During exercise, the energy consumed in muscle tissue is mainly supplied by carbohydrates (CHO) and fats. In regard to health promotion and athletic sports, it is important to regulate the metabolism of these two substrates. Fats are supplied by meals, and also by adipose tissues, but CHO must be acquired from meals before exercise because little is stored as glycogen in the liver or muscle tissue. Previous studies have been conducted on the ergogenic effects of CHO feeding before exercise (1–10). It was reported that CHO solution (such as glucose and maltodextrin) feeding before exercise can improve exercise performance through enhanced endurance exercise capacity (2, 4, 5, 10). Moreover, it was also reported that CHO mixed meal feeding before exercise can improve endurance exercise capacity (7, 9, 11).

The intake of CHO elevates the secretion of insulin from the pancreas into the blood. Glucose and insulin in plasma reach a peak approximately 30 min after the ingestion of CHO and thereafter gradually decrease to the basal level. Insulin facilitates glucose uptake into skeletal muscle cells via the translocation of glucose transporter 4 (GLUT4) and then increases CHO oxidation. On the other hand, insulin prevents fat oxidation in muscle tissue by inhibition of lipoprotein lipase activity (12). Hyperinsulinemia tends to supply energy with a predominance of CHO over fats as the energy source in the initial period during exercise (7). Previous studies have shown, by examining timing of carbohydrate intake before exercise, that a shorter time from intake to exercise increases blood glucose and insulin levels at the start of exercise and these factors gradually decrease after the start of exercise (9, 10). Moreover, Chryssanthopoulos et al. (5) have reported that glucose intake at 30 min before exercise causes a rapid reduction of blood glucose after the beginning of exercise. On the other hand, Coyle et al. (13) have shown that carbohydrate utilization during exercise is higher under the condition of meal intake at 4 h before exercise compared with the fasting condition. Therefore, regardless of the timing of carbohydrate intake, it is likely that carbohydrate intake before exercise promotes its utilization as a major energy source.
substrate during exercise. Some studies have shown a positive effect of CHO intake before exercise (2, 4, 5, 7–11), although other studies showed no effect (14, 15). Tokmakidis and Karamanolis (10) reported that the ingestion of a glucose solution (1 g·kg body mass⁻¹) at 15 min before exercise improves endurance running capacity. On the other hand, Devlin et al. (14) reported that when a snack (candy bar) was taken at 30 min before cycling exercise, there was no difference shown in the time to exhaustion between the ingestion of a snack and a placebo. These different results may have been caused by alterations in the insulin levels due to timing of the CHO intake. However, previous studies have investigated relatively short times between ingestion of CHO and exercise, and have looked at few variations in exercise time.

It has been reported that well-trained subjects have a high glucose uptake capacity compared with sedentary subjects (16–21). Seki et al. (20) examined the differences in capacity of glucose uptake and insulin sensitivity in sedentary and endurance-trained subjects. In their results, the GLUT4 mRNA level in the skeletal muscle was higher in endurance-trained subjects than the sedentary subjects and HOMA was also lower with endurance training (20), which suggests that the trained subjects have a high capacity of insulin sensitivity. Therefore, blood glucose and insulin levels after intake of carbohydrates (e.g. meal, drink and gel) pre-exercise do not rise in trained subjects compared with sedentary subjects. On the basis this background, we here hypothesized that energy metabolism during exercise in athletes with high insulin sensitivity does not affect fat oxidation during exercise soon or long after the last meal. To carry out the present study, we compared five conditions for aerobic exercise (lactate threshold (LT) intensity) in college athletes. The conditions are performance after a non-meal (water) and 1, 2, 3 and 4 h after identical meals on another day. The rate of CHO oxidation gradually increased along with the intensity of the exercise performed and drastically rose above the LT. In general, the LT is the exercise intensity that shows the largest amount of fat oxidation during aerobic exercise. Thus, exercise at that intensity is convenient for examination of changes in fat oxidation. We therefore investigated the effect of the timing of food intake on energy substrates at the LT intensity in well-trained young male college athletes.

**METHODS**

**Subjects.** Eight trained young male athletes belonging to the track and field team were recruited to participate in this study, which was approved by the ethics committee of Kyoto Prefectural University, and all of the subjects signed a consent form after reading information about the study and having the procedures explained to them. The present study was carried out in compliance with the Declaration Helsinki. One subject retired during the study due to deconditioning unrelated to the study. Therefore, data obtained from seven subjects (sprinters 2, long distance runners 3, jumpers 2) was analyzed.

**Table 1.** Subject characteristics.

| Age (y) | 20.0±0.0 |
| Height (cm) | 174.9±6.4 |
| Body weight (kg) | 63.8±5.8 |
| BMI (kg/m²) | 20.8±1.2 |
| LT (W) | 113.2±11.0 |
| HOMA-R | 0.86±0.40 |

Values are mean±SD for seven subjects. BMI: body mass index. LT: lactate threshold. The formula for calculating HOMA-R was [fasting insulin×fasting blood glucose×1/405].

**Table 2.** Macronutrients of test meal.

| Energy (kcal) | 1,022.6 |
| Protein (g) | 43.1 |
| Fat (g) | 22.8 |
| Carbohydrate (g) | 154.5 |
| P: F: C (%) | 17 : 21 : 62 |


The characteristics of the subjects are shown in Table 1. **LT determination.** LT was determined with reference to the method of Takizawa and Ishii (22). An incremental exercise test was performed in order to determine the experimental exercise intensity of each subject. This test was carried out using an electrically breached cycle ergometer (Aerobic 75 XLII, Combi Co., Tokyo, Japan). The test was commenced after a 3-min warm up period (10 W at 60 rpm); the workload gradually increased by 40 W at 4-min intervals. A blood sample of 5 µL was collected from the fingertip of each subject at the end of each phase and the blood lactate was measured by a lactate analyzer (Lactate Pro, Arkray, Kyoto, Japan). Thereafter, the workload was increased by 20 W/min after exceeding 4.0 mmol/L of blood lactate until the subjects reached exhaustion. LT was determined by using blood lactate level analyzing software (NEQNET LT Manager, Arkray).

**Experimental design.** Each subject started five trials after 12 h of fasting. The subjects were asked not to perform vigorous exercise on the day before each trial, and to refrain from drinking alcohol and smoking. After they arrived at laboratory, blood samples were collected from the subjects at rest and then the subjects consumed the test meal and drank water (500 mL). The subjects took water during the 1-h period before the exercise (the no-meal trial) or the meal taken 1, 2, 3, or 4 h before exercise (1h-Pre, 2h-Pre, 3h-Pre, and 4h-Pre, respectively). Each individual carried out the five experimental trials in random order. Exercise was performed at the LT intensity for 60 min using a cycling ergometer. The time that each meal was ingested was the same in all of the trials. During the time period after each meal intake until the time the exercise trials were conducted, the subjects were free to live as they liked, but were asked
not to drink or eat except for the drinking water supplied and not to do any exercise.

The subjects sat at rest during the 30-min period before the cycling exercise. After 10 min of cycling at 10 W for warm-up, the subjects performed the cycling exercise at the LT intensity for 60 min. During the exercise, the subjects were instructed to maintain pedal speed at 60 rpm. During the experimental trials, temperature and humidity in the laboratory were maintained at 24–26°C and 58–64%, respectively.

Test meal. The subjects were provided the test meal as breakfast. The nutritional content of the test meal...
meal is shown in Table 2. The test meal was a standard Japanese meal close to 1,000 kcal. Because Sugiura et al. (23) reported that the recommended energy intake of Japanese male track and field athletes is about 3,000 kcal/d (3,141 ± 592 kcal/d), the test meal was set at 1,000 kcal, one-third of 3,000 kcal/d. The macronutrient balance of the test meal was protein 17%, fat 21%, and CHO 62%.

Measurement of respiratory gas. The gas expired during the experimental trials was collected using the
Douglas bag method. The expired gases were collected before exercise, every 15 min during exercise (15, 30, and 45 min) and 2 min after the end of exercise. The gases collected in the Douglas bag were analyzed for oxygen consumption (\(\text{VO}_2\)) and carbon dioxide emissions (\(\text{VCO}_2\)) using indirect calorimetry (AR-1, Arco System, Kashiwa, Japan). The respiratory exchange ratio (RER; \(\text{VCO}_2/\text{VO}_2\)) was calculated from \(\text{VO}_2\) and \(\text{VCO}_2\). CHO oxidation and fat oxidation rates were calculated using a method shown in a previous study (24).

**Blood sampling and analysis.** Blood samples were collected from the antecubital vein before the test meal and before exercise, 30 min after the start of exercise and at the end of exercise. Ten milliliters blood samples were dispensed into the blood collection tube coated with ethylenediaminetetraacetate (EDTA) and sodium fluoride (NaF). The analysis of the blood samples was entrusted to Mitsubishi Chemical Medicine Corporation (Kyoto, Japan) for the measurements of blood glucose, serum insulin and serum free fatty acid (FFA).

**Statistical analysis.** Experiment data were expressed as means±SD. The data obtained from the blood samples and the expired gas during the five trials were analyzed for repeated-measures analysis of variance. The significant main effects were then located by the use of a post hoc test (Tukey). The significance level was set at \(p<0.05\).

**RESULTS**

**RER, and fat and CHO oxidation rates**

RER, and fat and CHO oxidation rates during exercise are shown in Fig. 1. No significant differences were demonstrated for RER, or fat or CHO oxidation rates during exercise among conditions.

**Blood glucose, serum insulin and serum FFA**

The blood glucose levels during exercise are shown in Fig. 2A. No significant differences were demonstrated in the blood glucose levels in any of the trials before the meals, or at 30 min after initiation of the exercise. However, the blood glucose levels at 60 min after the exercise was initiated were significantly higher in the 1h-Pre, compared with the no-meal trial. Additionally, they were significantly higher in the 4h-Pre trial, compared with the 1h-Pre trial at 60 min.

The serum insulin levels during exercise are shown in Fig. 2B. No significant differences were demonstrated in the levels of serum insulin in any of the trials before the meals. However, it was significantly lower in the no-meal trial, compared with the 1h-Pre, 2h-Pre, and 3h-Pre trials at before exercise, the 1h-Pre and 2h-Pre trials at 30 min during the exercise and 1h-Pre at 60 min during the exercise. In addition, it was significantly higher in the 1h-Pre, compared with the 2h-Pre and 4h-Pre trials before exercise, the 4h-Pre trial at 30 min during the exercise and the 3h-Pre and 4h-Pre trials at 60 min during the exercise.

Serum FFA during exercise is shown in Fig. 2C. No significant differences were demonstrated in the levels of serum FFA in any of the trials before the meals. However, the serum FFA levels before exercise, and at 30 and 60 min during exercise were significantly lower in the no-meal trial, compared with the 1h-Pre, 2h-Pre, 3h-Pre, and 4h-Pre trials.

**DISCUSSION**

The ingestion of CHO before exercise is important for supplementation of the energy substrate utilization during exercise. Any reduction in the blood glucose or muscle glycogen levels leads to a remarkable reduction in endurance. In addition, exercise while fasting causes an acute increase in the FFA in circulation, which increases the risk of cardiac failure (25). Therefore, the ingestion of a meal before exercise has been recommended in order to improve exercise performance and maintain health. Previously, it was reported that the intake of a CHO solution containing glucose and maltodextrin 1 h before exercise improved exercise performance (4). In contrast, Palmer et al. (26) have shown that the intake of a CHO solution immediately before exercise did not alter cycling time trial performance in well-trained subjects. Additionally, Devlin et al. (14) reported that when a snack (candy bar) was fed to the subjects 30 min before cycling exercise, there was no difference in the time to exhaustion between the snack ingestion and placebo trials. In explanation of these results, hyperglycemia and hyperinsulinemia resulting from CHO intake may have had an effect. The present study showed that fat oxidation during exercise was inhibited in the meal trials, compared with the no-meal trial, along with an increase in insulin concentration. Therefore, it is important to consider the timing of CHO ingestion before exercise in order to assure regulation of the energy metabolism during exercise, as well as to decrease the risk of cardiac failure due to an acute elevation of FFA.

As for the effect of blood insulin induced by food ingestion on the energy metabolism during exercise, a previous study reported an association with the glycemic index of CHO (2). When meals with a high or low glycemic index were consumed at 3 h before exercise, serum insulin before exercise was lower in the low-glycemic-index trial, compared with the high-glycemic-index trial, along with an acceleration of fat oxidation during the exercise. Different timing of CHO ingestion can also affect both the insulin level and the energy metabolism during exercise. Recently, several organizations, including the American Dietetic Association, the Dietitians of Canada, and the American College of Sports Medicine, have encouraged the consumption of a substantial meal at 2–4 h before exercise for athletes (27), but poor evidence was presented concerning the timing of the whole meal intake. To the best of our knowledge, the present study is the first conducted to investigate the effects of the timing of meal ingestion on the energy metabolism during exercise. As a result, no differences were shown in the CHO or fat oxidation rate among the various conditions, suggesting that the timing of the intake of a common meal taken within a 4-h period before exercise did not affect the energy metabolism during exercise at LT intensity. Although, Dumortier et al. (28) reported that when a meal of 550 kcal
(57% CHO, 26% protein, and 17% fat) was consumed 1 h or 3 h before exercise by diabetes patients, fat oxidation was higher after 3 h compared with 1 h during exercise. Moreover, the amount of carbohydrate intake in the present study was 2.4 g/kg body mass, which was higher than in previous studies (approximately 1.0–2.2 g/kg body mass) (4, 5, 7, 8). The meal form was also in contrast to many previous studies in which beverages or simple foods were used. Although ingested carbohydrate content was larger than in the previous studies, fat metabolism during exercise showed no difference among conditions, which may be influenced by the high capacity for insulin sensitivity in the subjects. Energy metabolism during exercise in athletes and diabetic patients cannot be easily compared because insulin sensitivity is higher in athletes compared with sedentary subjects and such patients, which may have led to the difference between the present study and a previous study (29). CHO absorbed from the intestines into blood after a meal is rapidly taken into muscle cells in athletes, which may have resulted in the phenomenon energy metabolism during exercise did not differ regardless of elapsed time after meal, because compared to the level shown immediately before exercise in the no-meal trial, the serum insulin level was elevated over a shorter interval in the meal trials after the exercise. The reason for the similar fat oxidation among the different intake timing trials, regardless of altered insulin level, remains to be clarified. One possibility may be the physical characteristics of the subjects. Trained athletes have a higher insulin sensitivity and can transport blood glucose into skeletal muscle more readily than sedentary subjects (16–19). The characteristics of the subjects, who were track and field athletes, may have accelerated the uptake of blood glucose and further led to low insulin secretion. In addition, athletes have a high capacity for insulin-independent glucose uptake (16–21), which may have also contributed to the decrease in blood glucose and insulin. In a previous study (20), it has been shown that HOMA is low in exercise-trained athletes compared to sedentary subjects. Similarly, the HOMA of the subjects in the present study (0.86±0.4, Table 1) was lower compared to data for sedentary subjects in a previous study (20), which suggests that the subjects in this study have high insulin sensitivity. Therefore, glucose uptake into cells after ingestion of carbohydrates is rapidly carried out, which causes a shorter high insulin state and fat oxidation inhibition during exercise. Furthermore, it is thought that athletes can constantly use the energy substrate regardless of blood insulin concentration, due to metabolic adaptation acquired through training. It is possible that because the athletes had a high amount of glycogen stored in the muscle tissue, they were able to maintain the energy utilization ratio corresponding to the exercise intensity even when the blood glucose decreased. On the other hand, sedentary people, with a low insulin sensitivity secrete large amounts of insulin after food ingestion, compared to trained subjects, and they require a longer time to return to the fasting level (18). Furthermore, the glycogen storage capacity in sedentary people is lower than that in trained subjects (21). These characteristics may have led to the inhibition of fat oxidation during exercise. Taken together, it is supposed that individual characteristics (e.g. insulin sensitivity) will affect the sensitivity to the timing of meal intake, and further studies are required to investigate these issues further, not only in athletic and active subjects, but also in sedentary subjects.

In conclusion, the present study demonstrated that metabolic substrate oxidation during exercise did not differ due to differences in the timing of meal intake, although all meal conditions lowered fat oxidation, compared to the fasting condition, suggesting that the timing of the meal intake had almost no effect on the energy metabolism at LT intensity in trained men. Further studies are required to clarify the significance of the timing of meal intake under various conditions, including subject characteristics and exercise intensity.

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