concurrence of oxygen uptake measurement between the Douglas bag method and the metabolic chamber measurement\(^4\).

On the MIE and non-exercise control days in the present study, the Douglas bag method was not used. Only the metabolic chamber was utilized for all measurements of oxygen uptake.

**Metabolic chamber.** An open-circuit indirect metabolic chamber (Fuji Human Calorimeter, Fuji Ika Sangyo, Chiba, Japan) was used to measure the subjects’ oxygen uptake. The temperature and relative humidity in the experiment room were controlled at 25°C and 50%, respectively; the internal area and volume of the chamber were 8.1 m\(^2\) and 18.5 m\(^3\), respectively. Fans were used to move air around the room. The chamber was a pull calorimeter (i.e., the flow was controlled and measured at the outlet at a rate of 80 L \cdot \text{min}^{-1}). To measure the outgoing flow, the chamber was equipped with a mass flow controller (CMQ02, Yamatake, Tokyo). The concentrations of gases in the outgoing air were measured with high precision by online process mass spectrometry (VG Prima dB; Thermo Fisher Scientific, Winsford, UK). The mass spectrometer measured the fractional concentrations of \(\text{O}_2, \text{CO}_2, \text{N}_2, \text{Ar}\), and \(\text{O}_3\).

The oxygen uptake was calculated with a modified Henning equation\(^{18,20}\). The accuracy and precision of the metabolic chamber for measuring oxygen uptake, as determined by the alcohol combustion test, was 100.6 ± 0.9% (mean ± SD) over 3 hr. The concentrations of gases in the outside air were measured every 1 hr to identify any effects of the changed concentration of incoming gases on the calculated oxygen uptake.

The respiratory quotient (RQ) or respiratory exchange ratio (R) is \(>1.0\) during HIIE and \(<0.7\) during the period immediately after HIIE. The RQ (R) during MIE such as that used in the present study may exceed 1.0. Therefore, the energy consumption in calories during HIIE and MIE and the following recovery period cannot be calculated using oxygen uptake data and the RQ or R. This is the case even with currently available formulas such as the Weir method\(^{19}\), which hypothesized that the range of RQ is 0.7–1.0. We thus calculated the energy expenditure after lunch by using the Weir equation\(^{19}\), because we observed that the subjects’ oxygen uptake, carbon dioxide production, and RQ (R) were stable, suggesting that respiratory oxygen consumption and carbon dioxide virtually reflect the oxygen consumption and carbon dioxide production in metabolically active organs/tissues/cells.

**Statistical analysis.** Values are shown as mean ± SD. We performed a repeated-measures two-way analysis of variance (ANOVA) to determine the degree of significance of among-group differences. The significance level for all comparisons was set at \(p < 0.05\). The sample sizes required to detect the HIIE effect on the change in diet-induced thermogenesis (ADIROU) in the Lunch experiments and the EPOC in the Fasting experiments were \(n = 8\) and \(n = 8\), respectively (G* Power ver. 3.1; Softpedia) (\(\alpha = 0.05\) and power = 0.8).

**Results**

**Accumulated oxygen uptake**

**Lunch experiments.** Figure 1 illustrates the subjects’ accumulated oxygen uptake values at every 30 min from the end of the MIE, HIIE, and on the control day to 16:00. Compared to the control day, the accumulated oxygen uptake on the MIE day was significantly higher from the end of the MIE to 11:00 and from 11:00 until 11:30 at \(p < 0.01\) and \(0.05\), respectively. No significant difference in accumulated oxygen uptake was noted from 11:30 to 12:00. Compared to the control day, the accumulated oxygen uptake was significantly higher from 12:30 to 13:00 and from 15:30 to 16:00 on the MIE day (\(p < 0.05\)).

In the Lunch experiments, the duration of exercise to exhaustion by the HIIE was 144.1 ± 10.0 sec. Compared to the control day, the accumulated oxygen uptake on the HIIE day was significantly higher from the end of the HIIE to 11:30 (\(p < 0.001\)). No significant difference in accumulated oxygen uptake between HIIE and the control day was noted from 11:30 to 12:00. The accumulated oxygen uptake on the HIIE day was significantly higher than that on the control day during the time period 12:30 to 16:00 (\(p < 0.05\)).

The accumulated oxygen uptake between the end of the exercise to 11:00, 11:00 to 11:30, and 13:00 to 13:30 were significantly higher on the HIIE day compared to the MIE day (\(p < 0.001\), 0.01 and 0.05, respectively).

The accumulated oxygen uptake from 10:34 to 16:00 for the HIIE day, MIE day, and control day was 1578.6 ± 74.3, 1459.0 ± 79.4, and 1399.9 ± 70.7 ml/kg, respectively. The accumulated oxygen uptake measured on the control day was significantly less than those of both the MIE and HIIE days (\(p < 0.001\)), and that on the MIE day was significantly less compared to the HIIE day (\(p < 0.01\)).

**Fasting experiments.** Figure 2 shows the accumulated oxygen uptake of each 30-min period from the end of the MIE to 16:00 in the Fasting experiments. Compared to the control day, the accumulated oxygen uptake after the MIE was significantly higher from the end of the MIE to 11:00 and from 11:00 to 11:30 at \(p < 0.01\) and 0.05, respectively. However, no significant difference in accumulated oxygen uptake was observed from 11:30 to 12:00.

The duration of exercise to exhaustion by HIIE in the Fasting experiments was 142.8 ± 10.3 sec, which is not
Fig. 1  Effect of lunch on the accumulated resting oxygen uptake after MIE and HIIE (mean ± SD) (Lunch experiments). ††p < 0.01, †p < 0.05, MIE vs. the non-exercise control day. ***p < 0.001, **p < 0.01, *p < 0.05, HIIE vs. the non-exercise control day. §§§p < 0.001, §p < 0.05, MIE vs. HIIE.

Fig. 2  The accumulated resting oxygen uptake after MIE and HIIE without lunch (mean ± SD) (Fasting experiments). ††p < 0.01, †p < 0.05, MIE vs. the non-exercise control day. ***p < 0.001, HIIE vs. the non-exercise control day. §§§p < 0.001, MIE vs. HIIE.
significantly different from that recorded in the Lunch experiments. Compared to the control day, the accumulated oxygen uptake on the HIIE day was significantly higher from the end of the HIIE until 11:30 (p < 0.001). No significant difference in accumulated oxygen uptake was noted from 11:30 to 16:00.

The accumulated oxygen uptake between 10:34 to 11:00 and between 11:00 and 11:30 was significantly less higher on the MIE day than the HIIE day at p < 0.01 and p < 0.05, respectively. However, no significant difference in accumulated oxygen uptake was observed after 11:30.

The accumulated oxygen uptake during the 10-min warm-up exercise for HIIE was 221.6 ± 14.0 mL/kg (Table 2). The accumulated oxygen uptake during the MIE was significantly higher than that measured during the HIIE. The EPOC was calculated as the difference between the accumulated oxygen uptake of the exercise day (MIE or HIIE) and the control day from 10:34 to 11:30. The EPOC calculated as the difference between the accumulated oxygen uptake of the HIIE day and the control day from 10:34 to 11:30 (139.0 ± 23.6 ml/kg) was significantly higher than that of MIE (50.8 ± 17.3 ml/kg) (p < 0.01).

The diet-induced increase in resting oxygen uptake (DIIROU) of the control day, calculated as the difference in the accumulated oxygen uptake from 12:00 to 16:00 between the Lunch day (1034.3 ± 52.6 ml/kg) and the Fasting day (957.1 ± 40.7 ml/kg) was 77.8 ± 40.7 ml/kg (Table 2). The DIIROU values of the HIIE day and MIE day were 132.7 ± 37.2 and 102.8 ± 48.0, respectively. The diet-induced increase in DIROU (ΔDIROU) of the MIE (25.0 ± 17.8 ml/kg), which was calculated as the difference in DIROU between the MIE and control day, was significantly less than that of the HIIE (55.0 ± 25.4 ml/kg) (p < 0.01).

**Accumulated carbon dioxide production**

*Lunch experiments.* The accumulated carbon dioxide production from 10:34 to 11:00 after the HIIE was significantly higher than that of the MIE and control days (Fig. 3). After this period, no significant difference was observed among the three conditions.

*Fasting experiments.* The accumulated carbon dioxide production from 10:34 to 11:00 after the HIIE was significantly higher than that of the MIE and control days (Fig. 4). After this period, no significant difference was observed among the three conditions.

**Respiratory quotient (RQ) and respiratory exchange ratio (R)**

*Lunch experiments.* The RQ (R) of the HIIE observed from 10:34–10:48 was significantly higher than that of the MIE and control days (p < 0.001) (Fig. 5). The RQ (R) of the HIIE measured from 10:48 to 12:00 was significantly less than that observed on the MIE and control days. No significant difference among the three conditions was observed after lunch.

### Table 2. Accumulated oxygen uptake (AOU), excess post-exercise oxygen consumption (EPOC), diet-induced increase in resting oxygen uptake (DIIROU), and ΔDIROU in the lunch and fasting experiments (n = 8)

|                      | HIIE     | MIE      | Control |
|----------------------|----------|----------|---------|
| AOU during 10-min warm-up exercise (ml/kg) | 221.6 ± 14.0 | 222.5 ± 15.3 |        |
| AOU during exercise (ml/kg) | 131.2 ± 24.3*** | 1083.6 ± 173.2 | 127.9 ± 18.0*** |
| AOU (ml/kg) 10:34-11:30 | 361.3 ± 31.6***,§§§ | 273.1 ± 29.7***,§§§ | 222.3 ± 18.0 |
| EPOC (ml/kg) 10:34-11:30 | 139.0 ± 23.6*** | 50.8 ± 17.3 | 134.9 ± 33.5*** |
| AOU (ml/kg) 12:00-16:00 | 1034.3 ± 52.6 | 957.1 ± 40.7 | 959.7 ± 43.7 |
| DIIROU (ml/kg) 12:00-16:00 | 132.7 ± 37.2***,§ | 102.8 ± 48.0 | 77.8 ± 40.7 |
| ΔDIROU (ml/kg) | 55.0 ± 25.4*** | 25.0 ± 17.8 |         |
**Fig. 3** Effect of lunch on the accumulated resting carbon dioxide production after MIE and HIIE (mean ± SD) (Lunch experiments). ***p < 0.001, HIIE vs. the non-exercise control day. §§§p < 0.001, MIE vs. HIIE.

**Fig. 4** The accumulated resting carbon dioxide production after MIE and HIIE without lunch (mean ± SD) (Fasting experiments). ***p < 0.001, HIIE vs. the non-exercise control day. §§§p < 0.001, MIE vs. HIIE.
Fig. 5 Effect of lunch on the RQ (R) after MIE and HIIE (mean ± SD) (Lunch experiments). †††p < 0.001, ††p < 0.01, MIE vs. the non-exercise control day. *** p < 0.001, *p < 0.05, HIIE vs. the non-exercise control day.

Fig. 6 The RQ (R) after MIE and HIIE without lunch (mean ± SD) (fasting experiments). †††p < 0.001, MIE vs. the non-exercise control day. ***p < 0.001, *p < 0.05, HIIE vs. the non-exercise control day.
Fig. 7 Effect of lunch on the energy expenditure after MIE and HIIE (mean ± SD) (Lunch experiments). †††p < 0.001, ††p < 0.01, †p < 0.05, MIE vs. the non-exercise control day. ***p < 0.001, **p < 0.01, *p < 0.05, HIIE vs. the non-exercise control day. §§§p < 0.001, §§p < 0.01, §p < 0.05, MIE vs. HIIE.

Fig. 8 The energy expenditure after MIE and HIIE without lunch (mean ± SD) (Fasting experiments). †††p < 0.001, ††p < 0.01, †p < 0.05, MIE vs. the non-exercise control day. ***p < 0.001, **p < 0.01, *p < 0.05, HIIE vs. the non-exercise control day. §§§p < 0.001, §§p < 0.01, §p < 0.05, MIE vs. HIIE.
**Fasting experiments.** The RQ (R) of the HIIE observed from 10:34 to 10:48 was significantly higher than that of the MIE and control days (p < 0.001) (Fig. 6). The RQ (R) of the HIIE measured from 10:48 to 12:00 was significantly less than that observed on the MIE and control days. No significant difference among the three conditions was noted after 12:00.

**Energy expenditure (kcal)**

**Lunch experiments.** The energy expenditure values during 12:30–13:00 (from 30 to 60 min after lunch) and at 13:00–13:30 (from 60 to 90 min after lunch) after the HIIE were significantly higher than that observed on the non-exercise control day, at p < 0.01 and 0.05, respectively (Fig. 7). The energy expenditure values during 12:30–13:00 (from 30 min to 60 min after lunch) after the MIE was significantly lower than that observe after the HIIE (p < 0.05). The energy expenditure during 15:30–16:00 after the HIIE was significantly higher than that observed on the non-exercise control day (p < 0.05).

**Fasting experiments.** No significant difference in energy expenditure among the three conditions was observed after lunch in the Fasting experiments (Fig. 8).

**Discussion**

Our findings demonstrated the ΔDIIROU after MIE and the ΔDIIROU measured after MIE were significantly less than that observed after HIIE. In addition to the resting oxygen uptake (Figs. 1, 2), Figures 3–6 show that from 12:00, the carbon dioxide production and the RQ (R) did not differ significantly among the three conditions of both the Lunch and Fasting experiments, suggesting that acute effect of both MIE and HIIE on resting metabolism wore off before noon. These results suggest that the difference in resting oxygen uptake after 12:00 among the three conditions is related to an event occurring after 12:00, which we speculate was the lunch.

Bahr and Sejersted reported that a 4.5MJ meal 2 hrs after the cessation of cycling (80 min at 75% $\text{VO}_2\text{max}$) did not produce a significant EPOC from a fasting trial. Broeder et al., Willms and Plowman also reported no apparent augmentation of post-exercise metabolism as a result of meals that were ingested immediately after exercise. However, several studies demonstrated effects of food on the resting oxygen uptake after moderate-intensity exercise. The existence of effects of diet on the resting oxygen uptake after exercise thus remains controversial.

In the present investigation, 1.5 hours after the MIE, lunch significantly increased the resting oxygen uptake. Since the EPOC was observed to have worn off before the subjects ate lunch, we speculate that this increase in resting oxygen uptake should not be called an increase in the EPOC. The effects of food on resting oxygen uptake were reported to start at least 20 min after eating. In the present study, we observed a higher post-MIE resting oxygen uptake after lunch. These results thus suggest that the elevation of the resting oxygen uptake at this time period (12:00–16:00) is attributable to diet, and that this elevation is augmented by the preceding MIE. We therefore use the term ΔDIIROU, which Broeder et al. defined as a post-exercise thermogenic effect (PETE) of food.

The ΔDIIROU that we observed after MIE might be explained by the differences in protocol and methodology used between the present and previous studies, which failed to find a ΔDIIROU after MIE. Since the MIE-induced ΔDIIROU itself was so small in the present study (25.0 ml/kg for subjects weighing 67.9 ± 7.7 kg), when the meal was ingested just after MIE, the effect of the meal on the resting oxygen uptake could have been masked by the prior MIE-induced EPOC, which was dominant in the period immediately after the MIE and was relatively large (Fig. 2). Since in the present investigation, lunch was served after the subjects’ resting metabolism (oxygen uptake, carbon dioxide production, and RQ (R)) after the MIE and HIIE had returned to the level observed on the non-exercise control day (Figs. 1–6), only a small ΔDIIROU could be detected.

Secondly, the method used to measure the oxygen uptake might also affect the results. In this study, the subjects’ resting oxygen uptake after lunch was evaluated by a metabolic chamber, which is capable of continuously and stably measuring the oxygen uptake, whereas previous studies used the Douglas bag method, which measures oxygen uptake intermittently for a specific duration (e.g., 5–10 min) during the post-exercise and post-meal period. Therefore, as observed for HIIE, the present investigation could detect MIE-induced ΔDIIROU, which is even smaller than that of HIIE.

Our findings demonstrated that the ΔDIIROU after MIE was less than that after HIIE. Since the oxygen uptake during MIE is far greater than that during HIIE, the energy deficit (which may have affected the present results) was far higher than that for HIIE. Therefore, it was not feasible to adjust the energy consumption and energy deficit during HIIE and MIE in the present study. However, since glycogen degradation after 30 min of MIE at an intensity of 70% $\text{VO}_2\text{max}$ is assumed to be similar to that after HIIE, we adjusted the amount of glycogen degradation (which is a major energy substrate for HIIE and MIE) and compared the ΔDIIROU values of HIIE to those of the MIE. The results demonstrated that the ΔDIIROU after HIIE was higher than that observed after MIE. Therefore, the energy balance per se is not thought to be a major factor affecting the ΔDIIROU. However, further research that matches the energy consumption (including the energy consumption during specific exercise and the EPOC) of MIE and HIIE should be conducted to determine the precise effects of the difference in the energy balance before lunch on the ΔDIIROU.

Diet-induced thermogenesis, which results in an in-
crease in resting oxygen uptake after meal, may be explained by two components of the thermic effect of meals—obligatory and facultative—which represent the energy necessary for nutrient digestion, absorption, transport, and storage\(^2\), and the energy modulated by factors such as parasympathetic nervous activity\(^26,29\) and glucose tolerance\(^30\), respectively. Consequently, the differences in these factors between the MIE and control days in the present study may account for the difference in DIIROU after lunch. First, it is reasonable to speculate that the effect of the obligatory component on the DIIROU is similar after meals irrespective of MIE, since our subjects ate the same meals on the MIE and non-exercise control days. Therefore, the ΔDIIROU after MIE could be explained by a prolonged elevation of insulin sensitivity\(^31\) and parasympathetic activity\(^2\) after MIE.

In the present investigation, MIE and HIIE enhanced the diet-induced thermogenesis (ΔDIT) (12:00–16:00) calculated using the Weir equation and expressed in kcal for our subjects weighing 67.9 ± 7.7 kg by 6.6 ± 8.4 kcal and 15.7 ± 10.4 kcal, respectively. The HIIE-induced ΔDIT after lunch and supper (12:00–23:00) (47.8 ± 32.0 kcal for 10 subjects whose mean weight was 64.4 ± 6.0 kg) in our previous study\(^13\) is higher than that induced only by lunch in the present study. Since insulin sensitivity is known to be high until the morning following exercise\(^33\), as in the previous study\(^13\), a further ΔDIT after dinner might be added to the extra energy consumption related to the MIE if the measurement is continued after subjects eat dinner.

The contributions of physical activity and MIE to the total daily energy expenditure have been discussed by referring to two factors (energy expenditure during physical activity/exercise and the EPOC). Demonstration of prior-MIE-induced ΔDIIROU, in our present findings, is important from the viewpoint of pure physiology and nutritional sciences, because our results suggest that another factor (ΔDIIROU) related to physical activity and MIE should be considered when the total daily energy expenditure is discussed in terms of physical activity and MIE. However, the ΔDIIROU of MIE was smaller that of HIIE, and was less than that during and after exercise. With the assumption of no change in dietary intake and based on metabolic efficiency and the caloric cost of weight gain (fat disposition), an increase in the total daily energy expenditure of approx. 50–150 kcal has been estimated as sufficient to prevent weight gain\(^33,34\). From this point of view, the ΔDIT calculated using the Weir equation (Figs. 7 and 8), after MIE in our present study, is limited even when the ΔDIT after dinner is considered. Therefore, for MIE, increasing the energy expenditure by increasing the exercise time is important for enhancing the energy expenditure to prevent weight gain, because a significant MIE-induced ΔDIT after meals is not expected.

Conclusions

The results of our experiments demonstrated that MIE elevated the DIIROU. The effects of MIE on daily energy consumption can thus be considered to include not only energy consumption during exercise and the EPOC, but also the ΔDIIROU, even though the energy expenditure (kcal) is relatively small compared to the daily total energy consumption. Our experiments also revealed that the effect of MIE on the ΔDIIROU is significantly smaller than that of HIIE.

Conflict of Interests

The authors have no conflict of interests to report. The results of the study are presented clearly, honestly, and without fabrication or inappropriate data manipulation.

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