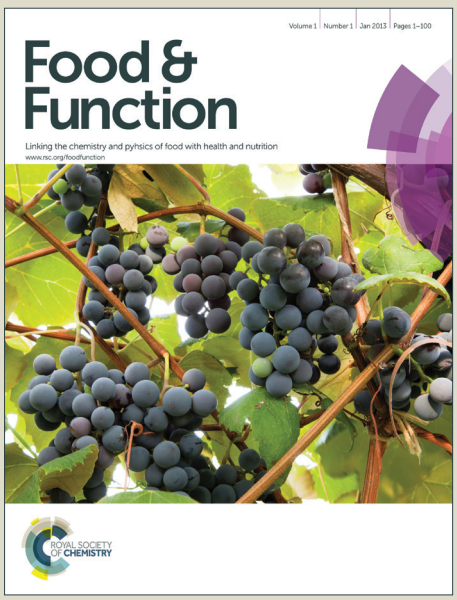
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Effect of taurine in chronic alcoholic patients

You-Liang Hsieh[1], Yen-Hung Yeh[2,3,*], Ya-Ting Lee[4], Chih-Yang Huang[1,5,6]

1. Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan, R.O.C.

2. School of Health Diet and Industry Management, Chung Shan Medical University, Taichung, Taiwan, R.O.C.

3. Department of Nutrition, Chung Shan Medical University Hospital, Taichung, Taiwan, R.O.C.

4. Department of Beauty Science, National Taichung University of Science and Technology, Taichung, Taiwan, R.O.C.

5. Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan, R.O.C.

6. Department of Chinese Medicine, China Medical University Hospital, Taichung, Taiwan, R.O.C.

Running Title: Taurine on alcoholic patients

Corresponding author:

* Corresponding author. Tel.: +886-4-24730022

E-mail address: yhyeh@csmu.edu.tw (Y. H. Yeh).

Abstract

A study was undertaken to investigate the dietary effect of taurine in chronic alcoholic patients. The 30 chronic alcoholic patients with 2 to 5 times over normal activities of aspartate transaminase (AST) or alanine transaminase (ALT) were selected and equally divided into taurine and control groups. In taurine group, each patient took 6 g taurine per day divided into 3 times for three months, and then stopped treatment for 1 month. In control group, patients took placebo without taurine for 4 months. It was found that AST and ALT activities and levels of cholesterol, triglyceride (TG), bilirubin, and thiobarbituric acid relative substances (TBARS) of serum plasma in the taurine group were all decreased, but increased alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities and serum vitamins concentrations. Except for level of TG, all of them showed significantly different after taking taurine for 2 or 3 months. It indicated that taurine plays an important role in the properties of antioxidation and has some improvements on the liver test of chronic alcoholic patients.

Keywords: Aspartate transaminase; Antioxidation; Alcoholic patient; Liver test; Taurine

1. Introduction

Alcoholic is a disease with powerful negative effects that impact not only the individual afflicted but also society at large. In the United States, alcoholic is the third leading cause of death and often leads to other sequela[1], such as cirrhosis, which are also leading causes of death[2]. Hepatic, pancreatic, and cardiovascular systems are the major targets of chronic alcohol abuse and alcoholic, alcoholic hepatitis is the single most common cause of mortality and morbidity from the liver diseases[3]. Chronic alcohol abuse is associated with wide-ranging neurological damage, such as Wernicke encephalopathy, dementia, delirium tremens and peripheral polyneuropathy. However, this area of inquiry will need to keep pace with the growing level of knowledge on how to best treat chronic alcoholic. The widespread effects of alcoholic necessitate its study with the goal of increasing treatment efficacy and thus reducing the cost to society.

In our previous papers, we found that dietary taurine could possess a protective liver test and detoxification action against oxidized oil and heavy metal induced injuries[4,5]. Taurine is a sulfur-containing amino acid that conjugates with bile acids in the liver[6] and an essential amino acid for cat[7]. It is well known that taurine is rich in fish products, especially in mollusks and fish liver[8]. Its physiological functions include bile acid conjugation, detoxification, osmoregulation, antioxidation, preventing lipid peroxidation, cell membrance stabilization, neuromodulation and calcium flux regulator[9,10]. Taurine is largely obtained from the diet, predominantly through eggs, meat, and seafood[11]. Additionally, taurine acts as an antioxidant and plays a role in detoxification, membrane stabilization a comma osmoregulation, neuromodulation, and brain and in retinal development. According to recent reports

by Yang et al.[12] and Chang et al.[13], taurine can accelerate cholesterol degradation to form bile acid thus increasing fecal bile acid excretion, and enhancing peroxisome proliferator-activated receptor-a (PPAR-α) expression which leads to an increase in hepatic lipid expenditure in high-fat/cholesterol dietary rodents. Chang et al.[13] also indicated that supplementing taurine can reduce serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) of high-fat/cholesterol dietary hamsters. At the same time, increased hepatic self-antioxidant capacities and decreased lipid peroxidation were also observed in high-fat/cholesterol dietary hamsters co-treated with taurine, taurine supplementation can lower liver triacylglycerol levels in alcohol fed rats via the downregulation of fatty acid synthase (FAS) and malic enzyme (ME) gene expressions and the upregulation of PPAR-α gene expression. Additionally, taurine supplementation also decreases TBARS values in sera and livers which result in lower hepatic TNF-α level and MMP-9 activity. Recently, Fang et al.[14] indicated that an acceleration of alcohol metabolism via upregulating alcohol dehydrogenase (ADH), catalase (CAT), and aldehyde dehydrogenase (ALDH) activities, less microvesicular steatosis, and necrotic cells in livers were observed when chronic alcohol-fed rats were supplemented with taurine. Hence, it prompted us to investigate the dietary effect of taurine on the chronic alcoholic patients. One of the promising research areas in biomedical science today is the focus on alcoholic and taurine. This article presents a brief overview of taurine, the results of taurine studies related to alcoholic. Therefore, it shows how nursing practice strategies can be derived from biomedical research related to taurine and alcoholic.

2. Materials and methods

103

104 *2.1. Experiment*

105 The 30 alcoholic patients with 2 to 5 times over normal activities of aspartate
106 transraminase (AST) or alanine transaminase (ALT) were selected and divided into
107 two groups, taurine group and control group. In taurine group, patients consisted of 8
108 men and 7 women and their average age and body weight were 59±13 years old and
109 62±11 kg, respectively. In control group, patients included each 9 men and 6 women
110 and their average age and body weight were 60±12 years old and 61±10 kg,
111 respectively. This study was carried out in accordance with the Helsinki Declaration
112 of the World Medical Association and was approved by the University's Ethics
113 Committee. Written informed consent was obtained from each subject after the study
114 was explained to them. All patients promised the treatment and then the experiment
115 was conducted out. In taurine group, each patient took 6 g taurine per day for three
116 months, and then stopped treatment for 1 month. The daily intake of 6 g taurine was
117 divided into three parts and given to each patient of taurine group after meal. In
118 control group, patients took placebo without taurine. Taurine was purchased from
119 Dokui Chemical Company (Taiwan), its purity was 99.5%. The blood samples of all
120 patients were collected per month and analyzed for blood characteristics, including
121 red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb) and platelet, by
122 using a Cell Hematology Analyzer (DYN 500, Sequoi-Turner, USA). The plasma
123 samples were collected by centrifugation (2,000 × *g*, 15 min) from blood and
124 examined for the levels of BUN, creatinine, bilirubin, cholesterol and TG and the
125 activities of AST and ALT in the plasma were assayed by a Vitalab Selectra (E. Merck,
126 Germany) with using an enzymatic kit. Serum concentrations of vitamin B_{12}, folate
127 and vitamin B_6 [15], were measured according to established published methods.

2.2. Determination of TBARS

Lipid peroxidation level was estimated by measuring thiobarbituric acid reactive substances (TBARS) according to Buege and Aust[16] and was expressed in terms of malondialdehyde (MDA) content which is the end product of lipid peroxidation. In brief, 125 µl of supernatants were homogenized by sonication with 50 µl of TBS, 125 µl of TCA-BHT in order to precipitate proteins and centrifuged (1,000 × *g*, 10 min, 4°C). 200 µl of obtained supernatant were mixed with 40 µl of HCl (0.6 M) and 160 µl of TBA dissolved in Tris and the mixture was heated at 80°C for 10 min. The absorbance of the resultant supernatant was read at 530 nm. The amount of TBARS was calculated by using an extinction coefficient of $156\times10^5 mM^{-1}\ cm^{-1}$.

2.3. Determination of total ADH activity

Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline (NDMA) as a substrate[17]. The reaction mixture (2 ml) contained 1.9 ml of a 26 µM solution of substrate in 0.1 M of sodium phosphate buffer, pH 8.5 and 0.1 ml of a mixture containing 0.25 M n-butanol and 5 mM NAD. The reduction of NDMA was monitored at 440 nm.

2.4. Determination of total ALDH activity

ALDH activity was measured using the fluorogenic method based on the oxidation of 6-methoxy-2-naphtaldehyde to the fluorescent 6-methoxy-2 naphtoate[18]. The reaction mixture contained 60 µl of substrate, 20 µl of 11.4 mM NAD and 2.8 ml of 50 mM of sodium phosphate buffer, pH 8.5. The mixture contained also 50 µl of a 12 mM solution of 4-methylpyrazole as a specific inhibitor of ADH activity. The

fluorescence was read at excitation wavelength 310 and emission wavelength 360 nm.

2.5. Statistical analysis

Statistical analysis for differences among patients in the experimental groups was performed by the 2-way analysis of variance procedure and Duncan's new multiple range tests. A P value < 0.05 was considered statistically significant.

3. Results

After treating with dietary taurine for 3 months, all indicators of blood characteristics in the alcoholic patients was not affected ($P > 0.05$). The data are similar to those in control group. The data of blood characteristics in normal people are as follows: 4.8-10.8 $\times 10^3$ cells/μl for WBC, 4.2-6.1 $\times 10^6$ cells/μl for RBC, 14-18 g/dl for Hb and 130-400 $\times 10^3$ cells/μl for platelet. All data of blood characteristics in chronic alcoholic patients in taurine and control groups were not different from each other. It indicates that the blood characteristics of alcoholic patients are the same as those normal people and dietary taurine supplement can not change them.

The dietary effect of taurine on AST, ALT, and bilirubin of chronic alcoholic patients is shown in Fig. 1. After treating with dietary taurine for 2 months, AST, ALT, and bilirubin of plasma in the chronic alcoholic patients was significantly decreased ($P < 0.05$). It was also found that the AST, ALT, and bilirubin of chronic alcoholic patients in control group are not changed during experimental period.

The dietary effect of taurine on BUN and creatinine of chronic alcoholic patients is shown in Fig. 2. After treating with dietary taurine for 3 months, There was no

177 significant difference (P>0.05) in the concentration on BUN and creatinine in the
178 plasma among various groups.

179 The dietary effect of taurine on cholesterol and TG level of chronic alcoholic
180 patients is shown in Fig. 3. After treating with dietary taurine for 3 months,
181 cholesterol and TG level of plasma in the chronic alcoholic patients was significantly
182 decreased ($P < 0.05$).

183 The dietary effect of taurine on ADH and ALDH activities of chronic alcoholic
184 patients is shown in Fig. 4. After treating with dietary taurine for 3 months, ADH and
185 ALDH activities of plasma in the chronic alcoholic patients was significantly
186 increased ($P < 0.05$). After stopping treatment at the fourth month, the ADH and
187 ALDH activity was also elevated. During the experimental period, the ADH and
188 ALDH activities of chronic alcoholic patients in control group was also not changed.

189 The dietary effect of taurine on TBARS and GSH level of chronic alcoholic
190 patients is shown in Fig. 5. After treating with dietary taurine for 2 months, TBARS
191 and GSH level of plasma in the chronic alcoholic patients was significantly decreased
192 ($P < 0.05$). It indicates that the peroxidation of blood plasma in alcoholic patients
193 could be availably ameliorated by dietary taurine.

194 The dietary effect of taurine on vitamin B_{12}, folate and vitamin B_6 of chronic
195 alcoholic patients is shown in Fig. 6. After treating with dietary taurine for 2 months,
196 vitamin B_{12}, folate and vitamin B_6 of plasma in the chronic alcoholic patients was
197 significantly increased ($P < 0.05$). Since stopping treatment at the fourth month,
198 vitamin B_{12}, folate and vitamin B_6 of plasma in the chronic alcoholic was not elevated.
199 However, the variation of vitamin B_{12}, folate and vitamin B_6 of plasma in the chronic

alcoholic patients without taurine supplement was not found during the experimental period. It indicates that the vitamins of blood plasma in alcoholic patients could be availably ameliorated by dietary taurine.

4. Discussion

In this study, the blood characteristics, activities of ALT, AST, ADH, and ALDH and levels of bilirubin, TG, cholesterol and TBARS in the plasma of chronic alcoholic patients without supplement of taurine during experimental period of 4 months were not changed. However the chronic alcoholic patients were treated with dietary taurine, the clinical symptoms including ALT, AST, ADH, and ALDH activities and bilirubin, TG, cholesterol and TBARS levels were significantly affected. ALT and AST activities in plasma serve as biomarkers for liver tests[19]. The value in normal human is as follows: 10-40 U/l for ALT and 5-45 U/l for AST. The ALT and AST activities in chronic alcoholic patients were higher than those of normal human. Among them, ALT activity was higher than AST activity in the chronic alcoholic patients. It means that alcoholic might injure liver test. Taurine significantly reduced the enzymatic activities of ALT and AST in the plasma of chronic alcoholic patients, indicating that the liver injury by alcoholic could be ameliorated by taurine. Wright et al.[20] pointed out that the function of taurine for preserving liver cells was presented by the high content of taurine in cell membrane.

Alcohol is mainly metabolized by ADH in cytosol, CYP2E1 inendoplasmic reticulum, and CAT in peroxisomes to form acetaldehyde, and further catabolized to acetic acid by ALDH[21]. However, during the alcohol metabolism by CYP2E1 the reactive oxygen species (ROS) also generates and increases the lipid peroxidation, i.e.

MDA in the liver[22-24]. However, the level of TBARS in the plasma is an additional indicator of liver injury. The level of TBARS in the plasma of alcoholic patients was significantly reduced when the patients were treated with the supplement of taurine. This result is the same as that reported previously[4,25]. Therefore, it is reasonable to assume that taurine may act as a good scavenger in reducing lipid peroxidation induced by drugs[26], heavy metals[5] and oxidized oil[4].

The levels of TG and cholesterol in the plasma of chronic alcoholic patients were not higher than those of normal human (120-200 mg/dl for cholesterol, 35-170 mg/dl for TG). However, the levels of TG and cholesterol in the plasma of alcoholic patients were significantly reduced when the patients were treated with the supplement of taurine. These results are the same as those reported previously[27-29]. The reduction of TG and cholesterol levels in the plasma may induce the decrease of lipid peroxidation, resulting in inhibiting production of TBARS. Judging from above data taurine plays an important role in the properties of antioxidation and has some improvements on the liver test of chronic alcoholic patients. Hence, taurine is a valuable dietary nutrient for chronic alcoholic patients.

The level of GSH in the liver of rats was reduced by chronic alcoholic patients, which was similar to that of other report[30,31]. In our previous study, the level of GSH in the liver of rats was raised significantly when the rats were fed with the supplement of taurine[4].

Alcohol has been known for a long time to interfere with the absorption of several nutrients, including vitamins, and to lead to mucosal damage of the upper small intestine, thereby contributing to the qualitative and quantitative malnutrition frequently observed in alcoholics[32,33]. Therefore, taurine is a safe dietary nutrient and an effective hepatoprotective agent to benefit chronic alcoholic patients.

Acknowledgement

This study was supported by the Asia University (100-A-06) and Chung Shan Medical University (CSMU-INT-102-12).

References

1. U.S. Department of Health and Human Services, National Institute on Alcohol Abuse and Alcoholism. 10th Special Report to the U.S. Congress on Alcohol and Health: Highlights from Current Research from the Secretary of Health and Human Services. 2000.
2. Carson VB, Arnold EN. The journey anesthetized by substance use. Mental health nursing: The nurse patient journey. Philadelphia: Saunders. 1996.
3. Lankisch PG, Banks PA. Pancreatitis. Berlin/Heidelberg/New York: Springer-Verlag. 1998.
4. Yeh YH, Chen MH, Lee YT, Hsieh HS, Hwang DF. Effect of taurine on toxicity of oxidized cholesterol and oxidized fish oil in rats. *J Food Drug Anal* 2008; 16: 76-85.
5. Yeh YH, Lee YT, Hsieh YL, Hwang DF. Dietary taurine reduces zinc induced toxicity in male Wistar rats. *J Food Sci* 2011; 76: 90-98.
6. Jacobsen JG, Smith LHJ. Biochemistry and physiology of taurine and taurine derivatives. Physiol Rev 1968; 48: 425-511.
7. Knopf K, Sturman JA, Armstrong M, Hayes KC. Taurine an essential nutrient for the cat. *J Nutr* 1978; 108: 773-778.

8. Sakaguchi M, Urata M. Seasonal variations of free amino acids in oyster whole body and adductor muscle. *Nippon Suisan Gakkaishi* 1989; 55: 2037-2041.

9. Balkan J, Kanbagli O, Aykac-Toker G, Uysal B. Taurine treatment reduces hepatic lipids and oxidative stress in chronically ethanol-treated rats. *Biol Pharm Bull* 2002; 25: 1231-1233.

10. Nandhini AT, Balakrishnan SD, Anuradha CV. Response of liver antioxidant system to taurine in rats fed high fructose diet. *Indian J Exp Biol* 2002; 40: 1016-1019.

11. Kadam SU, Prabhasankar P. Marine foods as functional ingredients in bakery and pasta products. *Food Res Int* 2010; 43: 1975-1980.

12. Yang SF, Tzang BS, Yang KT, Hsiao YC, Chang YY, Chan CH, Fu SG, Chen YC. Taurine alleviates dyslipidemia in hamsters fed a high-fat/cholesterol diet. *Food Chem* 2010; 120: 156-162.

13. Chang YY, Chou CH, Chiu CH, Yang KT, Lin YL, Weng WL, Chen YC. Preventive effects of taurine on development of hepatic steatosis induced by a high-fat/cholesterol-dietary habit. *J Agric Food Chem* 2011; 59: 450-457.

14. Fang YJ, Chiu CH, Chang YY, Chou CH, Lin HW, Chen MF, Chen YC. Taurine ameliorates alcoholic steatohepatitis via enhancing self-antioxidant capacity and alcohol metabolism. *Food Res Int* 2011; 44: 3105-3110.

15. Boers GHJ, Smals AGH, Trijbels FJM. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *N Engl J. Med* 1985; 313: 709-715.

16. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1984; 105: 302-310.

17. Skursky L, Kovar J, Stachova M. A sensitive assay for alcohol dehydrogenase

activity in blood serum. *Anal Biochem* 1979; 89: 65-71.

18. Jelski W, Zalewski B, Chrostek L, Szmitkowski M. The activity of class I, II, III and IV of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) in the colorectal cancer. *Dig Dis Sci* 2004; 49: 977-981.

19. Ronald L, Koretz MD. Chronic hepatitis: Science and superstition. In: Gitnick, G. (Ed.), Current Hepatology. Chicago: Mosby-Year. 1992.

20. Wright CR, Tallan HH, Lin YY. Taurine: Biological update. *Ann Rev Biochem* 1986; 55: 427-453.

21. Lieber CS. Biochemical factors in alcoholic liver disease. *Semin. Liver Dis* 1993; 13: 136-153.

22. Gill K, Amit Z, Smith BR. The regulation of alcohol consumption in rats: The role of alcohol-metabolising enzymes-Catalase and aldehyde dehydrogenase. *Alcohol* 1996; 13: 347-353.

23. Pari L, Suresh A. Effect of grape (*Vitis vinifera* L.) leaf extract on alcohol induced oxidative stress in rats. *Food Chem Toxicol* 2008; 46: 1627-1634.

24. Song Z, Zhou Z, Chen T, Hill D, Kang J, Barve S, McClain C. S-adenosylmethionine (SAMe) protects against acute alcohol induced hepatotoxicity in mice. *J Nutr Biochem* 2003; 14: 591-594.

25. Tadolini B, Gianfrance P, Gavino GP, Federico B, Flavia F. Effect of taurine and hypotaurine on lipid peroxidation. *Biochem Biophys Res Commun* 1995; 213: 820-826.

26. Waters E, Wag JH, Redmond HP, Wu QD, Kay E, Bouchier HD. Role of taurine in preventing acetaminophen-induced hepatic injury in the rat. Gastrointest. *Liver Physiol* 2001; 280: 1274-1279.

27. Huang CJ, Chuan NN, Sheu CT. The effects of taurine supplementation on plasma

and liver cholesterol level of rats fed diets containing high cholesterol food. *J Chin Nutr Soc* 1988; 13: 11-22.

28. Mizushima S, Nara Y, Sawamura M, Yamori Y. Effects of oral taurine supplementation on lipids and sympathetic nerve tone. *Adv Exp Med Biol* 1996; 403: 615-622.

29. Dawson R, Liu S, Eppler B, Patterson T. Effects of dietary taurine supplementation or deprivation in aged male Fishcher 344 rats. *Mech. Ageing Dev* 1999; 17: 73-91.

30. Azzalis LA, Junqueira VBC, Simon K, Giavarotti L, Silva MA, Kogare M, Simizu K, Barros SBM, Fraga C, Porta EA. Prooxidant and antioxidant hepatic factors in rats chronically fed an ethanol regimen and treated with an acute dose of lindane. *Free Radical Biol Med* 1995; 19: 147-159.

31. Bosch-Morell F, Colell A, Fernández-Checa JC, Marín M, Romero FJ. Chronic alcohol treatment promotes oxidative stress in rat peripheral nerve. Beneficial effects of antioxidants. *Pathophysiology* 1998; 5: 82.

32. Bode JC, Bode C. Alcohol malnutrition and the gastrointestinal tract. In Watson RR & Watzl B (eds) Nutrition and Alcohol. Boca Raton, FL: CRC Press. 1990.

33. Mezey E. Effect of ethanol on intestinal morphology, metabolism, and function. In Seitz HK & Kommerell B (eds) *Alcohol Related Diseases in Gastroentreology*. Berlin: Springer.1985.

Legends for Figures:

Fig. 1. Effect of taurine on the AST, ALT, and bilirubin of serum plasma in chronic alcoholic patients after 4 months. Data respresent significantly different when the letters are different (p<0.05). Taurine group: patients took 6 g taurine per day for three months. Control group: patients took placebo without taurine. a-b: values in the same week with different superscript are significantly different at P<0.05 by ANOVA (n=30).

Fig. 2. Effect of taurine on the BUN and creatinine of serum plasma in chronic alcoholic patents after 4 months. Taurine group: patients took 6 g taurine per day for three months. Control group: patients took placebo without taurine. a: values in the same week with different superscript are significantly different at P>0.05 by ANOVA (n=30).

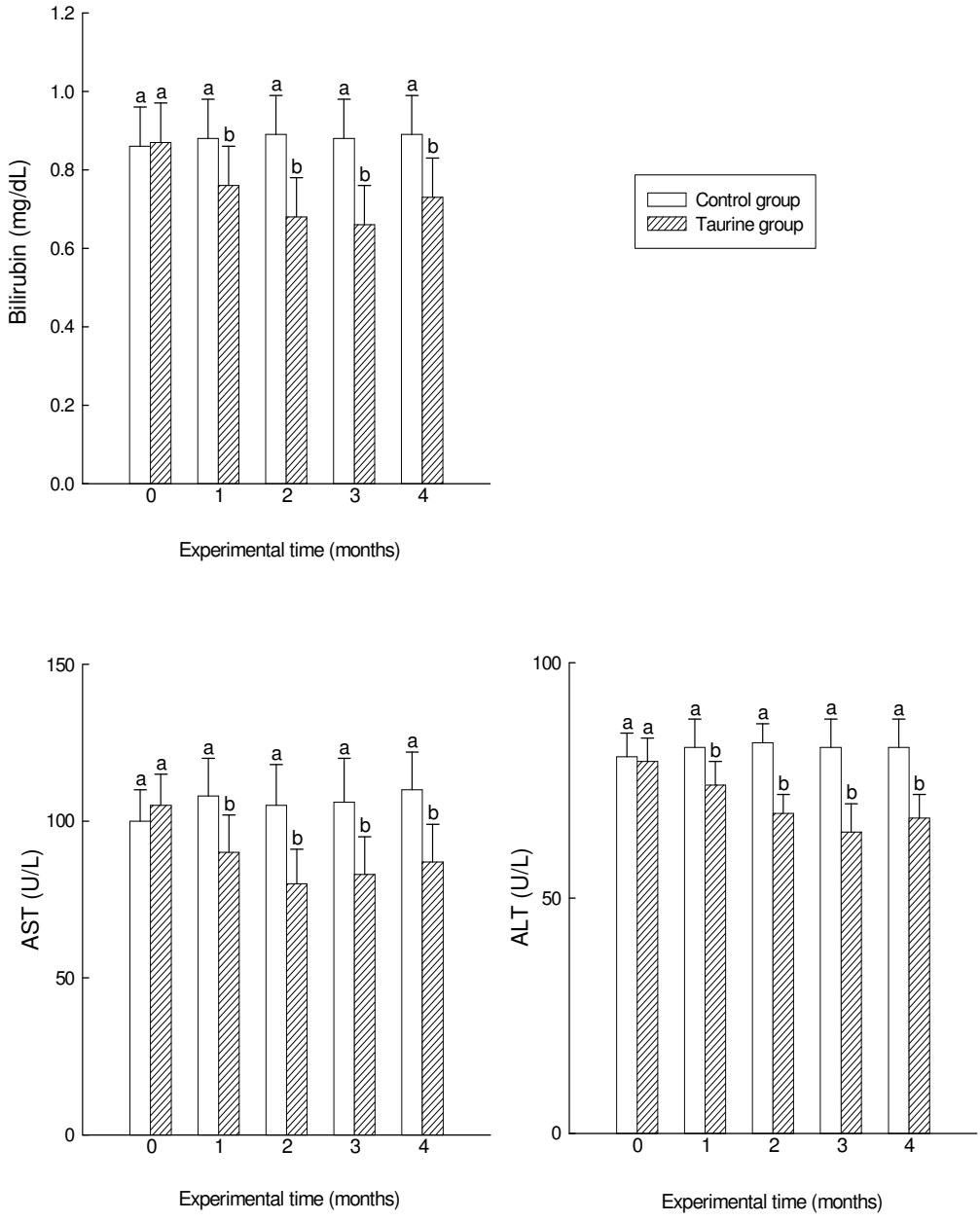
Fig. 3. Effect of taruine on the cholesterol and triglyceride of serum plasma in chronic alcoholc patients after 4 months. Taurine group: patients took 6 g taurine per day for three months. Control group: patients took placebo without taurine. a-b: values in the same week with different superscript are significantly different at P<0.05 by ANOVA (n=30).

Fig. 4. Effect of taruine on the ADH and ALDH of serum plasma in chronic alcoholic patients after 4 months. Taurine group: patients took 6 g taurine per day for three months. Control group: patients took placebo without taurine. a-b: values in the same week with different superscript are significantly different at P<0.05 by ANOVA (n=30).

Fig. 5. Effect of taurine on the TBARS and GSH of serum plasma in chronic alcoholic patients after 4 months. Taurine group: patients took 6 g taurine per day for three months. Control group: patients took placebo without taurine. a-b: values in the same week with different superscript are significantly different at P<0.05 by ANOVA (n=30).

Fig. 6. Effect of taurine on the vitamin B_{12}, vitamin B_6, and folate of serum plasma in chronic alcoholic patients after 4 months. Taurine group: patients took 6 g

taurine per day for three months. Control group: patients took placebo without taurine. a-b: values in the same week with different superscript are significantly different at $P<0.05$ by ANOVA (n=30).

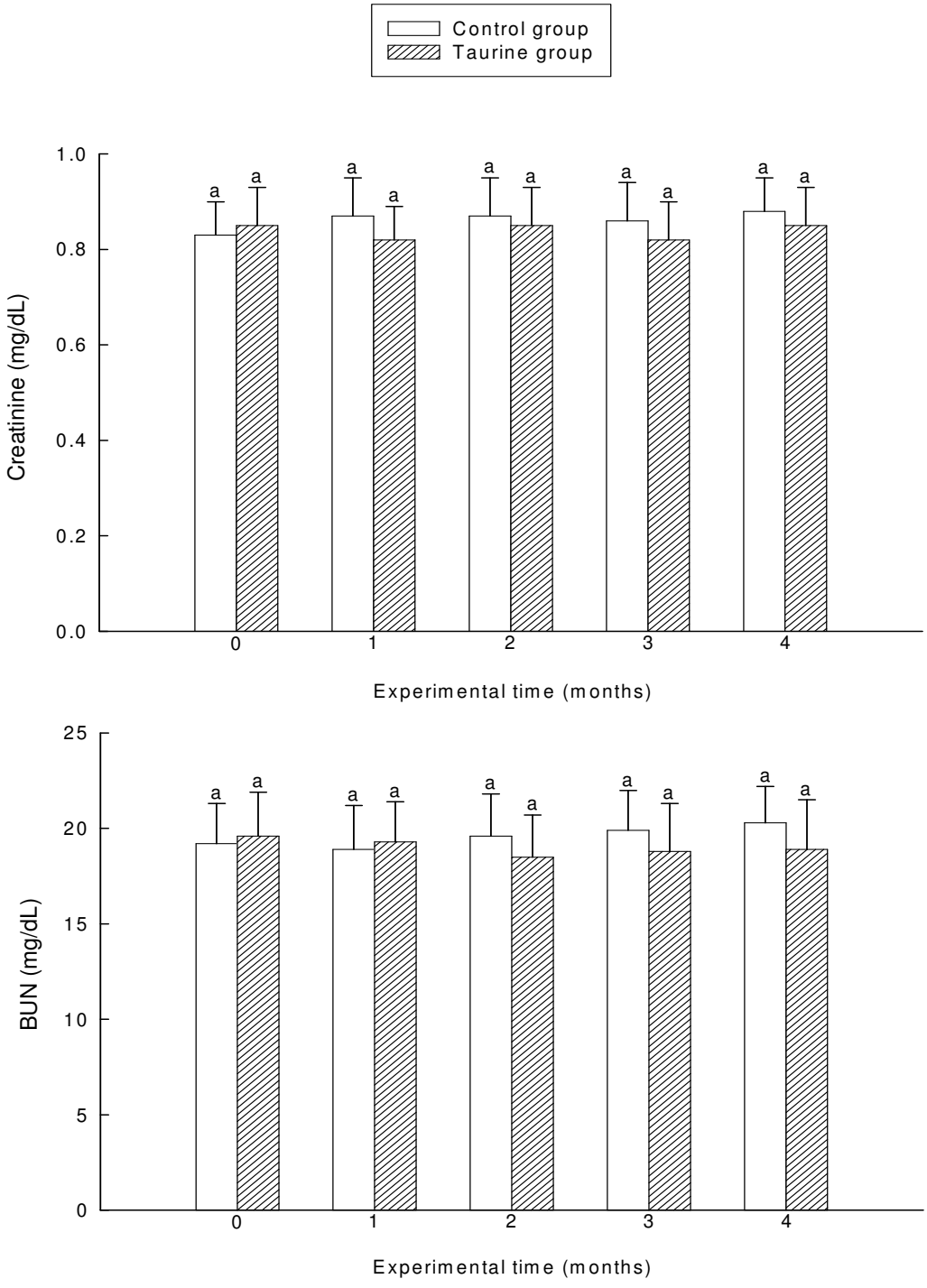


Fig, 1. Yeh et al.

388

389

390



391 Fig, 2. Yeh et al.

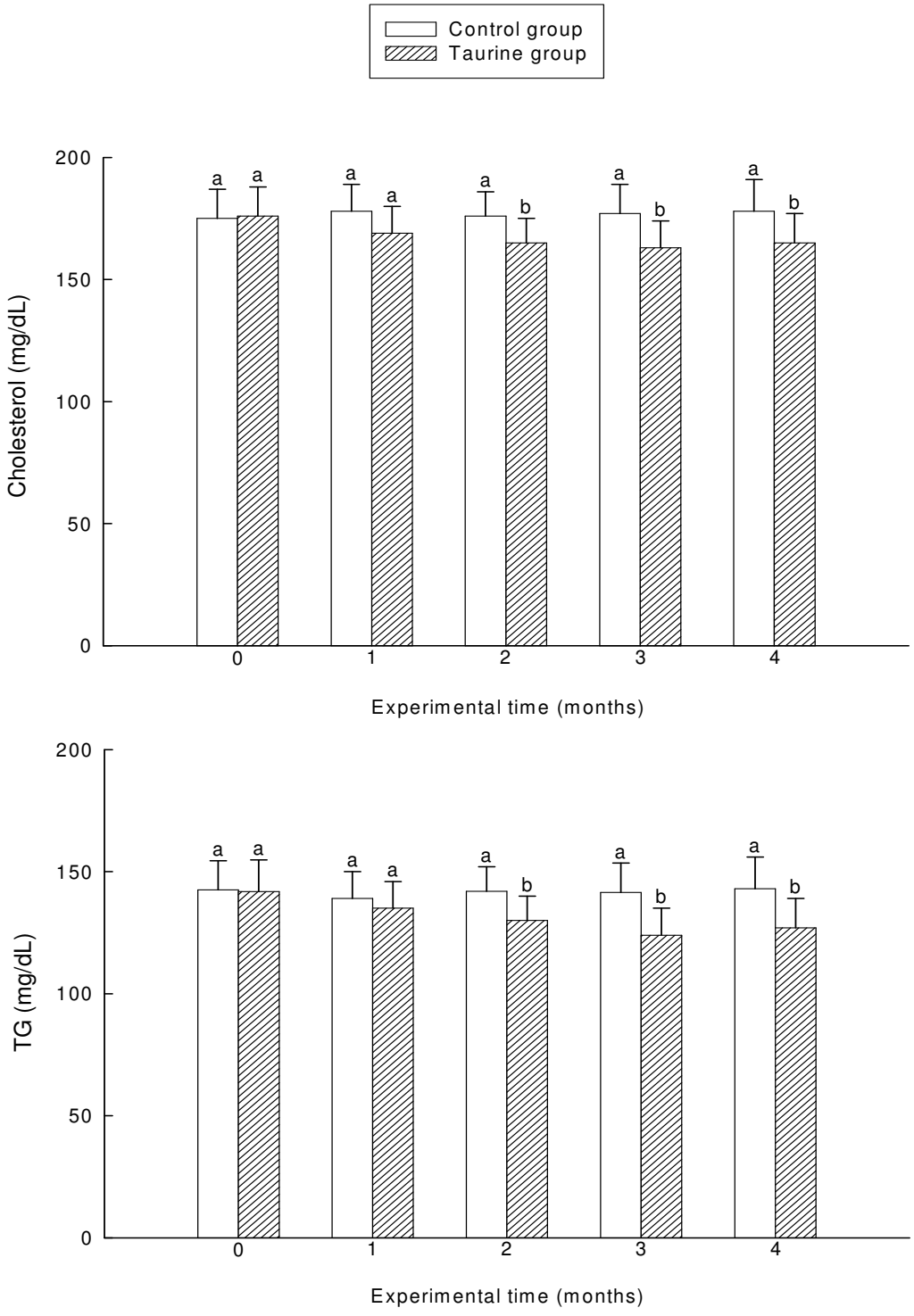
392

393

394

395

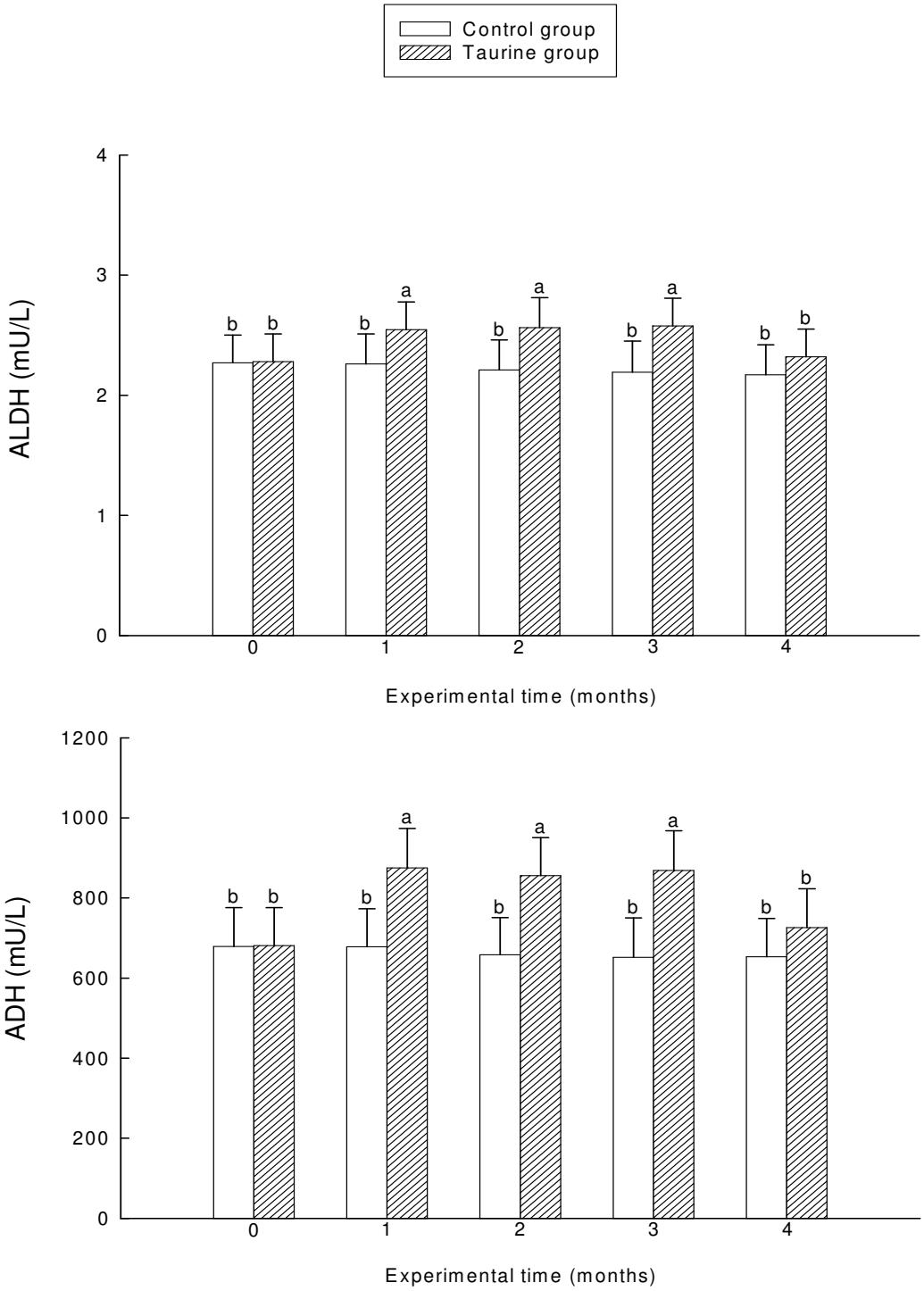
396



397

Fig, 3. Yeh et al.

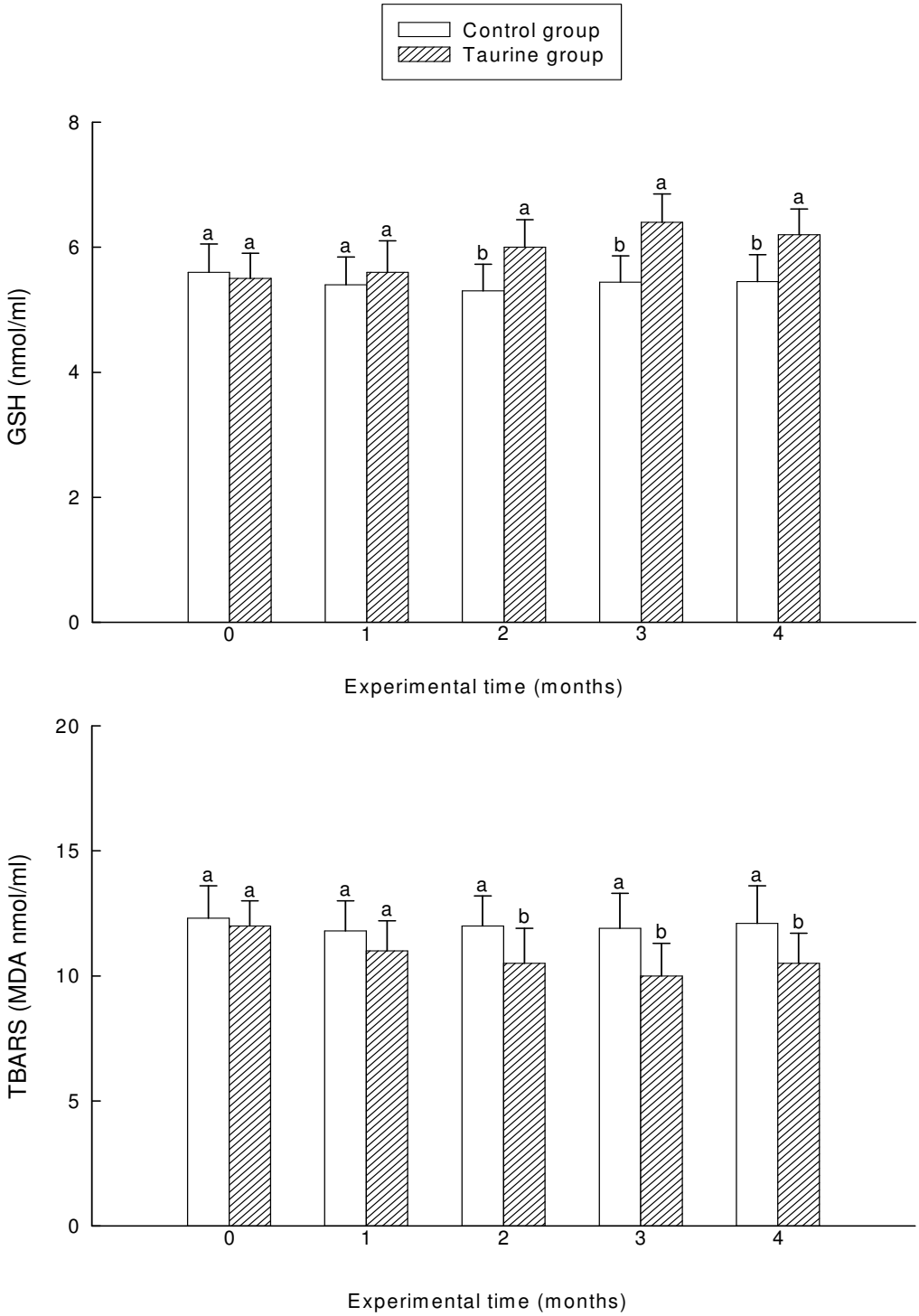
398
399
400



401
402

Fig, 4. Yeh et al.

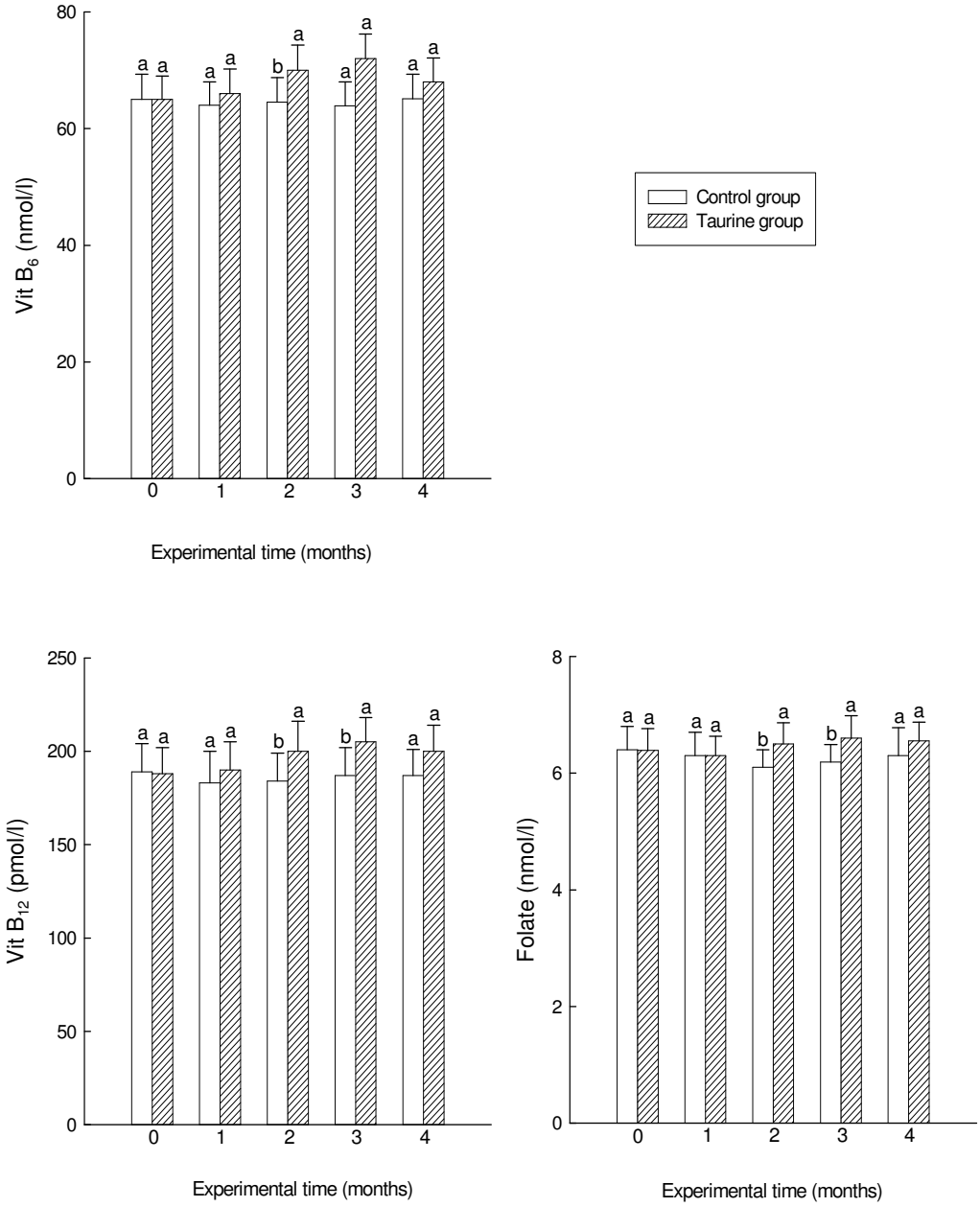
403
404
405



406 Fig, 5. Yeh et al.
407

408

409



Fig, 6. Yeh et al.

410

411

412

413

414