Effect of N-(α-Ribopyranosyl)taurine Sodium Salt Supplementation on the Hepatic Antioxidant System in Rat Models of Diet-Induced Obesity and Taurine Deficiency

So Young Kim, Jeong Soon You, Yun Ju Lee, Kyung Ja Chang, Hye Jeong Cho, and Sung Hoon Kim

Abbreviations

T-Rib  N-(α-Ribopyranosyl)taurine sodium salt
N     Normal diet
HF    High-fat diet
HFT   High-fat diet + taurine
HFA   High-fat diet + β-alanine
HFR2  High-fat diet + β-alanine + T-Rib (2 mmol/kg/day)
HFR4  High-fat diet + β-alanine + T-Rib (4 mmol/kg/day)
HFR6  High-fat diet + β-alanine + T-Rib (6 mmol/kg/day)
GOT   Glutamate oxaloacetate transaminase
GPT   Glutamate pyruvic transaminase
TBARS Thiobarbituric acid reactive substance
GSH   Glutathione
GPx   Glutathione peroxidase
SE    Standard error
H&E   Hematoxylin and eosin

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1 Introduction

Taurine (2-aminoethanesulphonic acid) is widely present in mammalian tissue and plays a role in many physiological processes, including development of the brain, osmoregulation, and cell membrane stabilization. It also has hypolipidemic effects, as well as anti-oxidant and hepatoprotective effects (Huxtable 1992). In particular, taurine supplementation inhibits hepatic lipid peroxidation and prevents membrane disintegration during hepatocarcinogenesis in rats (You and Chang 1997). Rats treated with β-alanine to induce taurine depletion were more susceptible to ethanol-induced hepatic dysfunction (Kerai et al. 2001).

Taurine has to be absorbed through its own transporter to have physiological activity. However, since antagonists such as β-alanine, β-aminobutyric acid, and gamma-aminobutyric acid have forms similar to that of taurine and compete with taurine for absorption into the cell, the amount of taurine absorbed is often limited. In order to overcome this disadvantage, numerous taurine derivatives were developed. There are many studies about taurine derivatives with various physiological functions. Taurine-chloramine and taurine-bromamine, formed by a reaction of taurine with HOCl and HOBr respectively, have anti-microbial and anti-inflammatory effects (Marcinkiewicz and Kontny 2014). Early taurine-chloramine supplementation in vivo delays the onset of collagen-induced arthritis (Marcinkiewicz et al. 1995). Furthermore, high doses of taurodilinone significantly inhibited advanced intraperitoneal tumor growth in rats (Braumann et al. 2005). Thiotaurine, the thiosulfonate analog of taurine, protects against diabetes-related biochemical changes in the plasma, heart, and aorta (Budhram et al. 2013).

N-(β-Ribopyranosyl)taurine sodium salt (T-Rib), expected to be absorbed through a carbohydrate transporter, was a synthetic derived from taurine and ribose (Fig. 1). In addition, T-Rib was reported to inhibit adipocyte differentiation in human pre-adipocytes (Cho et al. 2014).

The purpose of this study was to investigate the effect of T-Rib supplementation on the hepatic antioxidant system in rat models of diet-induced obesity and taurine deficiency.

Fig. 1 Structure of T-Rib
2 Methods

2.1 Animals and Diet

Fifty-six male Sprague-Dawley rats aged 4 weeks were purchased from Koatech (Pyeongtaek, Korea). They were each housed in a cage in the laboratory animal room at Inha University under the following conditions: a constant 12-h light and dark cycle (8 AM to 8 PM), controlled temperature of 22±2 °C, and 60±5 % humidity. Food and water were provided ad libitum. The composition of the experimental diet was based on AIN 93G (Reeves et al. 1993) as shown in Table 1.

2.2 Experimental Design

The experimental design is shown in Fig. 2. Following 1 week of acclimatization with a pelletized commercial diet, the rats were randomly assigned to the following seven groups with eight animals in each group for a period of 6 weeks: N group, normal diet; HF group, high-fat diet; HFT group, high-fat diet and 4 mmol/kg/day taurine; HFA group, high-fat diet and β-alanine; HFR2 group, high-fat diet, β-alanine, and 2 mmol/kg/day T-Rib; HFR4 group, high-fat diet, β-alanine, and 4 mmol/kg/day T-Rib; and HFR6 group, high-fat diet, β-alanine, and 6 mmol/kg/day T-Rib.

The taurine deficiency rat model was induced through β-alanine supplementation in drinking water (3 % w/v). Taurine and T-Rib were orally administered to the

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Experimental diets</th>
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<tr>
<td></td>
<td>Normal diet (g)</td>
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<tr>
<td>Casein</td>
<td>200</td>
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<tr>
<td>Corn starch</td>
<td>529.486</td>
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<tr>
<td>Sucrose</td>
<td>100</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
</tr>
<tr>
<td>Lard</td>
<td>0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70</td>
</tr>
<tr>
<td>AIN-93 mineral mixture</td>
<td>35</td>
</tr>
<tr>
<td>AIN-93 vitamin mixture</td>
<td>10</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
</tr>
<tr>
<td>Tert-butyl hydroquinone</td>
<td>0.014</td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
</tr>
</tbody>
</table>
HFT, HFR2, HFR4, and HFR6 groups, and the same amount of distilled water was orally administered to the N, HF, and HFA groups. Body weight (BW) was measured once per week.

### 2.3 Sampling and Chemical Analysis

After 6 weeks of treatment, the animals were deprived of food for 12 h and then sacrificed. Blood sample was collected by heart puncture, and the serum was separated by centrifugation at 1,650×g for 20 min. The isolated serum was immediately frozen in liquid nitrogen, and then stored at −70 °C before analysis. The liver, spleen, and kidney were quickly removed, washed in saline, and then weighed.

To analyze thiobarbituric acid reactive substance (TBARS) and glutathione (GSH) content and glutathione peroxidase (GPx) activity, liver extracts were prepared. Approximately 4 g of minced liver tissue was mixed with 8 ml of cold potassium phosphate buffer (154 mM KCl, 50 mM Tris-HCl, 1 mM EDTA buffer, pH 7.4) in a homogenizer (POTTER S, B. BRAUN, Hessen, Germany). The homogenate was centrifuged for 10 min at 1,000×g (VS 6000 CHN, Vision Scientific, Daejeon, Korea) to remove the precipitate. The above mentioned supernatant solution (1.2 ml) was stored for analyzing TBARS and GSH content, and the remaining supernatant solution was centrifuged for 20 min at 10,000×g (Ananti J-25 centrifuge, Beckman Coulter, Inc., CA, USA) to remove the cell debris. The cytosol was obtained by centrifuging the supernatant solution by using an ultracentrifuge for
60 min at 100,000×g to remove the microsome (Optima LE-80K Ultracentrifuge, Beckman Coulter, Inc., CA, USA). All procedures were carried out at 0–4 °C. After processing, all samples were immediately frozen in liquid nitrogen, and then stored at −70 °C until use.

Serum glutamine oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities were analyzed using an automatic analyzer (BPC BioSed srl, Italy). Standard serum (Asan Pharmaceutical, Korea) was used for calibration before every parameter was analyzed. All of the results were expressed as IU/L serum.

The hepatic lipid peroxide content was analyzed using thiobarbituric acid (TBA) in a method described by Ohkawa et al. (1979). In brief, 10% of the homogenate was introduced into a falcon tube containing 1.5 ml of 1% phosphoric acid and 0.5 ml of TBA reagent. After mixing, the tube was placed in a water bath for 45 min at 95 °C. The content was then cooled in an ice bath for 5 min in the dark, and then 2 ml of n-butanol was added. After mixing, centrifugation was performed for 15 min at 916×g. The supernatant was measured at 535 nm by using the Powerwave X microplate spectrophotometer (Biotek Instruments Inc., VT, USA), and the difference was used to calculate the TBARS concentration, which was expressed as nmol/mg protein. The standard curve was prepared using 1,1,3,3-tetraethoxypropane.

Hepatic GSH content was measured according to the method described by Ellman (1959). Briefly, 0.1 ml of the supernatant was mixed with the reaction mixture, which contained 0.1 ml of 0.2 M Tris buffer (pH 8), 4 ml of methanol, and 0.1 ml of 0.01 M 5,5'-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 15 min and then centrifuged for 30 min at 2,062×g. The absorbance of the supernatant was measured at 415 nm by using the Powerwave X microplate spectrophotometer (Biotek Instruments Inc., Winooski, USA). The GSH contents were expressed as nmol/mg protein.

Hepatic GPx activity was measured according to a procedure described by Tappel (1977). Briefly, 0.1 ml of cytosol was mixed with 1.55 ml of reaction solution containing 50 mM Tris-HCl, 0.1 mM EDTA, 0.25 mM GSH, 0.12 mM NADPH, and 1 U/ml glutathione reductase. The solution was placed in a water bath for 4 min at 37 °C and then 0.05 ml of cumene hydroperoxide was added. The difference in absorbance between 0 and 1 min was measured at 340 nm by using a UV-visible spectrophotometer (DU 650, Beckman Coulter, Inc., CA, USA). GPx activity was calculated by using the extinction coefficient of NADPH (0.00622 μM⁻¹ cm⁻¹) and expressed as nmol NADPH oxidized/min/mg protein.

The protein content in the hepatic tissue was estimated according to the Lowry method (Lowry et al. 1951). Bovine serum albumin was used as a standard.

### 2.4 Histological Examination of Liver Tissue

Histological examination was performed on sections of liver tissue. The liver tissues were fixed immediately with 10% buffered formalin after removal, and paraffin-embedded sections were stained with hematoxylin and eosin (H&E).
2.5 **Statistical Analysis**

All data were expressed as the means±standard error (SE) using the SPSS 17.0 program. Data were analyzed for significant differences by one-way analysis of variation followed by Duncan’s multiple range tests at p<0.05.

3 **Results and Discussion**

3.1 **Growth Curve and Major Organ Weights**

Since a high fat diet is the main cause of obesity (Kopelman 2000), a rat model of high-fat diet-induced obesity was used. A rat model of β-alanine-induced taurine deficiency was also used. The body weights of the rats were monitored for 6 weeks. There was no difference among the body weights of the groups at the start of the experiment; however, at the end of the experimental period, HF and HFA groups showed significantly higher weights than those shown by the N, HFR4, and HFR6 groups (Table 2). In addition, the weight of the HFT group tended to be lower than that of the HF group, but the difference was not significant.

Administration of taurine inhibited obesity; this finding was similar to that of a previous study (Chang et al. 2010), but T-Rib inhibited obesity more effectively than taurine.

The weights of major organs including the liver, spleen, and kidney were measured and relative organ weights were calculated. There were no significant intergroup differences in the relative weights of the liver, spleen, and kidney (Fig. 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>188.3±1.9⁷</td>
<td>354.4±12.7⁸</td>
</tr>
<tr>
<td>HF</td>
<td>187.3±6.0</td>
<td>402.7±6.6⁸</td>
</tr>
<tr>
<td>HFT</td>
<td>186.7±6.4</td>
<td>384.6±11.7⁹</td>
</tr>
<tr>
<td>HFA</td>
<td>191.6±5.9</td>
<td>405.3±16.2⁸</td>
</tr>
<tr>
<td>HFR2</td>
<td>190.5±4.6</td>
<td>370.5±13.4⁹</td>
</tr>
<tr>
<td>HFR4</td>
<td>190.2±4.5</td>
<td>364.1±5.9⁸</td>
</tr>
<tr>
<td>HFR6</td>
<td>187.0±5.0</td>
<td>355.1±6.1⁸</td>
</tr>
</tbody>
</table>

Table 2  Initial body weight and final body weight

Values are expressed as means±SE; N normal diet group, HF high-fat diet group, HFT high-fat diet + 4 mmol taurine group, HFA high-fat diet + β-alanine group, HFR2 high-fat diet + β-alanine + 2 mmol T-Rib group, HFR4 high-fat diet + β-alanine + 4 mmol T-Rib group, HFR6 high-fat diet + β-alanine + 6 mmol T-Rib group. Values with different superscripts within the column are significantly different at p<0.05 by Duncan’s multiple range test, ns is not significant.
**Fig. 3** Relative major organs weights. *N* normal diet group, *HF* high-fat diet group, *HFT* high-fat diet + 4 mmol/kg/day taurine group, *HFA* high-fat diet + 3 % β-alanine group, *HFR2* high-fat diet + 3 % β-alanine + 2 mmol/kg/day T-Rib group, *HFR4* high-fat diet + 3 % β-alanine + 4 mmol/kg/day T-Rib group, *HFR6* high-fat diet + 3 % β-alanine + 6 mmol/kg/day T-Rib group. Values are expressed as means ± SE; *ns* is not significant at p < 0.05, as determined by Duncan’s multiple range test.

### 3.2 Serum GOT and GPT Activities

Serum GOT and GPT activities are used as indicators of liver damage (such as fatty liver and hepatitis) since their activities increase in cases of liver damage (Angulo et al. 1999). The beneficial effects of taurine in decreasing GOT and GPT activity have been reported in rats (Chen et al. 2006) and obese children (Obinata et al. 1996) with non-alcoholic steatohepatitis.

There was no significant intergroup difference in serum GPT activity, but serum GOT activity tended to be higher in the HF and HFA groups than in the N group (Fig. 4). The taurine-administered groups showed significantly decreased serum GOT activity; in particular, the T-Rib-administered groups (HFR4 and HFR6) showed a dose-dependent decrease. These results suggest that taurine and T-Rib prevent liver damage.

### 3.3 Hepatic Antioxidant System

Since a high-fat diet is known to increase oxidative stress levels, which are also associated with cancer and aging, high-fat diets were reported to increase hepatic TBARS content, and decrease hepatic GSH content and GPx activity. In this study, a significant increase in hepatic TBARS content was observed in the HF group compared to that in the N group (Fig. 5). However, the taurine- and T-Rib-administered groups (HFR4 and HFR6) showed significantly smaller increases.
Fig. 4 Serum GOT and GPT activities. *N* normal diet group, *HF* high-fat diet group, *HFT* high-fat diet + 4 mmol/kg/day taurine group, *HFA* high-fat diet + 3 % β-alanine group, *HFR2* high-fat diet + 3 % β-alanine + 2 mmol/kg/day T-Rib group, *HFR4* high-fat diet + 3 % β-alanine + 4 mmol/kg/day T-Rib group, *HFR6* high-fat diet + 3 % β-alanine + 6 mmol/kg/day T-Rib group. Values are expressed as means ± SE; values with different superscripts are significantly different at *p* < 0.05, as determined by Duncan’s multiple range test; *ns* is not significant

Fig. 5 Hepatic TBARS contents. *N* normal diet group, *HF* high-fat diet group, *HFT* high-fat diet + 4 mmol taurine group, *HFA* high-fat diet + 3 % β-alanine group, *HFR2* high-fat diet + 3 % β-alanine + 2 mmol T-Rib group, *HFR4* high-fat diet + 3 % β-alanine + 4 mmol T-Rib group, *HFR6* high-fat diet + 3 % β-alanine + 6 mmol T-Rib group. Values are expressed as means ± SE; values with different superscripts are significantly different at *p* < 0.05, as determined by Duncan’s multiple range test.
GSH is an antioxidant in the body and is converted to GSSH (oxidative form) during oxidative stress (Franco et al. 2007). However, in this study, no significant differences were observed among the groups (data not shown).

It has been shown that obesity is related to a decrease in antioxidant capacity by lowering the activity of antioxidant enzymes in animal and human models. Hepatic GPx activity significantly decreased in the HF group and in the taurine- and T-Rib-administered groups (Fig. 6).

These results suggest that taurine and T-Rib is likely to play a role in the hepatic antioxidant system.

### 3.4 Histological Examination of Liver Tissue

It was reported that CCl₄-treated rats or high-fat diet-fed rats showed central vein disruption, ballooned lipid-laden hepatocytes, and dilated sinusoidal spaces (Desai et al. 2012). Liver tissue had a similar histological appearance in all groups (Fig. 7).
Fig. 7 Histological examination of liver tissue. Representative images of H&E stained sections of liver tissue from experimental groups. Scale bar = 100 μm. N normal diet group, HF high-fat diet group, HFT high-fat diet + 4 mmol/kg/day taurine group, HFA high-fat diet + 3% β-alanine group, HFR2 high-fat diet + 3% β-alanine + 2 mmol/kg/day T-Rib group, HFR4 high-fat diet + 3% β-alanine + 4 mmol/kg/day T-Rib group, HFR6 high-fat diet + 3% β-alanine + 6 mmol/kg/day T-Rib group.

4 Conclusion

It can be concluded from the present study that T-Rib as well as taurine supplementation seem to be beneficial to the hepatic antioxidant system in rat models of diet-induced obesity and taurine deficiency. In addition, T-Rib may be absorbed through a different transporter, not the normal taurine transporter. Further study is needed to investigate the mechanism of T-rib action.

Acknowledgement We thank Dong-A Pharmaceutical Co. for donating taurine.

References