Effects of Taurine Administration on Carbohydrate Metabolism in Skeletal Muscle during the Post-Exercise Phase

Yumiko TAKAHASHI, Yuki TAMURA, Yutaka MATSUNAGA,
Yu KITAOKA, Shin TERADA and Hideo HATTAT

Department of Sports Sciences, The University of Tokyo, 3–8–1 Komaba,
Meguro-ku, Tokyo 153–8902, Japan
(Received February 24, 2016)

Summary We previously reported that taurine (2-aminoethanesulfonic acid; dose: 0.5 mg/g body weight) administration after treadmill running at 25 m/min for 90 min increased the glycogen concentration in the skeletal muscle of ICR mice at 120 min after the exercise (Takahashi et al. 2014). In the current study, we further investigated the effects of taurine administration on glycogen repletion and carbohydrate metabolism in the tibialis anterior muscle after endurance exercise. The metabolomic profiles of the tibialis anterior muscle at 120 min after the exercise were analyzed by a capillary electrophoresis–time-of-flight mass spectrometry (n=6). Fructose-1,6-bisphosphate (F1,6P), a glycogenolytic/glycolytic intermediate produced by phosphofructokinase, was significantly lower in the taurine-treated group than that in the control group (p<0.01). Dihydroxyacetonephosphate (DHAP), a downstream product of F1,6P was lower (p=0.05) and glycerol 3-phosphate, a downstream product of F1,6P and DHAP, tended to be lower (p=0.09) in the taurine-treated group than in the controls. At that time, phosphorylated Ser293 on the E1α subunit of pyruvate dehydrogenase (PDH) tended to be higher in the taurine-treated mice than in the controls (p=0.09, n=5). There was a positive correlation between phosphorylation of the PDH E1α subunit at Ser293 and glycogen concentration (r=0.73, p<0.05). Our results showed that the enhanced glycogen repletion in skeletal muscle by taurine treatment during the post-exercise phase was accompanied by the lower levels of glycogenolytic/glycolytic intermediates.

Key Words skeletal muscle, post-exercise recovery, taurine, glycogenolysis/glycolysis, pyruvate dehydrogenase

Skeletal muscle glycogen level is considered a major determinant of performance during prolonged moderate- to high-intensity exercise (1–4). When athletes participate in multiple training sessions or competitions, recovery of skeletal muscle glycogen content after exercise is one of the determinants of subsequent exercise performance. As it was reported that oral administration of taurine (2-aminoethanesulfonic acid), which is a sulfur-containing β-amino acid that does not act as a substrate for energy metabolism, increased glycogen concentration in the liver after glucose loading (5), we previously examined whether taurine administration stimulates post-exercise glycogen repletion in skeletal muscle (6). Indeed, we observed enhanced glycogen repletion in skeletal muscle of the taurine-treated mice compared with that of the control mice after endurance exercise. Furthermore, we also found that taurine administration significantly increased voluntary wheel running activity after strenuous exercise in mice; voluntary wheel running activity is thought to indicate recovery from exercise-induced fatigue (7). These results suggest that post-exercise taurine treatment enhanced glycogen repletion in skeletal muscle and then improved performance during subsequent exercise.

Taurine is thought to be involved in various physiological processes including antioxidant processes, plasma membrane stabilization, osmoregulation, ion channel regulation, and the release and uptake of Ca²⁺ by sarcoplasmic reticulum (8). However, the mechanism by which oral taurine administration enhances post-exercise glycogen repletion in skeletal muscle has not been fully determined. We previously reported that the increase in blood glucose concentration after post-exercise glucose administration was attenuated by taurine administration (6). As changes in blood glucose concentration after post-exercise glucose administration are primarily influenced by glucose uptake into the skeletal muscle, this result suggests that taurine enhances skeletal muscle glucose uptake. In addition, we also found higher serum free fatty acid (FFA) concentrations in the taurine-treated group compared with the control group during the post-exercise phase. At rest, blood FFA concentration was negatively correlated with the respiratory exchange ratio (9, 10). This observation indicates that a higher blood FFA concentration leads to the increased fat oxidation concomitant with the decreased carbohydrate utilization as an energy fuel. There is a possibility

*To whom correspondence should be addressed.
E-mail: hatta@idaten.c.u-tokyo.ac.jp
that taurine administration may lead to sparing carbohydrate toward glycogen repletion rather than utilization as a fuel, resulting in higher glycogen concentration in skeletal muscle during post-exercise recovery. In this study, we investigated the effect of taurine administration after treadmill running on carbohydrate metabolism in skeletal muscle during the post-exercise phase. First, we identified the levels of metabolites involved in glycogenolysis/glycolysis and the tricarboxylic acid (TCA) cycle and high energy phosphates in skeletal muscle by metabolome analysis using capillary electrophoresis–time-of-flight mass spectrometry (CE-TOFMS) as described in detail previously (11, 12). As changes in the maximal enzyme activities and levels of key proteins do not necessarily reflect energy metabolism during the recovery after exercise, we considered it appropriate to use metabolome analysis. In addition, to investigate the effect of taurine administration on carbohydrate utilization as energy fuel during the post-exercise recovery more deeply, we also measured the phosphorylation state of pyruvate dehydrogenase (PDH), which is generally considered as a regulatory enzyme of carbohydrate oxidation.

MATERIALS AND 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, given free access to a standard chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan), and allowed to aclimatize for 1 wk. It has previously been shown that skeletal muscle taurine concentration remains within the physiologically normal range in ICR mice fed MF chow (8, 13). All procedures performed in this study involving animals were in accordance with the ethical standards of the Committee on Animal Care and Use, The University of Tokyo, and all protocols of research on animals were approved by this committee (approval number: 24-4).

Experimental procedures. We set the dark phase at 9AM–9PM and all experimental treatments were performed during the dark phase, when the mice were active. The exercise protocol used in this study was the same as that used in our previous studies (6, 7). Three days before the experimental day, all mice were familiarized with running on the treadmill (MK-6080, Muromachi Kikai Co., Inc., Tokyo, Japan) at a speed of 25 m/min for 10 min. The mice were divided into a taurine-treated group and a control group with similar mean body weights. On the day of the experiment, the mice were fasted for 4 h to avoid a postprandial state and then run for 90 min at 25 m/min on a treadmill. The volume of each ingestion for mice was 0.01 mL/g body weight.

Experiment 1: The mice were given oral glucose solutions twice during the post-exercise phase and no other food was provided. After treadmill running, mice in the taurine-treated group (n=6) received 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%) to a volume equal to 0.01 mL/g body weight. This amount of glucose approximates the optimal carbohydrate intake for maximizing post-exercise glycogen repletion (14). The mice were administered with the solutions immediately after treadmill running and again 60 min later, and then sacrificed by cervical dislocation at 120 min after the first oral administration. One side of the tibialis anterior muscle was used for metabolome analysis and the other side was used for measuring glycogen concentration. Experiment 2: To measure the phosphorylation state of the PDH E1α regulatory site by Western blotting, the tibialis anterior muscle at 120 min after the exercise was harvested from mice as previously described (6). Briefly, immediately after treadmill running, mice in the taurine-treated group (n=5) were orally administered 0.5 mg/g body weight taurine, while mice in the control group (n=5) received physiological saline (0.9%) using a sonde. After administration, the mice were placed in individual standard cages and fed ad libitum. Mice were sacrificed by cervical dislocation at 120 min after exercise. Tibialis anterior muscles were harvested, frozen in liquid nitrogen, and stored at −80°C.

Measurement of glycogen concentrations. Glycogen concentrations in the tibialis anterior muscles at 120 min after exercise were measured using the phenol-sulfuric acid method as previously described (15).

Capillary electrophoresis–time-of-flight mass spectrometry. The metabolomic profiles of the tibialis anterior muscles were investigated using capillary electrophoresis–time-of-flight mass spectrometry (CE-TOFMS) performed as previously reported (11, 12, 16). Briefly, 50 mg of tibialis anterior muscle was transferred to 2,100 μL of 50% (v/v) acetonitrile containing 20 μM of internal standards for cation and 5 μM of internal standards for anion. Samples were homogenized at 1,500 rpm for 120 s five times at 4°C using BMS-M10N21 (Bristol-Myers Squibb, Tokyo, Japan). The homogenates were then centrifuged at 2,300 × g for 5 min at 4°C. The two 400 μL supernatant fractions were each transferred into an Ultrafree-MC PLHCC HMT Centrifugal Filter Device with a 5-kDa cutoff membrane. They were centrifuged at 9,100 × g for 120 min at 4°C for ultrafiltration. The filtrates were desiccated, and then dissolved in 50 μL of Milli-Q water and subjected to CE-TOFMS analysis using the Agilent CE-TOFMS system (Agilent Technologies, Palo Alto, CA). Separations were conducted in fused silica capillary tubes (inner diameter, 50 μm; length, 80 cm). Sample solutions were injected at 50 mbar over 10 s. For cation analysis, the CE voltage was set at 27 kV and the MS capillary voltage was set at 4,000 V; for anion analysis, the CE voltage was set at 30 kV and the MS capillary voltage was set at 3,500 V. The scan range was 50 to 1,000 m/z. Peak values detected by CE-TOFMS were obtained using the automatic integration software MasterHands (developed at Keio University, Japan). Each metabolite was identified and quantified based on its peak information including m/z, migration time, and peak area.

Protein isolation and Western blotting. Protein isolation from the tibialis anterior muscle and Western
Post-Exercise Taurine Administration and Carbohydrate Metabolism

 blotting were performed as previously described (17, 18). The tibialis anterior muscles were homogenized in lysis buffer (1% Triton X-100, 50 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 50 mM sodium fluoride, 10 mM sodium β-glycerol phosphate, 5 mM sodium pyrophosphate, 2 mM dithiothreitol, 1 mM Na orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL each of aprotinin, leupeptin, pepstatin A, pH 5.7.5). After centrifugation at 2,000 x g for 15 min at 4°C, the supernatants were collected and their protein concentrations were determined by the Bradford assay (Quick Start™ Bradford Dye Reagent 1x; Bio-Rad, Hercules, CA). The supernatant was diluted with buffer A (1 mM EDTA, 10 mM 2-amino-2-hydroxymethyl-1,3-propanediol, pH=7.4). For Western blotting, proteins (10 μg) and prestained molecular weight markers (Bio Dynamics Laboratory Inc., Tokyo, Japan) were run on 12% SDS-PAGE gels for 60 min at 150 V. The proteins were then transferred from the gel to Hybond-P polyvinylidene difluoride transfer membranes (GE Healthcare Japan, Tokyo, Japan) for 75 min at 100 V. The membranes were blocked with TBS-T (20 mM Tris Base, 137 mM NaCl, 0.1 mM HCl, 0.1% (vol/vol) Tween 20, pH=7.5) containing 5% skim milk for 60 min at room temperature. Membranes were incubated with the primary antibody against the pyruvate dehydrogenase (PDH) E1α subunit (459400; Invitrogen, Carlsbad, CA), the PDH E1α subunit phosphorylated at Ser 293 (ab92696; Abcam, Cambridge, UK) in TBS-T (1 : 4,000 dilution) overnight at 4°C. Subsequently, the membranes were incubated for 60 min at room temperature with goat-anti-rabbit IgG (American Qualex, San Clemente, CA) or goat-anti-mouse IgG (American Qualex) in TBS-T (1:4,000 dilution). Antibodies were visualized using Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific) detected using the Chemidoc system (Bio-Rad). Densitometric analyses of the captured images were performed using Bio-Rad Quantity One software version 4.6.1. All blots on the membranes were stained by Ponceau-S solution (P7170–1L; Sigma-Aldrich, St. Louis, MO) to ensure proteins were equally loaded.

Statistical analysis. All values are expressed as means±SE. The difference in tibialis anterior muscle glycogen concentrations, protein levels and phosphorylated states of protein between the two groups were assessed using Welch’s t-test. **p<0.01 vs. the control group.
the metabolome analysis, between-group differences in the relative levels of metabolites in the tibialis anterior muscle were analyzed using Welch’s t-test. Correlation between PDH E1α subunit phosphorylation at Ser293 and the glycogen concentration was determined using least squares linear regression and the Pearson correlation coefficient was calculated. Statistical significance was set at $p<0.05$.

**RESULTS**

**Experiment 1**

**Skeletal muscle glycogen concentration.** The tibialis anterior muscle glycogen concentration was 40% higher in the taurine-treated group than in the control group at 120 min after exercise (2.81±0.15 mg/g in the taurine-treated group, 2.01±0.29 mg/g in the control group; $p<0.05$).

**The glycogenolytic/glycolytic and TCA cycle intermediates in skeletal muscle.** We performed metabolome analysis of the tibialis anterior muscle at 120 min after the exercise when glycogen concentration was significantly higher in the taurine-treated group compared with that of the control group. We identified 88 cation metabolites and 62 anion metabolites in the tibialis anterior muscle via capillary electrophoresis–time-of-flight mass spectrometry (CE-TOFMS), and expressed their quantities as relative levels between the taurine-treated and the control group. We described the metabolome pathway map of glycogenolytic/glycolytic and tricarboxylic acid (TCA) cycle intermediates in the tibialis anterior muscle at 120 min after the exercise (Fig. 1). The level of fructose-1,6-bisphosphate (F1,6P), which is an intermediate metabolite produced by phosphofructokinase (PFK) during glycogenolysis/glycolysis, was 56% lower in the taurine-treated group than in the control group ($p<0.01$). The concentration of dihydroxyacetonephosphate (DHAP), which is a downstream product of F1,6P during glycolysis, was 38% lower in the taurine-treated group than in the control group ($p=0.05$). Glycerol 3-phosphate (G3P), a downstream product of F1,6P and DHAP, tended to be 20% lower in the taurine-treated group than in the control group ($p=0.09$). There were no significant differences between the two groups in the high-energy phosphate content at 120 min after exercise (Fig. 2).

**Levels of taurine and other metabolites related to carbohydrate metabolism in skeletal muscle.** The taurine level in the tibialis anterior muscle of the taurine-treated group did not significantly differ from that of the control group (0.97±0.02 in the taurine-treated group, 1.00±0.04 in the control group). We also analyzed amino acids levels because some amino acids are utilized as precursors for gluconeogenesis and converted into intermediates in the TCA cycle (Fig. 3). Threonine, which is one of the precursors for acetyl-CoA, was significantly lower in the taurine-treated group than in the control group ($p<0.05$). There were no significant between-group differences in the levels of the other amino acids levels. We did not observe any significant differences in the levels of NADH (0.71±0.10 in the taurine-treated group, 1.00±0.13 in the control group) or NAD$^+$ (0.88±0.04 in the taurine-treated group, 1.00±0.06 in the control group).
group) between the two groups.

Experiment 2

Food consumption during 120 min of post-exercise recovery. As we previously reported (6), no significant difference was observed in total food consumption between the two groups during 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 concentration. The tibialis anterior muscle glycogen concentration in the taurine-treated group was 94% higher compared with that in the control group (4.62±0.81 mg/g in the taurine-treated group, 2.39±0.41 mg/g in the control group; *p*, 0.05) (6).

Phosphorylation of a pyruvate dehydrogenase E1α subunit regulatory site. We observed that the levels of glycogenolytic/glycolytic intermediates in the tibialis anterior muscle were lower in the taurine-treated group than those in the control group but no significant between-group differences were observed in TCA intermediates at 12 min after the exercise. As TCA cycle intermediate levels are influenced by not only glycogenolysis/glycolysis but also β-oxidation of fatty acids and the conversions of amino acids, we needed to focus on the effect of post-exercise taurine administration on carbohydrate utilization as an energy fuel. PDH, which catalyzes the irreversible conversion of pyruvate into acetyl-CoA, is considered a regulatory enzyme of carbohydrate oxidation. We investigated the possibility that post-exercise taurine administration increased the phosphorylated (inactivated) state of PDH. Phosphorylation of the PDH E1α subunit at Ser293 tended to be higher in the tibialis anterior muscle of the taurine-treated group than that of the control group (*p* = 0.09; Fig. 4A). In addition, there was a positive correlation between PDH E1α subunit phosphorylation at Ser293 and the glycogen concentration in skeletal muscle at 120 min after exercise (*r* = 0.73, *p* < 0.05, Fig. 4B).

**DISCUSSION**

We previously demonstrated that oral taurine administration enhanced glycogen repletion in skeletal muscle and was associated with higher serum free fatty acid (FFA) concentrations during the post-exercise phase in mice (6). As it has been indicated that elevation of blood FFA levels leads to an increase in fat oxidation (9, 10), the possibility exists that taurine administration may lead to sparing carbohydrate toward glycogen repletion in skeletal muscle during post-exercise recovery. We identified the levels of metabolites involved in glycogenolysis/glycolysis and the TCA cycle and high energy phosphates in the tibialis anterior muscle by metabolome analysis using CE-TOFMS since metabolomic profiles would reflect energy metabolic states during recovery from exercise better than the maximal activities and the contents of proteins. We found that the F1,6P level was significantly 56% lower in the taurine-treated group than in the control group. F1,6P is produced by PFK, which is one of the rate-limiting enzymes for glycogenolysis/glycolysis. Furthermore, the relative level of DHAP (a downstream product of F1,6P) was 38% lower and G3P (a downstream product of F1,6P and DHAP) tended to be 20% lower in the taurine-treated group than in the control group. These results indicate that glycogenolysis/glycolysis in skeletal muscle during the post-exercise phase would be suppressed by taurine administration. The decreased glycogenolytic/glycolytic intermediates by post-exercise taurine administration
are in line with a previous study showing that taurine depletion by the inhibitor of taurine transporter (TAUT) resulted in a rise in glycogenolytic/glycolytic flux with the higher levels of F1,6P and DHAP in the heart (19). Furthermore, the whole body TAUT knockout mouse, which has significant lower taurine levels in various tissues including skeletal muscle, showed a higher lactate concentration in skeletal muscle at rest and blood during moderate-intensity exercise (20). On the other hand, taurine treatment attenuated the increase of blood lactate level during the exercise, which is considered as an indicator of activation of glycogenolysis/glycolysis in skeletal muscle (21). Results from previous studies and the present study suggest that taurine is involved in the regulation of glycogenolysis/glycolysis in skeletal muscle at rest, during exercise and in the post-exercise recovery. To date, however, the mechanisms by which taurine is involved in the regulation of glycogenolysis/glycolysis have not been clarified. We did not observe any significant between-group differences in either the activator (AMP) or inhibitors (ATP and citrate) of PFK in skeletal muscle at 120 min after exercise. Further experiments would be required to investigate the effect of taurine administration on the regulation of glycogenolysis/glycolysis. Suppression of glycogenolysis/glycolysis may enhance glycogen repletion in skeletal muscle during the post-exercise phase. Studies administering acetic acid in rats previously showed that enhanced glycogen repletion after food deprivation was associated with a lower F1,6P/fructose-6-phosphate ratio, which is an indicator of the activity of PFK in skeletal muscle (22, 23). Recent studies suggested that during the post-exercise recovery, reducing carbohydrate utilization as a fuel effectively enhances skeletal muscle glycogen repletion. For example, oral administration of hydroxycitrate, which increases energy reliance on fat oxidation during the post-exercise phase, improved glycogen repletion in skeletal muscle (24). In contrast, accelerating carbohydrate oxidation by exposure to hot conditions impaired post-exercise glycogen resynthesis (25). The present study suggests that taurine administration is effective for glycogen recovery in skeletal muscle by sparing carbohydrate after exercise.

PDH, which catalyzes the irreversible conversion of pyruvate into acetyl-CoA and is considered a regulatory enzyme of carbohydrate oxidation, is activated by dephosphorylation of regulatory sites and inactivated by phosphorylation of regulatory sites. As Ser\textsuperscript{293} on the PDH E1α subunit is a major regulatory site, we measured its phosphorylation. In the tibialis anterior muscle of the taurine-treated group, phosphorylation of Ser\textsuperscript{293} on the PDH E1α subunit tended to be higher than that of the control group at 120 min after the exercise. In addition, we found a positive correlation between phosphorylation of Ser\textsuperscript{293} and glycogen concentration in skeletal muscle. These results are consistent with a recent study demonstrating that mice with the higher phosphorylated PDH content had a higher glycogen resynthesis rate in skeletal muscle during the post-exercise phase (26). It is possible that the increased phosphorylation of Ser\textsuperscript{293}, a PDH regulator, is related to the reduced utilization of carbohydrate as an energy fuel and the increase in skeletal muscle glycogen during the post-exercise phase. In our previous study, we showed that the serum concentration of FFA was significantly higher in the taurine-treated group than in the control group at 60 min after exercise (6). Since FFA is converted into acetyl-CoA and then utilized as a substrate in the TCA cycle, taurine-treated mice might have higher acetyl-CoA availability in skeletal muscle compared with the control mice. In addition, mice in the taurine-treated group had a lower relative level of threonine, which is one of the precursors of acetyl-CoA. Although the role that the conversion of threonine into acetyl-CoA plays in energy metabolism has not yet been fully clarified, there is a possibility that the lower skeletal muscle threonine level in the taurine-treated group may be due to conversion of threonine into acetyl-CoA for use as an energy substrate. The decreased threonine level in skeletal muscle by taurine treatment was observed in a previous rat study (27). In that study, they also found lower levels of other acetyl-CoA precursors such as serine, glycine, and alanine. However, to date, the mechanism by which taurine treatment alters amino acids levels in skeletal muscle remains unclear.

At 120 min after exercise, there was no significant difference in the taurine levels of the tibialis anterior muscle between the two groups. A previous study in rats showed that orally ingested taurine was detected in the blood and many tissues, including the liver and urinary bladder within 10 min after administration (28). In addition, taurine has been detected in skeletal muscle 15 min after intravenous injection in rats (29). Based on these results, orally administered taurine is expected to be detectable in the blood within 30 min in mice. There is a possibility that orally administered taurine was taken up by skeletal muscle via TAUT and the mice in the taurine-treated group had higher skeletal muscle taurine levels at an early time point (before sample collection). As some of effects of taurine administration may occur at the plasma membrane—these include antioxidant processes on the plasma membrane (30, 31), excitation-contraction coupling (32), and regulation of cellular osmolality through influences on ion channels (33)—it is also possible that orally administered taurine indirectly influenced energy metabolism in skeletal muscle by affecting some cellular functions outside of tissue. Moreover, taurine administration could be involved in the regulation of metabolism in other tissues. For example, pre-exercise taurine administration in humans increased whole body fat oxidation during exercise (34) and we previously observed higher serum free fatty acid level during post-exercise recovery (6). These effects of taurine administration seem to be due to an activation of lipolysis in adipocytes (35). In addition, as 2 and 3 wk of taurine administration altered levels of precursors of gluconeogenesis (27), the possibility exists that taurine administration may influence gluconeogenesis in the liver. Further investigation is needed to elucidate how and where taurine administration affects...
energy metabolism during the post-exercise phase.

In conclusion, we found that higher glycogen concentration in the tibialis anterior muscle of taurine-treated mice at 120 min after endurance exercise was accompanied by lower levels of glycogenolytic/glycolytic intermediates compared with those of the control group, evidenced by metabolome analysis with CE-TOFMS. At that time, we also observed that phosphorylation of Ser\(^{293}\) on the PDH E1\(\alpha\) subunit tended to be higher in the taurine-treated group than in the control group in skeletal muscle and there was a positive correlation between phosphorylation of Ser\(^{293}\) and glycogen concentration in skeletal muscle. The results of the current study suggest that the enhanced post-exercise skeletal muscle glycogen repletion occurring with taurine administration is associated with reduced utilization of carbohydrate as an energy fuel.

Acknowledgments

This study was supported by Taisho Pharmaceutical Co., Ltd., Tokyo, Japan. Yumiko Takahashi was the recipient of a Grant-in-Aid for JSPS Fellows from the Japan Society for the Promotion of Science. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

26) Fritzen AM, Lundsgaard AM, Jeppesen J, Christiansen ML, Bienro R, Dyck JR, Pilegaard H, Kiens B. 2015. 5′-AMP activated protein kinase α2 controls substrate


