Post-exercise taurine administration enhances glycogen repletion in tibialis anterior muscle

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Abstract Previous studies suggested that taurine (2-aminoethanesulfonic acid) administration enhances glucose uptake, one of the rate-limiting factors for glycogen synthesis. In this study, we investigated the effects of post-exercise taurine administration on glycogen repletion in skeletal muscle in ICR mice. In experiment 1, we orally administered either taurine (0.5 mg/g body weight) solution or physiological saline immediately after treadmill running at 25 m/min for 90 min. The serum free fatty acid (FFA) concentration at 60 min after the exercise was significantly higher in the taurine-treated group compared with the control group ($p < 0.05$). At 120 min after the exercise, the tibialis anterior muscle glycogen concentration in the taurine-treated group was significantly higher than that in the control group ($p < 0.05$). In experiment 2, we orally administered either glucose (1 mg/g body weight) solution or glucose solution containing taurine immediately after and at 60 min after the exercise. The area under the curve (AUC) for blood glucose concentration from 0 to 60 min after the exercise was significantly smaller in the taurine-treated group compared with the control group ($p < 0.01$). Our results show that post-exercise taurine administration enhances glycogen repletion in skeletal muscle. Higher skeletal muscle glycogen concentration by taurine administration may be partly due to the acceleration of glucose uptake. In addition, as the elevation of blood FFA level leads to an increase in fat oxidation, it is possible that a higher serum FFA concentration by taurine treatment is related to the sparing of carbohydrate for glycogen repletion.

Keywords : taurine, free fatty acid, treadmill running, glycogen resynthesis, glucose

Introduction

Exercise-induced fatigue is thought to be caused by various central and peripheral factors¹⁾. Concerning energy metabolism, depletion of glycogen in skeletal muscle is one of the major causes of fatigue during exercise. Many researchers have demonstrated that higher glycogen content in skeletal muscle before exercise results in improved performance in both prolonged endurance exercise and high-intensity intermittent exercise²⁻⁵⁾. Recent *in vitro* studies also have shown that higher glycogen content before contraction is associated with the attenuation of force reduction during repeated tetanic contractions⁶⁻⁹⁾. Therefore, during the post-exercise phase, glycogen repletion in skeletal muscle should be one of the most important factors for performance in the subsequent exercise.

In our previous study, we showed that oral taurine (2-aminoethanesulfonic acid) administration after prolonged exercise increased the amount of voluntary wheel running during the post-exercise phase, which has been used as an indicator of recovery from exercise-induced fatigue¹⁰⁾. These results suggest that taurine administration

has some positive effects during the post-exercise phase. Some rodent studies have demonstrated the possibility that taurine administration enhances glucose uptake, one of the rate-limiting factors for glycogen resynthesis in skeletal muscle¹¹⁻¹⁷⁾. Moreover, Kulakowski et al. showed that a single dose of taurine was able to increase glycogen content in the liver at resting state in non-diabetic rats¹¹⁾. Therefore, we hypothesized that one possible effect of taurine administration, during recovery from fatigue induced by exercise, is facilitating glycogen resynthesis in skeletal muscle. In this study, we administered taurine (0.5 mg/g body weight) solution or physiological saline in mice immediately after treadmill running at 25 m/min for 90 min, and then measured the concentrations of metabolites including glycogen in tissues during the post-exercise phase.

Methods

Animals. Six-week-old male ICR mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). Mice were housed in a room maintained at 23°C, and were acclimatized for 1 week. Mice were given free access to a standard chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan). A previous

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study using ICR mice fed with MF chow showed that the concentrations of taurine in skeletal muscles remained within the physiologically normal range^{18,19}. Ethical approval for this work was obtained from the Committee on Animal Care and Use, The University of Tokyo.

Experimental procedure. We set the dark phase at 9AM–9PM and all experimental treatments were performed in the dark phase when mice were active. Three days before the experimental day, all mice were familiarized with running on the treadmill (MK-680, Muromachi Kikai Co., Inc., Tokyo, Japan) at a speed of 25 m/min for 10 min. On the day of the experiment, after mice were fasted for 4 h to avoid a postprandial state, they ran on the treadmill at 25 m/min for 90 min. *Experiment 1:* Mice were divided into either a taurine-treated group ($n = 5$ or 6) or a physiological saline-treated group (control group; $n = 5$ or 6) with similar mean body weights. Immediately after the treadmill running, mice in the taurine-treated group were orally administered 0.5 mg/g body weight taurine dissolved in physiological saline, while mice in the control group received physiological saline (0.9%) using a sonde (the volume of ingestion: 0.01 ml/g body weight). After administration, mice were individually put in standard cages and fed *ad libitum*. Mice were sacrificed by cervical dislocation immediately after the exercise and at 60 or 120 min after exercise. Blood was taken from the open chest. Serum, liver, and tibialis anterior muscle were frozen in liquid nitrogen and stored at -80°C . Food consumption was determined by the differences in chow weight at the start and end of the experiment. *Experiment 2:* After treadmill running, mice in the taurine-treated group ($n = 6$) were orally administered solutions containing 1 mg/g body weight glucose and 0.5 mg/g body weight taurine, while mice in the control group ($n = 6$) received 1 mg/g body weight glucose dissolved in physiological saline (0.9%) using a sonde (the volume of ingestion: 0.01 ml/g body weight). We administered solutions to mice at immediately after treadmill running and at 60 min after the first oral administration. Mice were sacrificed by cervical dislocation at 120 min after the first oral administration. Blood was taken from the open chest. Serum, liver, and tibialis anterior muscle were frozen in liquid nitrogen and stored at -80°C . In this experiment, five mice performed two experimental tests (taurine treatment and control treatment) as repeated-measures with randomized crossover design to measure blood glucose and plasma insulin concentrations. Blood samples were collected in heparinized capillary tubes from the tail vein before administration (0) and 20, 60 (before the second administration), 80, and 120 min after the first administration. The interval between the two experimental tests was set at 1 week. The heparinized capillary tubes were centrifuged and then plasma samples were stored at -80°C . The areas under the curves (AUCs) for blood glucose and plasma insulin concentrations were calculated with the trapezoidal rule.

Analytical methods. Blood glucose concentrations were measured using an auto analyzer (Glutest Ace; Arkray Inc., Kyoto, Japan). Serum free fatty acid concentrations were measured using a kit (Wako NEFA C test kit; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Muscle and liver glycogen concentrations were measured using the phenol–sulfuric acid method²⁰. Plasma insulin concentrations were measured using an ELISA assay kit (Mouse Insulin ELISA Kit; Mercodia AB, Uppsala, Sweden).

Statistical analysis. All values are expressed as mean $\pm$ SE. We used Prism 5 software (Graph Pad Software, San Diego, CA) for the analyses. The changes in each metabolite during the experiment were compared by one-way analysis of variance (ANOVA). When differences were found to be significant, comparisons were made using Tukey's post-hoc (HSD) test. We used an unpaired t-test to determine differences in concentration of metabolites in tissues between the taurine-treated and control groups. In experiment 2, we used a two-way (treatment $\times$ time) repeated-measures ANOVA to determine differences in blood glucose and plasma insulin concentrations between the taurine-treated and control groups. We used an unpaired t-test to determine differences in concentration of skeletal muscle and liver glycogen and serum FFA between the two groups. The differences in AUCs between treatments were analyzed with a paired t-test. Statistical significance was set at $p < 0.05$.

Results

Experiment 1

Body weight and food consumption

No significant differences in body weight of mice between the two groups were observed (at 60 min: 35.1 ± 0.5 g in the taurine-treated group vs. 34.9 ± 0.7 g in the control group; at 120 min: 35.9 ± 1.0 mg/g body weight in the taurine-treated group vs. 37.0 ± 0.7 mg/g body weight in the control group). There were also no significant differences in total food consumption between the two groups during both 0–60 min (0.5 ± 0.1 g in the taurine-treated group vs. 0.6 ± 0.1 g in the control group) and 0–120 min (1.0 ± 0.2 g in the taurine-treated group vs. 1.2 ± 0.2 g in the control group) of post-exercise phases.

Skeletal muscle glycogen, liver glycogen, blood glucose and serum FFA concentrations

Glycogen concentration in the tibialis anterior muscle at the end of treadmill running was 0.78 ± 0.20 mg/g, which was about 40% of that at baseline (resting and fed state; 1.96 ± 0.35 mg/g) (Fig. 1A). In both groups, skeletal muscle glycogen concentration recovered to the baseline level at 60 min after the exercise. At 120 min after exercise, skeletal muscle glycogen concentration in the taurine-treated group was 94% higher compared with that in the control group ($p < 0.05$). Skeletal muscle glycogen

Fig. 1 Tibialis anterior muscle glycogen (A), liver glycogen (B), blood glucose (C), and serum free fatty acid (FFA) (D) concentrations in mice orally administered taurine solution (taurine-treated) or saline (control) immediately after the exercise. Values are means $\pm$ SE, $n = 5$ or 6 per group, * $p < 0.05$ vs. the control group at the same time, † $p < 0.05$ and †† $p < 0.01$ vs. baseline.

concentration in the taurine-treated group at 120 min after exercise was 136% higher than the baseline level ($p < 0.05$). In contrast, the glycogen content in skeletal muscle in the control group at 120 min after exercise did not differ from the baseline level. Liver glycogen concentration at the end of exercise was 36% of that at baseline (2.74 ± 0.42 mg/g at the end of exercise vs. 7.67 ± 1.67 mg/g at baseline, $p < 0.05$) (Fig. 1B). In both groups, liver glycogen concentration recovered to the baseline level at 60 min after exercise and was above the baseline level at 120 min ($p < 0.01$). No significant difference was found in liver glycogen concentration between the taurine-treated and control groups at any time. Exercise significantly decreased the blood glucose concentration compared with the baseline level (2.49 ± 0.30 mmol/l at the end of exercise vs. 8.65 ± 0.66 mmol/l at baseline, $p < 0.01$) (Fig. 1C). In both groups, blood glucose concentration at 60 min after the exercise was significantly higher than that at baseline ($p < 0.01$), and returned to the baseline level at 120 min after exercise. There was no difference in blood glucose concentration between the two groups at any time. Exercise significantly increased serum FFA concentration compared with the baseline level (1.56 ± 0.26 mEq/l at the end of exercise vs. 0.45 ± 0.07 mEq/l at

baseline, $p < 0.05$) (Fig. 1D). Serum FFA concentration was significantly higher in the taurine-treated group than in the control group at 60 min after exercise ($p < 0.05$).

Experiment 2

Blood glucose and plasma insulin concentrations during post-exercise glucose tolerance test

There was no significant difference in blood glucose concentration between the two groups (Fig. 2A). The AUC for blood glucose concentration from 0 to 60 min after exercise was 28% smaller in the taurine-treated group than that in the control group ($p < 0.01$) (Fig. 2B). There was no significant difference in the AUC for blood glucose concentration from 0 to 120 min after exercise between the two groups (Fig. 2C). No significant differences were observed in both plasma insulin concentration and the AUCs for plasma insulin concentration during the post-exercise phase between the two groups (Fig. 3A-C).

Concentrations of skeletal muscle and liver glycogen and serum FFA

Tibialis anterior muscle glycogen concentration in the taurine-treated group was 40% higher than that in the control group at 120 min after exercise ($p < 0.05$) (Fig. 4A).

Fig. 2 (A) Blood glucose concentration in mice after oral administration of glucose solution containing taurine (taurine-treated) or glucose solution (control) at immediately after and 60 min after the exercise. The area under the curve (AUC) for blood glucose concentration during 60 min (B) or 120 min (C) of post-exercise phase. The AUCs were calculated in accordance with the trapezoidal rule. Values are means $\pm$ SE, $n = 5$ per group, ** $p < 0.01$ vs. the control group.

Fig. 3 (A) Plasma insulin concentration in mice after oral administration of glucose solution containing taurine (taurine-treated) or glucose solution (control) at immediately after and 60 min after the exercise. The area under the curve (AUC) for plasma insulin concentration during 60 min (B) or 120 min (C) of post-exercise phase. The AUCs were calculated in accordance with the trapezoidal rule. Values are means $\pm$ SE, $n = 5$ per group.

We did not observe any significant difference between the two groups in liver glycogen concentration (Fig. 4B) and serum FFA concentration (Fig. 4C).

Discussion

In our previous study, we found a significant effect of taurine administration on the amount of voluntary wheel running activity after 90 min of treadmill running as an indicator of recovery from endurance exercise¹⁰. The results suggested that taurine administration has some positive effects on recovery from exercise-induced fatigue. Glycogen repletion in skeletal muscle after exercise is one of the major determinants of performance during subsequent exercise since higher pre-exercise glycogen content in skeletal muscle resulted in improving performance during both prolonged endurance and high-intensity intermittent exercise²⁻⁵. Some rodent studies have shown the possibility that taurine administration enhances glucose uptake in tissues¹¹⁻¹⁷, which is one of the limiting factors for glycogen synthesis in skeletal muscle. Therefore, we hypothesized that taurine administration enhanced post-exercise glycogen repletion in skeletal muscle. Indeed,

we found that the glycogen content in the tibialis anterior muscle was significantly higher in the taurine-treated group than that in the control group at 120 min after treadmill running (Fig. 1A and 4A). This result represents the positive effect of post-exercise taurine administration on glycogen repletion in skeletal muscle. Results from this and our previous studies indicate that higher voluntary wheel running activity following endurance exercise by taurine treatment is partly due to the enhancement of glycogen repletion in skeletal muscle during the post-exercise phase.

In experiment 2, we observed that taurine treatment attenuated the increase of blood glucose concentration after post-exercise oral glucose administration (Fig. 2B). This effect of taurine treatment is in agreement with some rodent studies that showed taurine administration attenuated the increase of blood glucose concentration during glucose tolerance testing¹¹⁻¹⁷. In general, most glucose (>80%) is taken up by skeletal muscle²¹. Therefore, changes in blood glucose concentration after post-exercise glucose administration will be largely influenced by glucose uptake in skeletal muscle. Although we should consider that there are possibilities that taurine admin-

Fig. 4 Tibialis anterior muscle glycogen (A), liver glycogen (B), and serum free fatty acid (FFA) (C) concentrations in mice at 120 min after exercise with twice oral administrations of glucose solution containing taurine (taurine-treated) or glucose solution (control). Values are means $\pm$ SE, $n = 6$ per group, * $p < 0.05$ vs. the control group.

istration influences other functions involved in glucose homeostasis such as glucose absorption in the small intestine or glucose production from the liver etc., taurine administration may have a positive effect on glucose uptake in skeletal muscle during the post-exercise phase. As glucose uptake, as well as the activity of glycogen synthase, is one of the limiting factors of glycogen synthesis in skeletal muscle²²⁾, higher skeletal muscle glycogen concentration by taurine treatment might partly be due to enhancement of glucose uptake in skeletal muscle during the post-exercise phase. Another possible mechanism of smaller AUC for blood glucose concentration by taurine

treatment is stimulation of insulin secretion. Some studies demonstrated that taurine administration improved glucose tolerance in rodents without altering the plasma insulin concentration^{11,13)}, whereas others reported that taurine administration increased insulin secretion from islets^{14,16,17)}. In our experiment, no significant difference was observed in the plasma insulin concentration between the two groups (Fig. 3A-C). Therefore, our results suggest that taurine administration may improve insulin sensitivity rather than stimulate insulin secretion during the post-exercise phase.

Serum FFA concentration at 60 min after exercise was significantly higher in the taurine-treated group than in the control group (Fig. 1D). This result is keeping with recent studies, which showed taurine treatment increased fat oxidation during prolonged exercise in humans²³⁾. This result is also supported by an *in vitro* study reporting that taurine treatment to adipocytes increased catalytic activity of cAMP-activated protein kinase A, which is an important enzyme for lipolysis in adipocytes, and then supplying free fatty acid into the blood²⁴⁾. At resting state, the plasma FFA concentration was negatively correlated with the respiratory exchange ratio^{25,26)}. This result indicates that a higher FFA concentration in blood is associated with an increase in fat oxidation. Thus, it is possible that a higher serum FFA concentration by taurine treatment may result in enhancement of fat oxidation during the post-exercise phase. In the present study, carbohydrate storage in tissues at the end of endurance exercise was largely lower than those at the baseline level. During recovery from such exercise, enhancement of fat oxidation should be important in sparing exogenous carbohydrate for glycogen repletion. Therefore, a higher serum FFA concentration by taurine treatment may be related to higher glycogen content in skeletal muscle.

In conclusion, we found that oral taurine administration following prolonged glycogen depleting exercise enhanced post-exercise glycogen repletion in skeletal muscle. Consistent with previous studies, we observed that taurine treatment attenuated the increase in blood glucose concentration after post-exercise glucose administration. This effect of taurine treatment may reflect the acceleration of glucose uptake in the skeletal muscle. Therefore, it is possible that higher muscle glycogen repletion by taurine treatment is partly due to the enhancement of glucose uptake. Furthermore, we also found that serum FFA concentration in the taurine-treated group was significantly higher than that in the control group at 60 min after exercise. As elevation of the blood FFA level leads to an increase in fat oxidation^{25,26)}, it is possible that a higher serum FFA concentration by taurine treatment may lead to the sparing of carbohydrate for glycogen repletion due to the suppression of carbohydrate utilization as a fuel.

Acknowledgments

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Conflict of interest

We declare that there is no conflict of interest.

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