

Original Article

Resistance Imparted by Vitamin C, Vitamin E and Vitamin B₁₂ to the Acute Hepatic Glycogen Change in Rats Caused by Noise

Bei-Wei Zhu^{a,b}, Mei-Lan Piao^b, Yu Zhang^a, Song Han^b,
Qing-Da An^c, Yoshiyuki Murata^d and Mikiro Tada^{d*}

^aCollege of Bio & Food Technology, Dalian Institute of Light Industry, Dalian 116034, China,

^bGraduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan,

^cCollege of Chemical & Engineering, Dalian Institute of Light Industry, Dalian 116034, China,

and ^dDepartment of Agriculture, Okayama University, Okayama 700-8530, Japan

The effects of vitamin C, vitamin E and vitamin B₁₂ on the noise-induced acute change in hepatic glycogen content in rats were investigated. The exposure of rats to 95 dB and 110 dB of noise acutely reduced their hepatic glycogens. Vitamin C (ascorbic acid) and vitamin E (α -tocopherol) attenuated the noise-induced acute reduction in the hepatic glycogen contents. This result suggests that antioxidants could reduce the change *via* reactive oxygen species. Vitamin B₁₂ (cobalamin) delayed the noise-induced change, a finding that suggests that vitamin B₁₂ could postpone the acute change via compensating for vitamin B₁₂ deficiency.

Key words: α -tocopherol, ascorbic acid, cobalamin, hepatic glycogen, noise

Noise pollution has become so significant that people now must pay much more attention to noise stress than in the past. Chronic exposure to noise can cause hearing loss, annoyance, sleep disturbance and decreased performance in animals, including humans [1, 2]. Recently, a few studies demonstrated the following noise-induced acute change. Noise acutely reduced the glycogen, lactic acid and cholesterol contents in the liver of the rat, and noise rapidly increased the activities of serum glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP) and creatine kinase (CK) in rats [3, 4]. Corticosterone and serum cholesterol, glutamic-oxaloacetic transaminase (GOT) and glutamic pyruvic

transaminase (GPT) in albino rats increased after 30 min of noise stress, while the serum triglyceride level dropped [5]. Moreover, the Chinese traditional medicines *Astragali*, *Rhodiolae* and *Ligusticum* suppressed the acute physiological and biochemical changes [3, 4]. However, the mechanism by which the harmful effects were reduced remains to be elucidated.

On the other hand, hearing loss induced by noise stress has been energetically studied. Vitamin C (Ascorbic acid) can affect the oxidative system thus leading to a reduction in noise-induced hearing loss [6, 7]. Vitamin E (α -tocopherol) can attenuate the noise-induced cochlear damage and enhance recovery [8, 9]. The vitamin B₁₂ level of patients with noise-induced hearing loss was significantly lower than that of the control [10]. However, there have been few studies on the acute changes in the physiological and

Received March 10, 2005; accepted October 13, 2005.

*Corresponding author. Phone: +81-86-251-8309; Fax: +81-86-251-8309
E-mail: mtada@cc.okayama-u.ac.jp (M. Tada)

biochemical conditions of a rat subjected to noise, and on the suppression of these acute changes by vitamins.

The effects of vitamin B₁₂, which is reduced by noise stress, as well as antioxidant vitamins, vitamin C and vitamin E, on the acute change in the hepatic glycogen contents in rats were investigated.

Materials and Methods

Animals. Female Wistar rats aged 2 months and weighing 180–200 g were used in the experiments as described previously [3, 4]. The rats were provided by the Experimental Animals Laboratory of Dalian Medical University. The rats were treated in accordance with European Union Directives (86/609/EEC) for laboratory animal care. They fasted for 60 min before the exposure to noise. The physiological saline water containing each vitamin was injected into the abdomen of each rat. The concentrations of vitamin C (ascorbic acid), vitamin E (α -tocopherol) and vitamin B₁₂ (cobalamin) were 300 mg/kg body weight, 100 mg/kg body weight and 300 μ g/kg body weight, respectively. The injection was rendered before the noise application. The intravenous drip with vitamin C and vitamin B₁₂ was purchased from Shandong Reyoung Pharmaceutical Ltd. (Shandong, China), and the intravenous drip with vitamin E was purchased from Zhejiang Medicine Co. Ltd. (Zhejiang, China). The drips were diluted to the desired concentration with physiological saline water before each vitamin was injected into the rats.

Noise application and sample preparation. The equipment used to produce the noise was kindly provided by the Acoustics Institute of Nanjing University. The rats were divided into a non-exposed group and noise-exposed group, with the non-exposed group not being subjected to noise stimulation. The noise-exposed group was divided into 12 sub-groups that were stimulated by a 2–4 kHz noise at 95 or 110 dB for 30, 60, 90 or 120 min. Each group was composed of 10 rats. Soon after the noise had been applied for the indicated time, the rats were sacrificed, and their livers were excised. The livers were transferred into liquid nitrogen. The frozen liver (0.6 g) was homogenized in 5 ml of 20 mM Tris-HCl (pH 7.6) with a Teflon-glass homogenizer. The homogenate was centrifuged at 5000 \times g for 60 min at

4°C, the supernatant being transferred to new tubes and stored at -70°C for subsequent glycogen analysis.

Hepatic glycogen analysis. Hepatic glycogen contents were analyzed as shown previously [3]. The hepatic glycogen in the supernatant was dissolved in KOH with Na₂SO₄ and precipitated with ethanol. The precipitated glycogen was then dissolved in distilled water to a final volume of 5 ml. The amount of glycogen was determined by the phenol-sulfuric acid method from the absorbance at 490 nm.

Protein measurement. The protein concentration was determined by the modified Bradford method, using bovine serum albumin (BSA) as a standard [11].

Statistics. All statistical analyses were performed by the Dunnett's multiple comparison test using EXCEL software. Values of $p < 0.05$ are considered to show statistically significant differences.

Results

Effect of noise stress on the hepatic glycogen contents in rats and the ameliorating effect of antioxidant vitamins. The hepatic glycogen content in rats injected with vitamin C (\triangle) and vitamin E (\blacktriangle) under noise stress at 95 dB (Fig. 1A) or 110 dB (Fig. 1B) was measured.

The application of 95 dB and 110 dB of noise for 30 min reduced the amount of hepatic glycogen (\circ), which subsequently returned to the level observed before the noise application. The injection of vitamin C and vitamin E into the rats significantly suppressed the acute reduction in hepatic glycogen. The levels of hepatic glycogen in the rats injected with vitamin C or vitamin E under noise stress were close to those in the rats under non-stress (\blacklozenge). The effect of these vitamins could be classified within a time-dependent manner. When vitamin C was injected, the amounts of hepatic glycogen slightly decreased throughout the duration of the noise application. When vitamin E was administered, the amount of hepatic glycogen dropped at 30 min after the noise application and the amount of hepatic glycogen slightly increased throughout the duration of the noise application. However, there were no significant differences between the amounts of hepatic glycogen before and after the noise application.

Ameliorating the effect of vitamin B₁₂ on noise-induced acute reduction in hepatic glycogen. The hepatic glycogen levels in rats injected with vitamin B₁₂ (△) under noise stress at 95 dB (Fig. 2A) or 110 dB (Fig. 2B) were measured. When vitamin B₁₂ was injected into the rats, the amounts of hepatic glycogen gradually decreased up to 120 min under noise stress. However, there are no significant differences between the amounts of hepatic glycogen in the rats injected with vitamin B₁₂ (△) under noise stress and those in the rats under non-stress (◆).

Discussion

Effect of noise stress on the hepatic glycogen contents in rats and the ameliorating effect of antioxidant vitamins. The application of noise temporarily reduced the amount of hepatic glycogen, which then returned to the level observed before the noise application (Fig. 1). These results are consistent with our previous data [3].

Cellular lipids, protein and DNA are vulnerable to reactive oxygen species (ROS) activity [12]. The generation of ROS is considered to be part of the

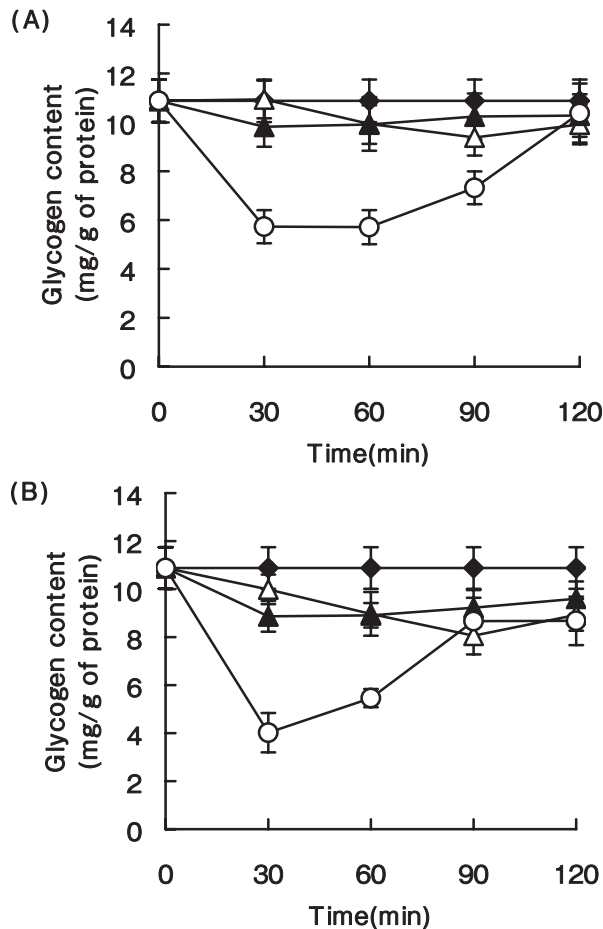


Fig. 1 Effect of vitamins on the content of hepatic glycogen in rats subjected to 95 dB (A) and 110 dB (B) noise stress. The hepatic glycogen content is shown in the exposed rats that received vitamin C (△) or vitamin E (▲) and in those that received neither (○). Rats not subjected to any noise were used as a control (◆). Each value is presented as the mean ± S.D. for 10 rats.

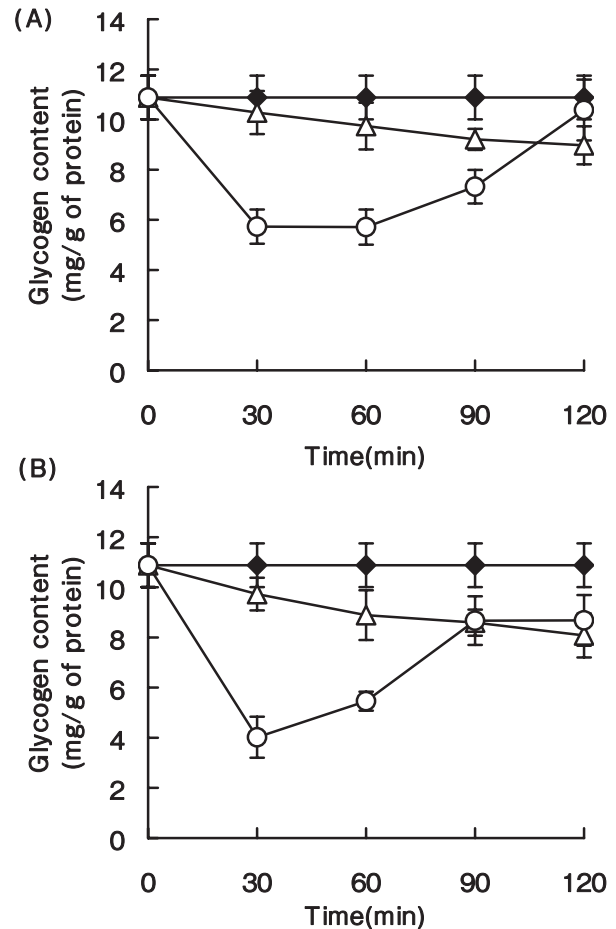


Fig. 2 Effect of vitamin B₁₂ on the hepatic glycogen content in rats subjected to 95 dB (A) and 110 dB (B) noise stress. The hepatic glycogen content is shown in exposed rats that were administered vitamin B₁₂ (△) and in rats that did not receive the vitamin (○). Rats that were not subjected to any noise were used as a control (◆). Each value is presented as the mean ± S.D. for 10 rats.

second mechanism that also includes ischemia, excitotoxic damage, metabolic exhaustion and ionic imbalance in the inner ear fluids. Vitamin C and vitamin E are known to be potent antioxidants. The administration of vitamin C inhibited lipid peroxidation and oxidative damage of proteins in rabbits and also reduced noise-induced hearing loss [6, 7]. Dietary vitamin E reduced temporary threshold shifts (TTS), and intraperitoneal injection of vitamin E protected against noise-induced hearing loss in guinea pigs [8, 9]. Taken together, vitamin C and vitamin E might suppress the acute reduction of hepatic glycogen induced by noise stress via scavenging reactive oxygen species (ROS) produced during the noise application.

Chinese medicinal herbs are increasingly becoming the subject of pharmacological research. Radices of *Astragali* and *Rhodiolae* prevented the noise-stress-induced reduction in the glycogen contents in the liver in rats [3]. *Astragali* and *Rhodiolae* had antioxidant activities that reduced some harmful effects [13, 14]. These results suggest that treatment with *Astragali* or *Rhodiolae* could alleviate the noise-induced effects via scavenging ROS, and this suggestion is supported by the results of the present paper.

Ameliorating effect of vitamin B₁₂ on noise-induced acute reduction in hepatic glycogen.

It has been reported that noise stress induces hearing loss by reducing the vitamin B₁₂ level [10]. These results suggest that noise might acutely induce a vitamin B₁₂ deficiency and thus lead to the reduction of hepatic glycogen.

Quaranta *et al.* demonstrated that vitamin B₁₂ administration had a protective effect via increasing the serum vitamin B₁₂ concentration in the experimental group [15]. Exogenous vitamin B₁₂ might have delayed the acute change in the hepatic glycogen, which suggests that the gradual decrease in hepatic glycogen might be attributed to a decrease in the exogenous vitamin B₁₂ due to the noise application.

Conclusion. Vitamin C, vitamin E and vitamin B₁₂ could suppress the decrease in the hepatic glycogen contents in rats exposed to 95 or 110 dB of noise, a finding which suggests that the administration of these vitamins could alleviate the harmful effects of noise. Little is known about the molecular responses to high-intensity noise exposure, but Cho

et al. (2004) reported on gene expression under experimental conditions [16]. Gene expression analysis including the use of DNA microarray technology could clarify a mechanism for reducing the harmful effects of noise exposure.

Acknowledgments. The authors are grateful for financial support from the State Administration of Foreign Experts Affairs, the P. R. of China (CG 2004210207) and the National Natural Science Fund of China. The authors thank Han Jie of Dalian Medical University for measuring the biochemical parameters.

References

1. Passchier-Vermeer W and Passchier WF: Noise exposure and public health. *Environ Health Perspect* (2000) 108: 123-131.
2. Berglund B, Hassmen P and Job RF: Sources and effects of low-frequency noise. *J Acoust Soc Am* (1996) 99: 2985-3002.
3. Zhu BW, Sun YM, Yun X, Han S, Piao ML, Murata Y and Tada M: Reduction of noise-stress-induced physiological damage by radices of *Astragali* and *Rhodiolae*: glycogen, lactic acid and cholesterol contents in liver of the rat. *Biosci Biotechnol Biochem* (2003) 67: 1930-1936.
4. Zhu BW, Sun YM, Yun X, Han S, Piao ML, Murata Y and Tada M: Resistance imparted by traditional Chinese medicines to the acute change of glutamic pyruvic transaminase, alkaline phosphatase and creatine kinase activities in rat blood caused by noise. *Biosci Biotechnol Biochem* (2004) 68: 1160-1163.
5. Prabhakaran K, Suthanthirarajan N and Namasivayam A: Biochemical changes in acute noise stress in rats. *Indian J Physiol Pharmacol* (1988) 32: 100-104.
6. Dereköy FS, Köken T, Yılmaz D, Kahraman A and Altuntaş A: Effects of ascorbic acid on oxidative system and transient evoked otoacoustic emissions in rabbits exposed to noise. *Laryngoscope* (2004) 114: 1775-1779.
7. McFadden SL, Woo JM, Michalak N and Ding D: Dietary vitamin C supplementation reduces noise-induced hearing loss in guinea pigs. *Hear Res* (2005) 202: 200-208.
8. Hou F, Wang S, Zhai S, Hu Y, Yang W and He L: Effects of α -tocopherol on noise-induced hearing loss in guinea pigs. *Hear Res* (2003) 179: 1-8.
9. Scholik AR, Lee US, Chow CK and Yan HY: Dietary vitamin E protects the fathead minnow, *Pimephales promelas*, against noise exposure. *Comp Biochem Physiol C Toxicol Pharmacol* (2004) 137: 313-323.
10. Gok U, Halifeoglu I, Canatan H, Yildiz M, Gursu MF and Gur B: Comparative analysis of serum homocysteine, folic acid and Vitamin B₁₂ levels in patients with noise-induced hearing loss. *Auris Nasus Larynx* (2004) 31: 19-22.
11. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* (1976) 72: 248-254.
12. Van Campen LE, Murphy WJ, Franks JR, Mathias PI and Toraason MA: Oxidative DNA damage is associated with intense noise exposure in the rat. *Hear Res* (2002) 164: 29-38.
13. Xuejiang W, Ichikawa H and Konishi T: Antioxidant potential of Qizhu Tang, a Chinese herbal medicine, and the effect on cerebral oxidative damage after ischemia reperfusion in rats. *Biol Pharm*

- Bull (2001) 24: 558–563.
14. Ip SP, Che CT and Leung PS: Association of free radicals and the tissue renin-angiotensin system: prospective effects of Rhodiola, a genus of Chinese herb, on hypoxia-induced pancreatic injury. *JOP* (2001) 2: 16–25.
 15. Quaranta A, Scaringi A, Bartoli R, Margarito MA and Quaranta N: The effects of 'supra-physiological' vitamin B₁₂ administration on temporary threshold shift. *Int J Audiol* (2004) 43: 162–165.
 16. Cho Y, Gong TW, Kanicki A, Altchuler RA and Lomax MI: Noise overstimulation induces immediate early genes in the rat cochlea. *Brain Res Mol Brain Res* (2004) 130: 134–148.