A Thiamin Derivative Inhibits Oxidation of Exogenous Glucose at Rest, but Not during Exercise

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Summary
Thiamin (vitamin B₁) is known to activate carbohydrate metabolism in part through activation of pyruvate dehydrogenase. The purpose of this study was to investigate the effect of thiamin tetrahydrofurfuryl disulfide (TTFD), a thiamin derivative, on utilization of exogenous glucose by measuring oxidation of ¹³C-glucose at rest and during prolonged exercise in mice under normal dietary conditions. Mice orally ingested TTFD (0.1 mg/g BW [body weight]) and ¹³C-glucose (0.8 mg/g BW) or ¹³C-lactate (0.1 mg/g BW) plus glucose (0.8 mg/g BW) at rest or before endurance running. The average percent of ¹³C atoms in total ¹³C+¹²C (¹³C atom%) in expired air after ingestion of ¹³C-glucose at rest was significantly lower in the TTFD group than in the control group. No significant difference was found in ¹³C atom% in expired air after ingestion of ¹³C-glucose and prolonged exercise. In addition, no significant effect of TTFD was found in expired ¹³C atom% after ingestion of ¹³C-lactate plus glucose at rest. TTFD also had no effect on concentrations of muscle or liver glycogen at rest. These results suggest that TTFD, which is a thiamin derivative, decreases oxidation of exogenous glucose at rest, but not during exercise.

Key Words thiamin, glucose, rest, prolonged exercise, oxidation

Thiamin (vitamin B₁) is known to act as a cofactor in numerous reactions of carbohydrate metabolism including activation of pyruvate dehydrogenase (PDH) (1–3). PDH is a mitochondrial enzyme that catalyzes the conversion of pyruvate to acetyl-CoA and the entry of carbohydrate derived substrates into the citric acid cycle for oxidation (4). It has been reported that in thiamin deficiency, production of lactate is increased because of decreased oxidation of carbohydrate due to reduced activity of PDH (5). Therefore administration of thiamin in the deficient state can affect carbohydrate metabolism (5, 6). However, it is not clear whether ingestion of thiamin under non-deficient conditions affects carbohydrate metabolism at rest or during exercise.

Lactate had been regarded as an end-product of carbohydrates and as the main cause of muscle fatigue. However, recent studies have shown that lactate is not a waste product but an oxidizable fuel and that muscle fatigue is not necessarily caused by the accumulation of lactate (7, 8). Thiamin is often used to prevent fatigue, not only muscle fatigue but also feelings of fatigue, particularly in Japan (9, 10). The rationale for the use of thiamin as an anti-fatigue reagent has been that it can inhibit production of lactate because of increased PDH activity by thiamin, and the decreased production of lactate seen after ingestion of thiamin in a thiamin-deficient state has been seen as evidence of this (6). However, it is not established that thiamin actually decreases production of lactate under normal dietary conditions. Therefore, the mechanism of the effect of thiamin on fatigue has not been determined.

The purpose of this study was to investigate the effect of thiamin tetrahydrofurfuryl disulfide (TTFD), a thiamin derivative, on glucose metabolism using ¹³C-glucose (0.8 mg/g BW [body weight]) at rest and during and after prolonged exercise in mice under normal dietary conditions. ¹³C-lactate (0.1 mg/g BW) was used as a marker of carbohydrate metabolism with ingestion of glucose (0.8 mg/g BW). We found that TTFD decreased oxidation of exogenous glucose at rest, but not during exercise.

METHODS

Experimental conditions. Male ICR mice (CLEA Japan, Inc., Japan), which were purchased at 8 wk old, were used at 9 wk old (n=5–6 for each groups) for the experiments. They were housed in a room maintained at 23°C with three mice in a standard cage. Mice were given standard lab chow (MF, Oriental Yeast Co., Ltd., Japan) ad libitum. The content of thiamin in the food was 1.91 mg/100 g food, which is considered sufficient to fulfill daily requirements.

Three sets of experiments were carried out (Fig. 1). In all experiments, mice in the TTFD group ingested TTFD (0.1 mg/g BW) and control mice ingested water using a sonde. In experiment 1, TTFD and control mice received [U-¹³C]-glucose (0.8 mg/g BW) (CLM-1396, 99%, Cambridge Isotope Laboratories, USA) using a sonde. After
the ingestion, they were kept at rest for 60 min. In experiment 2, TTFD and control mice ran at a speed of 20 m/min for 50 min using a treadmill after 10 min of rest following ingestion of TTFD or water and [U-13C]-glucose (0.8 mg/g BW). In experiment 3, TTFD and control mice received glucose (0.8 mg/g BW) plus [1-13C]-sodium lactate (0.1 mg/g BW) (CLM-1577, 99%, Cambridge Isotope Laboratories). Mice were kept at rest for 60 min. In all experiments, mice were placed in a tightly sealed metabolism chamber containing a treadmill belt (MK-680AT, Muromachi Kikai, Japan) immediately after ingestion of 13C-substrate (13C-glucose or 13C-lactate). Expired air was collected using a pump (5.2 L/min) at 10 min intervals after administration of 13C-substrate at rest or during prolonged exercise for 60 min. In experiments 1 and 3, mice were sacrificed by cervical dislocation and blood from the open chest, liver and muscles of lower limb were taken immediately, and were frozen by liquid nitrogen, and kept at −80°C until further analysis. In experiment 2, blood was taken from the tail vein to measure blood concentrations of glucose and lactate immediately after the exercise. Ethical approval for this work was obtained from the Committee on Animal Care and Use of the University of Tokyo.

Analytical methods. Blood concentrations of glucose (Glutest-Ace, Arkray, Japan) and lactate (Lactate-Pro, Arkray) were measured using auto analyzers. The concentration of plasma free fatty acid (FFA) was measured using a kit (FFA-C test, Wako Pure Chemical Industries, Ltd., Japan). Concentrations of muscle and liver glycogen were measured using phenol-sulfuric acid (11). The air in the metabolism chamber was collected using a pump (flow rate 5.2 L/min). Air was collected at first in a Douglas bag for a 10 min interval. After mixing the content of the Douglas bag, air was sampled into an aluminum bag (0.5 L). The ratio of 13C/12C (=13CO2/12CO2) in the bag was measured using a mass analyzer (Delta V, Thermo Fisher Scientific) and was calculated and expressed as a percent of 13C atoms (13C atom%) in the total 12C plus 13C (=13C/(12C+13C) ×100), which is 1.09% in a normal natural environment.

Statistical analysis. Values are reported as means±SE. All data were analyzed using one-way ANOVA (Statcel 2, OMS Publishers, Japan). Statistical significance was considered to be p<0.05.

RESULTS

Blood substrate concentrations

In experiment 1, no significant differences were found in the blood concentrations of glucose or lactate between the control and TTFD groups at rest (Figs. 2A and 3A), while concentrations of plasma FFA was significantly lower in the TTFD mice than in the control mice (control: 0.39±0.03 mEq/L, TTFD: 0.28±0.02 mEq/L). In experiment 2, no significant differences were found in the blood concentrations of glucose or lactate between the two groups immediately after exercise (Figs. 2B and 3B).

Glycogen concentrations

Concentrations of muscle glycogen (gastrocnemius, control: 1.43±0.34 mg/g, TTFD: 0.95±0.26 mg/g; tibialis anterior, control: 1.92±0.41 mg/g, TTFD: 2.00±0.43 mg/g) and liver (control: 38.0±2.5 mg/g, TTFD: 38.1±2.4 mg/g) also showed no significant effects of
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In experiment 1, the $^{13}$C atom% in expired air was significantly lower in the TTFD group than in the control group both at 10–20 min and 20–30 min after ingestion of $^{13}$C-glucose and the accumulated area of $^{13}$C atom% for 0–40 min was also significantly lower in the TTFD mice (Fig. 4). In experiment 2, no significant difference was found between the two groups in the $^{13}$C atom% in expired air after ingestion of $^{13}$C-glucose (Fig. 5). In experiment 3 also, no significant effects of TTFD were seen in the $^{13}$C atom% in expired air after ingestion of $^{13}$C-lactate at rest (Fig. 6).

DISCUSSION

Thiamin is known to activate PDH (1, 3). PDH can play a pivotal role in the oxidation of carbohydrates because it is one of the rate limiting steps in this process (2). It has been shown through administration of dichloroacetate, which is an activator of PDH, that activation of PDH can increase oxidation of carbohydrates and lactate at rest and during exercise (12). However, it is not known whether supplemental administration of thiamin has effects on carbohydrate metabolism in the absence of thiamin deficiency. In this study, mice had free access to standard mice chow, so it can be assumed that they were not in a state of thiamin deficiency (1, 5). The amount of thiamin in the food used in this study (1.91 mg/100 g food) was considerably higher than that often used in a normal chow for rats and mice (around 0.6 mg/100 g food). So the amount of thiamin taken by mice was considered to be sufficient even in control mice in experiment 2 with 50 min of exercise.

The dose of TTFD ingested (0.1 mg/g BW) was chosen as being close to maximum amount that mice could digest without side effects in a single dose. It has been reported that orally administered TTFD is readily absorbed and converted to thiamin, so that ingestion of TTFD increases the blood thiamin level (9). It is also reported administration of TTFD is better than administration of thiamin itself in order to increase the blood thiamin level because of faster absorption of TTFD than that of thiamin (13). Although we cannot exclude the possibility that the effect found in this study is a unique effect of TTFD, it is suggested that the effect was mainly due to increase in thiamin level after ingestion of TTFD.

We expected that oral administration of TTFD would increase oxidation of glucose by activation of PDH. However, the results showed that oxidation of exogenous glucose was not increased but rather decreased at rest after administration of TTFD. We measured oxidation of $^{13}$C-lactate as a marker of carbohydrate oxidation at rest after ingestion of glucose because lactate is a substrate for oxidation derived from glucose and glycogen (8, 12). Although some portion of lactate could be converted to glucose and oxidized, the major portion of lactate was assumed to be oxidized directly (8). There was no significant effect of TTFD on oxidation of exogenous lactate in mice at rest following glucose ingestion. These data suggest that oxidation of endogenous lactate and possibly overall oxidation of carbohydrate is not changed by oral ingestion of TTFD and glucose. Therefore, we speculate that oxidation of exogenous glucose was decreased without decreasing total oxidation of carbohydrates. On the other hand, we did not find a sig-

**Fig. 4.** $^{13}$C atom% in expired air after ingestion of $^{13}$C-glucose at rest with water or TTFD in experiment 1. *p<0.05, **p<0.01.

**Fig. 5.** $^{13}$C atom% in expired air after ingestion of $^{13}$C-glucose before exercise for 50 min with water or TTFD in experiment 2.

**Fig. 6.** $^{13}$C atom% in expired air after ingestion of $^{13}$C-lactate at rest with water or TTFD in experiment 3.
significant effect of TTFD on muscle or liver glycogen concentrations at rest after ingestion of glucose in experiment 1. A likely explanation of why oxidation of exogenous glucose was decreased without significant changes in the muscle or liver glycogen is that utilization of muscle and liver glycogen was increased by the activation of muscle PDH by thiamin with increased glycogen synthesis from ingested glucose. Therefore we speculate that the carbohydrate metabolism through glucose–glycogen–oxidation is activated after administration of a thiamin derivative at rest without changing overall oxidation of carbohydrates. It is possible that the amount of glucose ingested (0.8 mg/g BW) was not enough to detect changes in muscular glycogen synthesis at rest.

We did not measure actual volume of CO₂ expired, but if we estimated VCO₂ of mice at rest (15 mL/kg·min) and exercise (70 mL/kg·min), then about 7% for control mice and about 4.5% for TTFD mice of 13C-glucose ingested was calculated to be recovered as 13CO₂ at rest for 60 min in experiment 1 and about 40% of 13C-glucose was recovered as 13CO₂ in both groups in experiment 2. We have no answer about the fate of 13C-glucose not expired as 13CO₂ at rest by TTFD compared to control mice. It is reported that in diabetic conditions or in high glucose conditions that administration of thiamin derivative can activate glucose disposal other than direct oxidation through the glycolytic pathway, such as the pentose phosphate pathway in the liver and adipose tissue (14–16), whose activation might change production of 13CO₂ from uniformly labeled 13C-glucose (17). We used normal mice that were not diabetic but the physiological state of the mice after ingestion of glucose was somewhat similar to the diabetic condition in that mice had a high blood glucose concentration. Further investigation is needed to uncover the precise mechanism of how oxidation of exogenous glucose is inhibited by TTFD at rest.

In conclusion, this study suggests that ingestion of TTFD, which is a derivative of thiamin, inhibits oxidation of ingested glucose at rest without affecting muscle or liver glycogen concentrations, but not during exercise.

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REFERENCES