Increased *N*-Acetyltaurine in the Skeletal Muscle After Endurance Exercise in Rat

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Abstract Taurine is metabolized to a novel metabolite, *N*-acetyltaurine (NAT), through N-acetylation with acetate. Furthermore, NAT production increases when the endogenous production of acetate is elevated in some situations, such as alcohol catabolism and endurance exercise. We have previously reported that both the serum concentration and urinary excretion of NAT from humans were increased after endurance exercise, and that NAT was secreted by cultured skeletal muscle cells exposed to both acetate and taurine. The present study evaluated the hypothesis that NAT is synthesized in the skeletal muscle after endurance exercise. Normal rats were loaded to a transient treadmill running until exhaustion. Serum, skeletal muscle, and liver were collected immediately after the exercise. The NAT concentration in the plasma and in the soleus muscle from the exercised rats was significantly increased compared to that in the samples from the sedentary control rats. There was a significant positive correlation in the NAT concentration between the plasma and soleus muscle. The NAT concentration in the liver was unchanged after the endurance exercise. These results confirm that the significantly increased NAT in both the serum and urine after endurance exercise is derived from NAT synthesis in the skeletal muscle.

Keywords Treadmill running • *N*-Acetylation • Acetate • Acetyl-CoA • Energy production

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Abbreviations

ACS2	Acetyl-CoA synthetase 2
EX group	Exercise group
IS	Internal standard
NAT	*N*-acetyltaurine
PDH	Pyruvate dehydrogenase
SED group	Sedentary group
TCA	Trichloroacetic acid

1 Introduction

Taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid-like compound found in various mammalian tissues, including skeletal muscle (Awapara 1956; Jacobsen and Smith 1968; Huxtable 1980). Many physiological and pharmacological actions of taurine have previously been established, such as stabilization of the cellular plasma membrane, osmoregulation, anti-oxidant effects, and detoxification (Pasantes et al. 1998; Huxtable 1992; Miyazaki and Matsuzaki 2014; Nakamura et al. 1993; Miyazaki 2010; Nieminen et al. 1988). The most established activity of taurine is the conjugation with bile acids in the liver to increase the excretion of bile acid into the bile through enhancement of the solubility of hydrophobic bile acids, which reduces the toxicity of hydrophobic bile acid (Danielsson 1963; Sjovall 1959). Based on the conjugation with bile acids, taurine has the potential to react with many substances.

In 2012, Shi et al. reported that taurine was metabolized to *N*-acetyltaurine (NAT) in the liver by acetylation with acetate (Shi et al. 2012), which is a metabolite in ethanol detoxification (Buckley and Williamson 1977). NAT is transported from the liver to the circulation, and then, is excreted into the urine. Because acetate produced through alcoholic catabolism is finally metabolized to H_2O and CO_2 in extrahepatic tissues, mainly in skeletal muscle (Zakhari 2006), it is suggested that taurine might enhance the excretion of acetate in urine before metabolism to H_2O and CO_2.

Acetate is also produced through hepatic lipid oxidation when energy production is in high demand in the skeletal muscle, such as during endurance exercise. We have previously reported that both the serum concentration and urinary excretion of NAT were significantly increased after endurance running by humans (Miyazaki et al. 2015). In addition, we have confirmed that NAT was excreted into the culture medium from cultured skeletal muscle cells incubated with both acetate and taurine (Miyazaki et al. 2015). Thus, our previous studies indirectly suggested that the increased NAT in both serum and urine after endurance

exercise might be derived from the skeletal muscle. The present study aimed to evaluate the NAT concentration in the skeletal muscle *in vivo* after endurance exercise.

2 Methods

2.1 Experimental Animal and Exercise

Male Fischer 344 rats (Japan SLC, Shizuoka, Japan), 10 weeks of age, were randomly divided into two groups after 1 week acclimatization: a sedentary group (SED: n = 7, body weight 195 ± 4 g; mean ± SD) and an exercise group (EX: n = 8, 189 ± 4 g). Rats in the EX group were habituated to treadmill running for 5 days by gradual increases in both the running speed and duration for up to 45 m/min and 10 min, respectively. After 1 day following the habituation period, rats in the EX group were fasted for 3 h, and, put on the treadmill for 15 min. Thereafter, the rats ran on the treadmill at 40 m/min until exhaustion based on the justification point previously reported (Dohm et al. 1980; Miyazaki et al. 2004). We found that the running time until exhaustion was 40.1 ± 3.0 min. Immediately after the exercise, the rats were euthanized by cervical dislocation under anesthesia with pentobarbital (*i.p.* 64.8 mg/kg body weight). In the SED group, the rats were euthanized following placement on the treadmill for 15 min without exercise. Plasma, liver, and soleus muscle that contain abundant taurine, were collected, and kept at −80 °C until analysis.

2.2 Taurine and NAT Analyses

For taurine quantification, muscle and hepatic tissues were homogenized with a 15-fold volume (*w/v*) of 5% trichloroacetic acid (TCA) solution, and centrifuged at 6200 × *g*, 4 °C for 20 min. In addition, 100 μL of plasma was mixed with the TCA solution, and centrifuged at 12000 × *g*, 4 °C for 15 min. Thereafter, the supernates were used for taurine quantification using an automatic amino acid analyzer. The NAT concentration was quantified by an HPLC-MS/MS system according to our previously reported method. Standard and internal standard (IS) NATS were synthesized from taurine and 2-aminoethane-d4 sulfonic acid (taurine-d4), respectively, by reaction with acetic anhydride. Muscle and hepatic tissues were homogenized with a tenfold volume (*w/v*) of 1% formic solution. After centrifugation at 3500 × *g* at 4 °C for 10 min, the supernate was collected for analysis. Five μL of plasma and of the supernate of the homogenized tissue were mixed with 1 ng NAT-d4 as an IS

in 50 μL of acetonitrile-water (19:1, *v/v*), and centrifuged at 2000 × *g* for 1 min. The supernate was evaporated to dryness at 80 °C under a nitrogen stream. The residue was redissolved in 60 μL of 1% formic acid, and an aliquot (5 μL) was analyzed by HPLC-MS/MS.

2.3 Statistic Analysis

Statistical significance was determined by the unpaired Student's *t*-test. Each value was expressed as the mean ± SEM. Differences were considered as statistically significant when the calculated *P*-value was less than 0.05. Correlations between plasma and tissue levels were analyzed by the Pearson's correlation coefficient.

3 Results

3.1 Taurine Concentrations in Plasma, Skeletal Muscle, and Liver From Rats after Transient Endurance Running Exercise

After the endurance treadmill exercise, the plasma taurine concentration in the EX group was significantly increased compared to that in the SED group (Fig. 1). On the other hand, the taurine concentration in the soleus muscle was significantly

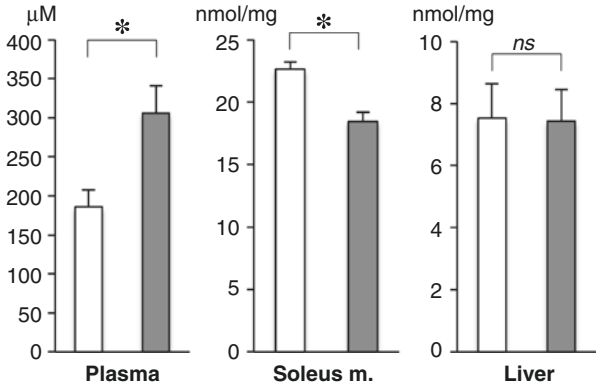

Fig. 1 Taurine concentration in plasma, skeletal muscle, and liver after a transient endurance running exercise until exhaustion. Opened and closed columns show the sedentary (n = 7) and exercise (n = 8) groups, respectively. Tissue taurine concentration is expressed as per tissue wet weight. Data are the mean ± SEM. **P* < 0.05 by unpaired Student's *t*-test. *Soleus m.* soleus muscle, *ns* no significant difference

lower in the EX group than that in the SED group. There was no significant difference in the hepatic taurine concentration between the two groups.

3.2 *NAT Concentration in Plasma, Skeletal Muscle, and Liver From Rats after Transient Endurance Running Exercise*

Similar to the taurine concentration, the NAT concentration in plasma was significantly higher in the EX group than that in the SED group (Fig. 2). Furthermore, the NAT concentration in the soleus muscle in the EX group was also significantly increased compared to that in the SED group. In the liver, there was no significant difference in the NAT concentration between the two groups.

This study found that there was a significant positive correlation in the NAT concentration between the plasma and skeletal muscle ($y = 4.1\times + 43.7$, $R^2 = 0.2437$, $P < 0.05$).

4 Discussion

In the present study, the NAT concentration in both plasma and skeletal muscle of rats was significantly increased after transient endurance treadmill running with a significant positive correlation to the exercise. Previously, we reported in humans that serum NAT concentration was significantly increased after an endurance exercise (Miyazaki et al. 2015). We also confirmed in the cell culture study that NAT

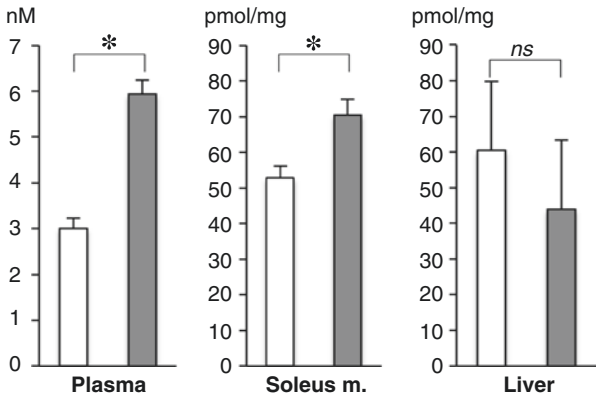

Fig. 2 NAT concentration in plasma, skeletal muscle, and liver after a transient endurance running exercise until exhaustion. Opened and closed columns show the sedentary (n = 7) and exercise (n = 8) groups, respectively. Tissue NAT concentration is expressed as per tissue wet weight. Data are the mean ± SEM. *$P < 0.05$ by unpaired Student's *t*-test. *NAT N*-acetyltaurine, *Soleus m.* soleus muscle, *ns* no significant difference

was synthesized in skeletal muscle exposed to both acetate and taurine in the media. Our previous and present results in human, rat, and cell culture suggest that NAT is synthesized from both acetate and taurine in the skeletal muscle and is excreted into the circulation with endurance exercise.

We have reported a significant decrease in the taurine concentration in rat skeletal muscle dependent on the exercise duration up to exhaustion (Matsuzaki et al. 2002). In the present study, the taurine concentration was significantly decreased in the soleus muscle in which the NAT concentration was significantly decreased after endurance exercise. It is suggested from these results that the significant decrease in muscular taurine concentration after endurance exercise might be partly due to utilization of taurine for NAT synthesis.

During endurance exercise, lipid metabolism for energy production is activated dependently on exercise duration and/or intensity. With β-oxidation of fatty acids in the liver, acetyl-CoA is metabolized to ketone bodies that are an energy source for extrahepatic tissues, mainly skeletal muscle and brain. In the ketogenic conditions during endurance exercise, acetate is also metabolized from the acetyl-CoA in the liver by the acetyl-CoA hydrolase, and then, it is reconverted to acetyl-CoA by the acetyl-CoA synthetase 2 (ACS2) in the mitochondria of the skeletal muscle for energy production (Fig. 3) (Fukao et al. 2004; Luong et al. 2000; Sakakibara et al. 2009). Because the metabolism from acetate of acetyl-CoA by the ACS2 is carried

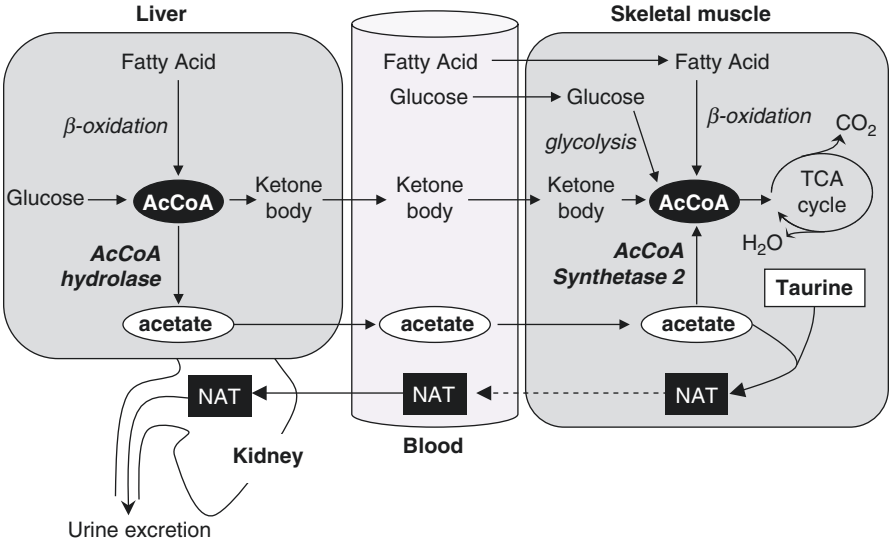

Fig. 3 Putative metabolic pathways of acetate and NAT during/after endurance exercise between the liver and skeletal muscle. In endurance exercise, NAT is likely to be synthesized from taurine and acetate in the skeletal muscle. By synthesis of NAT, taurine may play a role to prevent from acetate-induced acetyl-CoA accumulation in the mitochondrial, which is a possible cause of metabolic abnormality or delayed metabolic normalization in the recovery period after endurance exercise. *AcCoA* acetyl-CoA, *NAT N*-acetyltaurine

out in one reaction, acetate is easier to convert to energy when compared with other nutrients, including carbohydrates, fatty acids, ketone bodies, and amino acids, which require multiple metabolic reactions to form acetyl-CoA in skeletal muscle.

Although the energy expenditure declines after endurance exercise, acetate needs to be continuously broken down into H_2O and CO_2 via acetyl-CoA in the skeletal muscle. These findings suggest that acetate could cause excess acetyl-CoA accumulation in the skeletal muscle during the recovery period after endurance exercise. Excess acetyl-CoA accumulation in the mitochondria of the skeletal muscle leads to a metabolic imbalance. Mitochondrial acetyl-CoA accumulation inhibits negative feedback of the activity of the pyruvate dehydrogenase (PDH) complex that catalyzes an irreversible reaction from pyruvate to acetyl-CoA, through upregulation of the pyruvate dehydrogenase kinase 4 gene (Kerbey et al. 1976; Sugden and Holness 2003; Wu et al. 1998). The reduction of PDH activity induces a delayed flux through glycolytic reactions, and consequently, causes insulin resistance (Hoy et al. 2009; Fueger et al. 2007; Furler et al. 1991; Furler et al. 1997; Katz et al. 1991). Indeed, our previous study confirmed that the excess acetyl-CoA accumulation in the mitochondria induced by exogenous treatment of acetyl-carnitine that is the end product of fatty acid β-oxidation significantly decreased insulin-dependent glucose uptake in cultured C2C12 myotubes (Miyamoto et al. 2016). Therefore, it is necessary to eliminate the acetate from the skeletal muscle as soon as possible after the endurance exercise.

Furthermore, Takahashi et al. have reported a mouse study where taurine administration immediately after a transient endurance treadmill test significantly facilitated the recovery of muscle glycogen concentration at 120 min after the exercise (Takahashi et al. 2014). The effect of taurine administration on glycogen recovery might be due to the acceleration of glucose uptake in the skeletal muscle, because post exercise taurine administration significantly inhibited the elevation of the blood glucose concentration following oral glucose ingestion during the recovery period (Takahashi et al. 2014). The acceleration of glycogen recovery and glucose uptake in the skeletal muscle by taurine administration after the endurance exercise likely prevents excess mitochondrial acetyl-CoA accumulation induced by acetate through the conversion of taurine to NAT. Thus, taurine might play a role in eliminating acetate through NAT synthesis in the skeletal muscle to prevent mitochondrial acetyl-CoA accumulation and to normalize muscular energy metabolism after the exercise.

5 Conclusion

In summary, the present study shows that the NAT concentration in rat skeletal muscle was significantly increased by transient endurance exercise and showed a significant positive correlation with the plasma NAT concentration. These findings suggest that taurine in skeletal muscle might react with the acetate that is supplied from hepatic tissue during exercise, and thus the metabolite NAT would be excreted into the urine in order to prevent intramuscular excess accumulation of acetyl-CoA that might induce a metabolic imbalance after exercise.

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