Effects of carbohydrate ingestion on intermittent fuel metabolism during prolonged walking

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To study the effects of carbohydrate (CHO) ingestion on intermittent fuel metabolism changes during prolonged treadmill walking, seven male subjects performed 120 minutes walking on a treadmill at a speed of 80 m/min with (GLU) and without (CONT) glucose ingestion 1 hour prior to the exercise. During this experiment, blood samples were collected to analyze the concentrations of plasma glucose, lactate and triglyceride. Respiratory gas was carried into the gas analyzer to measure oxygen uptake (VO₂), elimination of carbon dioxide (VCO₂) and pulmonary ventilation (VE). As a result, plasma glucose was significantly higher (p<0.05) in GLU than CONT from the onset of the exercise to the period of 60 min. More contribution of fat oxidation was found in CONT than GLU during the exercise. The cross points that the contribution rates of CHO/fat oxidation were equal came at 40 min and 120 min in CONT and GLU, respectively. The oxidation rates were approximately 300 mg/min in both experiments and there were no significant difference in the volumes of whole oxidation rates between GLU and CONT during the exercise. These findings suggest that during prolonged walking at moderate speed a glucose ingestion prevents from consuming fat even after 120 min exercise.

Keywords: carbohydrate 炭水化物, fat 脂肪, oxidation 酸化, fuel metabolism 燃料代謝, prolonged walking 長時間歩行

INTRODUCTION

It is well documented that carbohydrate (CHO) and free fatty acids (FFA) are the dominant fuels oxidized by the muscle for energy production during exercise and that the absolute and relative contribution of these fuels can be influenced by diet¹,²,³, muscle glycogen content ⁴,⁵, exercise intensity ¹, ⁶, ⁷, ⁸, ⁹, ¹⁰, duration ⁸, and training status ¹¹,¹²,¹³.

It is generally known that FFA starts to increase after about 15 min from the initiation of exercise¹⁴,¹⁵. Pruett (1970) showed that the FFA increase during prolonged work was dependent on the relative severity of the work to the individual’s maximal aerobic power, rather than upon total energy expenditure ¹⁶. He also reported that at 20 % of Vo₂max the increase in FFA levels was small but significant. Ahlborg et al. (1974) demonstrated a progressive increase in serum FFA
during four hours of cycling at approximately 30% of VO$_{2\text{max}}$. These investigations suggest that the main fuel of energy production gradually shifts to FFA from CHO during moderate exercises.

As above, CHO and fat have been considered as the most important fuels of energy production so that ingestion of CHO must have much influence on fuel metabolism during exercise. Now it is well established that the CHO ingestion during prolonged exercise can postpone fatigue and enhance endurance capacity $^{18,19,20,21}$. The benefits of ingesting CHO during exercise have been attributed to the maintenance of plasma glucose concentrations and high rates of CHO oxidation in the latter half of the exercise when muscle and liver glycogen levels are low. $^{19,22,23,24,25,26}$.

Ingesting CHO surely have positive effects over the performance during exercise as many investigations demonstrated before like above. However, it could cause storing certain amount of fat which was supposed to be consumed instead of the CHO ingested at a moderate intensity exercise. It is hypothesized that temporary ingestion of CHO may increase CHO oxidation and decrease fat oxidation. In prolonged exercise at moderate intensity, however, CHO would not be always considered as a main fuel provider and fat would take over the place instead. Now assuming ingestion of CHO as diet, which is normally taken in our life, it would be able to keep us excessive energy. This suggests that ingestion of CHO can prevent from consuming fat effectively.

There have been many studies approaching on fuel metabolism during prolonged exercise, however, none of them has evaluated the oxidation rates of CHO and fat intermittently under this experimental conditions. Furthermore, most of the studies have focused only on the performances and the utilization of carbohydrate. Therefore, present study was designed to clarify the effects of CHO ingestion on fuel metabolism during prolonged treadmill walking at a moderate speed by using the latest calculation method of CHO/fat oxidation from respiratory functions.

**METHODS**

**Subjects**

Seven healthy, active men who moderately play some sports [volleyball, basketball, boxing, softball or jogging] participated in this study. None of them were highly trained, but all were physically active in their daily routines. No subject had any evidence of metabolic or cardiovascular disorders nor were any of them taking prescription medications. All subjects were fully informed of the possible risks associated with this study and signed an informed consent form before participation. The characteristics of the subjects are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Physical Characteristics of Subjects</th>
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<tr>
<td>Age (yrs)</td>
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<tr>
<td>Height (cm)</td>
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<td>Weight (kg)</td>
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<td>Body mass index (kg/m$^2$)</td>
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<td>Body fat (%)</td>
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<td><strong>Values are means ± SE. (n = 7)</strong></td>
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**Experimental protocol**

Subjects arrived at the Health Science Laboratory at the Hiroshima University in the morning (before 9:45 AM). All subjects were instructed to have only a light breakfast, and to refrain from the consumption of beverages containing alcohol and caffeine during the 12 hours preceding the trials. The time of glucose ingestion and starting trials allocated to each subject remained consistent for all experiments to avoid any influence of circadian variance.

On arrival in the laboratory, subjects were asked to empty their bladder before body mass and body fat were recorded. And then, blood samples were taken to see concentration of plasma glucose, lactate and triglyceride as standard data before the exercise. Thereafter subjects received glucose drink or plain water and were instructed to drink it immediately at 10 a.m. when it is 1 hour before the onset of the exercise. The glucose drink consisted of 500ml water and 1g of
glucose per kg of body mass whereas plain water consisted of 500 ml water.

Each subject completed two exercise trials, each consisting of 120 min of walking on the treadmill (Tread-Mill NT-type12, Nishikawa Iron Work Co. LTD.) at a speed of 80 m/min (approximately 25% of Vo2max) started at 11 a.m. soon after 5 min rest with the standing position. Each experimental trial was carried out with 7 days interval at least in random order to minimize any intervention. All exercise trials were performed under normal and standard environmental conditions (ambient temperature 20°C; relative humidity 60%).

**Physiological measurements**

During experiments, blood samples were collected into auto analyzers for analysis of concentrations of plasma glucose (Medi-Safe Mini GR-102, TERUMO), lactate (Lactate Pro LT-1710, KDK. CO.) and triglyceride (Refrotron, S, BOEHRINGER MANNHEIM). Concentrations of plasma glucose and triglyceride were measured every 15 min and plasma lactate was measured every 30 min. Respiratory functions including the respiratory exchange rate (RER), VO2, VCO2 and pulmonary ventilation were continuously measured during the exercise (Aero Monitor AE300S, Minato Medical Science Co.). Heart rate (HR) was monitored by using a heart rate monitor (Accurex Plus, Polar Electro Oy.) throughout the experiment.

**Calculation of oxidation rates**

From VCO2 and VO2 (l/min), total CHO and fat oxidation rates (mg/min) were calculated by using the respiratory gas measurements, with the assumption that protein oxidation during exercise was negligible. The following calculations (Jeukendrup JE and Wallis GA, 2005) were used to assess the rates of oxidation during exercise intermittently.

CHO oxidation (mg/min)

\[
= 4.21 \times VCO_2 - 2.962 \times VO_2
\]

Fat oxidation (mg/min)

\[
= 1.695 \times VO_2 - 1.701 \times VCO_2
\]

**Statistical analysis**

Means and standard errors (means ± SE) were calculated for all variables. A paired Student’s t-test was used to compare the difference between the two experiments in each variable. Statistical significance was set at *p < 0.05, **p < 0.01.

**RESULTS**

Fig. 1 shows the changes in HR during the exercise. The HR gradually increased throughout the exercise. There was no significant difference between GLU and CONT until 60 min. Thereafter HR in CONT, however, became significantly higher than in GLU until termination of the exercise.

The changes in plasma concentration of glucose during the exercise are presented in Fig. 2. There were great differences in plasma glucose concentrations between GLU and CONT until 60 min. Immediately prior to the onset of the exercise, plasma glucose concentrations in GLU was significantly higher (p<0.05) than that in CONT. These differences persisted for 60 min during the exercise, but thereafter no significant differences were observed between the experimental conditions. For the first 30 min of the exercise, the plasma glucose concentration in GLU decreased, while the plasma glucose concentration in CONT were constant and no great change was found during the exercise.

The change in the concentration of triglyceride during the exercise is illustrated in Fig. 3. There was a sharp fall in GLU for the first 30 min. However, it became relatively constant after 30 min until termination of the exercise. In CONT, there was no significant change in the concentration of triglyceride and it was relatively constant throughout the exercise. Compared GLU to CONT, the concentrations of triglyceride were always higher in CONT during the
exercise except the onset of the exercise, although no significant difference was found between them during the exercise.

Fig. 4 shows the changes in VO$_2$ during the exercise. The VO$_2$ in both of experimental conditions were relatively constant from the onset of the exercise until 60 min. However, thereafter there were found significant differences between them.

The changes in RER during the exercise is shown in Fig. 5. The RER decreased linearly in both of experimental conditions during the exercise. The regression line of GLU shifted upward than that of CONT and the RER in GLU was higher than that in CONT with the difference of approximately 0.05.

Fig. 6 shows calculated the oxidation rates of CHO and fat in GLU during the exercise. The oxidation rates of CHO decreased linearly to about half of the first measurement at the start of the exercise, while those of fat increased gradually up to about twice as much as the first measurement. The peak oxidation rates of CHO and fat were reached at the onset of the exercise and at the end of the exercise, respectively. The substrate contribution rates of CHO oxidation were much higher than that of fat oxidation at the onset of the exercise. They became almost equal (the oxidation rates were approximately 300 mg/min) at the end of the exercise.

Fig. 7 shows calculated the oxidation rates of CHO and fat in CONT during the exercise. The oxidation rates of CHO decreased linearly to a quarter of the first measurement, while those of fat increased constantly up to twice as much as the first measurement throughout the exercise. The peak oxidation rates of CHO and fat were reached at the onset of the exercise and at the end of the exercise, respectively. The substrate contribution rates of CHO oxidation were higher than that of fat oxidation at the onset of the exercise, although they became equal (the oxidation rates were approximately 300 mg/min) at 40 min and reversed thereafter.
Fig. 4. The changes in VO₂ during the exercise
Values are means.
* p<0.05; ** p<0.01 (Significant difference between GLU and CONT)

Fig. 5. The changes in RER during the exercise
Values are means.
* p<0.05; ** p<0.01 (Significant difference between GLU and CONT)

Fig. 6. The oxidation rates of CHO and fat in
GLU during the exercise
Values are means.

DISCUSSION

The main finding of this study is that the fat oxidation rates in CONT were intermittently higher than that in GLU while the CHO oxidation rates were higher in GLU than CONT during the exercise. This has brought the difference of timing, the substrate contribution rates of CHO and fat oxidation become equal without difference in whole volume of CHO and fat oxidation in the two experimental conditions, which came 40/120 min in CONT/GLU, respectively.

The HR showed relative but not significant differences between GLU and CONT after 60 min during the exercise and this seems to be related with the differences of change in VO₂ between the conditions. It is simple that more HR indicates necessity of more VO₂. Higher HR and VO₂ could suggest that the exercise intensity was set at the same throughout the experiment but relatively lower in GLU than CONT as many previous studies reported about effects of CHO ingestion on performance. However there were not found any significant changes in these two measurements so that more study is required to investigate the relationship of the changes between HR and VO₂.

The plasma glucose concentrations were different in
the two experimental conditions. Before starting the exercise, the concentration in GLU was much higher than that in CONT due to prior glucose ingestion. This difference remained for 60 min from the start, however, there were found no significant differences thereafter. This finding means that the increase in plasma glucose concentration during the exercise found to be directly proportional to the magnitude of plasma glucose concentration. There was great drop in GLU, although, no great change was found in CONT. It remained unchanged throughout the exercise. But Ahlborg et al. (1974) reported that plasma glucose concentration during exercise at a work load of 30% of VO₂max showed a gradual decline occurred after 40 min (17). Pruett (1970) reported that a work load of 20% of VO₂max did not cause a drop in plasma glucose concentration during 6 hours exercise, whereas the work loads of 50% and 70% of VO₂max caused a drop (10). M. Yamasaki (1985) demonstrated prolonged treadmill walking at a work load of 25% of VO₂max for 3 hours and there was found no change in the plasma glucose concentration (28). Therefore it can be suggested that no great change in CONT was related with low work intensity and the prior glucose ingestion influenced the plasma glucose concentration just temporary up to 60 min.

The RER in GLU remained higher than that in CONT throughout the exercise and it was constantly significant as Fig. 5 showed. It is reported that CHO ingestion generally increase RER, indicating that whole body CHO metabolism is increased, with values of up to 2 g/min during the later stages of exercise (23). However, in some studies, this effect has not been shown, a discrepancy which could be explained by a short exercise bout, or subjects commencing exercise with muscles and liver that are filled with glycogen (19,20). At present study, the RER and the CHO oxidation rates definitely increased during the exercise, however, significant differences in RER of the two experimental conditions appeared from the start of this exercise, which is in disagreement with some studies above. Yet this could be explained due to 1 hour interval between CHO ingestion and start of the exercise.

The concentration of plasma triglyceride in both experiments showed no significant difference. In GLU, it remained relatively constant during the exercise except a great drop at the first 30 min. This result was fairly anticipated before this experiment, because a glucose ingestion reduces a fat oxidation rate and prevents triglyceride from resolving into FFA and glycerol (29). In CONT, the concentration of plasma triglyceride was constant and a bit higher than that in GLU, but not significant throughout the exercise. M. Yamasaki (1985) reported that during 3 hours walking the concentration of plasma triglyceride decreased linearly after 60 min to the end of the exercise and the FFA increased sharply throughout the exercise (28). In other words, more triglyceride is supposed to be consumed in CONT than GLU for the amount of CHO ingestion, however, this result was contrary to that idea. These results could suggest that more fat were consumed in GLU than CONT.

Yet we have to persistently take account of that there was no significant difference in this result by blood sampling and that calculated oxidation rates by respiratory gas sampling indicated the different result. Having a look at Fig. 6, 7, it is obvious that more fat were consumed in CONT than GLU for the amount of CHO ingestion, however, this result was contrary to that idea. These results could suggest that more fat were consumed in GLU than CONT.

In conclusion, the ingestion of glucose increased the CHO oxidation rates and decreased the fat oxidation rates with an increase of the RER during the exercise. This is because that most of CHO is resolved into energy through the glycolytic process without oxygen uptake, whereas the fat is mostly resolved...
through the aerobic process with oxygen uptake and production of CO₂ under this experimental condition. These facts indicate that timing of exercise is a very important factor to consume fat. This suggests that it is more effective to have an exercise before or long enough after having a diet than soon after having a diet. In this study blood sampling, the concentrations of plasma triglyceride and glucose, were not enough useful to investigate the CHO/fat oxidation metabolism clearly. However, the method by using calculation from VO₂ and VCO₂ did a great work on investigating of CHO/fat oxidation rates intermittently. And this latest method is worth being used for incoming studies which is investigating not only fuel metabolism, but also performance as well so that more sophisticated considerations would be done in near future.

REFERENCES
14) Carlson L.A. and Pernow B., 1961, Studies on blood lipids during exercise 2. The arterial plasma tree fatty acid concentration during and after