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Effect of Taurine on Toxicity of Oxidized Cholesterol and Oxidized Fish Oil in Rats

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ABSTRACT

Taurine (2-aminoethanesulfonic acid) occurs naturally in food, especially in seafood and meat. The aim of our investigation was to evaluate the effect of dietary taurine on oxidized cholesterol and oxidized fish oil induced toxicity in male Wistar rats. Thirty male wistar rats were fed with diets supplemented with 5% taurine, 2% oxidized cholesterol or 3% oxidized fish oil for 6 weeks. After feeding such diet, taurine could increase the high density lipoprotein-cholesterol (HDL-C) level in plasma, and glutathione (GSH) level in plasma, and decrease the activities of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) in plasma, and the levels of low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), triglyceride, total cholesterol in plasma, and relative ratios of liver weight to body weight and thiobarbituric acid-reactive substances (TBARS) level in the rat liver caused by oxidized cholesterol and oxidized fish oil. It could reduce the biochemical parameters characteristic in the plasma and rat liver caused by oxidized cholesterol and oxidized fish oil. It was also found that taurine possessed a good recovering effect and a short-term preventing effect from the toxicity of oxidized cholesterol and oxidized fish oil in rats. The results suggest that taurine may play an important role in suppressing effect by oxidized cholesterol and oxidized fish oil induced toxicity in rats.

Key words: taurine, oxidized cholesterol, hepatotoxicity, oxidized fish oil, rat

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is a sulfur-containing amino acid present in high concentrations in mammalian plasma and cells such as heart, retina, skeletal muscle, brain, and leukocytes⁽¹⁾. Taurine is a protective and restorative molecule for the cardiovascular system by alleviating dyslipidemic and atherosclerotic states. It also plays an important role in several essential biological processes such as development of the central nervous system and the retina, calcium modulation, membrane stabilization, reproduction, and immunity⁽²⁻⁵⁾.

Oxidized cholesterols as well as fatty acid oxidation in food system have been a concern for human diseases. A variety of oxidized cholesterols have been detected in processed foods such as meat products⁽⁶⁾, commercial sweet baked foods⁽⁷⁾ and sea foods⁽⁸⁾. Therefore,

consumption of oxidized cholesterols is inevitable in our usual diet. The formation of oxidized cholesterols was accelerated by PUFAs present in lipids⁽⁹⁾. Oxidized cholesterols have been known to be more injurious to arterial cells than pure cholesterol and are more directly connected to the development of atherosclerosis, coronary heart disease and possibly carcinogenic effects⁽¹⁰⁻¹²⁾.

It is well known that n-3 PUFAs and taurine are rich in marine fish^(13,18). We have found that PUFAs inhibited the acute induction of hypertriglyceridemia and liver enlargement by a single mega dose of retinyl palmitate in rats⁽¹⁴⁾. On the other hand, these PUFAs are easily oxidized when fish livers are cooked in the air. The oxidized fish oil is a hazardous substance to human and animals⁽¹⁵⁻¹⁷⁾.

Recently, we reported that taurine played an important role in reducing the toxic effect of oxidized fish oil in rats⁽¹⁸⁾. Marine foods usually contain cholesterol and PUFAs and produce taurine. When people consume these kinds of marine foods, the oxidized cholesterol and

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oxidized fish oil were not usually prevented to diets, the oxidized cholesterol and oxidized fish oil could cause harm for human health. In addition, taurine plays an important role in lipid metabolism to produce the bile acid conjugates in the liver; it can protect the liver against lipid peroxide. Therefore, the aim of the present study was to evaluate the effect of taurine on oxidized cholesterol and oxidized fish oil induced toxicity in male Wistar rats.

MATERIALS AND METHODS

I. Reagents

Taurine was purchased from Dokui Chemical Company (Taiwan), purity of 99.5% to add of 5% in the feed.

II. Preparation of Oxidized Fish Oil

Fish oil was obtained from Kozein Company (Taiwan), 1 g fish oil contents of 30% (EPA = 180 mg; DHA = 120 mg; vitamin E = 1 mg). Fish oil was heated at $60 \pm 2^\circ\text{C}$ for 12 hr in a water-bath with air pumping into it to produce oxidized fish oil⁽¹⁹⁾. The oxidized fish oil was characterized as follows: POV 302.8 meq/kg oil, AV 9.13 mg/g oil and TBA 11.63 mg/kg oil. The fresh fish oil was characterized as follows: POV 14.8 mg/kg oil, AV 0.35 mg/g oil and TBA 0.24 mg/kg oil.

III. Preparation of Oxidized Cholesterol

Cholesterol (99.9% purity, Wako Pure Chemical, Osaka, Japan) was heated at 150°C for 12 hr to produce oxidized cholesterol⁽²⁰⁾. The oxidized cholesterol was characterized as follows: POV 50.2 meq/kg oil, AV 1.74 mg/g oil, and TBA 1.58 mg/kg oil.

IV. Animals

Male weanling Wistar rats (weight 220 ± 20 g) were purchased from the National Laboratory Animal Center. The rats were kept in an air-conditioned room ($23 \pm 1^\circ\text{C}$, 50-60% humidity) lit for 12 hr/day (7 AM to 7 PM). Experimental protocol was approved by the Institutional Animal Care and Use Committee of Toko University. After acclimatizing for 2 wk with a commercial non-purified diet (rodent Laboratory Chow 5001, Purina Co., USA), rats were randomly assigned to one of the following five groups ($n = 6$ per group). A basal diet (no added cholesterol, oxidized cholesterol, fish oil, oxidized fish oil, or taurine with a formulation based on AIN)⁽²¹⁾. Group A = received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B = oxidized fish oil + cholesterol diet (3% oxidized fish oil + 2% cholesterol), Group C = oxidized fish oil + cholesterol + taurine diet (3% oxidized fish oil + 2% cholesterol + 5% taurine), Group

D = oxidized fish oil + oxidized cholesterol diet (3% oxidized fish oil + 2% oxidized cholesterol), Group E = oxidized fish oil + oxidized cholesterol + taurine diet (3% oxidized fish oil + 2% oxidized cholesterol + 5% taurine). The composition of the basal and all treatment diets is listed in Table 1. The concentration of taurine in fish oil was 5%⁽²²⁾, therefore 5% taurine was added to the treatment diets.

Rats were fasted for 10 hr before the experiment in the 2, 4 and 6 weeks, respectively. On weeks 2 and 4 blood was obtained by tail vein puncture and the rats were weighed and euthanized (with diethyl ether) on the 6th week. The blood samples which obtained by heart puncture with syringes were subjected to RBC count, Hct, WBC count and Hgb by using a Cell Hematology Analyzer (DYN 500, Sequoi-Turner, USA). Plasma was obtained by centrifugation ($1,000 \times g$ for 15 min) and subjected to AST, ALT and ALP activities. Creatinine, blood urea BUN, HDL-C, cholesterol, triglyceride and LDL-C+VLDL-C levels in the plasma were determined by enzymatic assay using commercially available kits (Merck, Germany).

V. Assays of Enzymatic Activities

The plasma was determined for AST, ALT and ALP activities by using enzymatic kit with Selectra Analyser (Merck Co. Ltd, Germany).

VI. TBARS Production

Lipid peroxidation activities in the liver were assayed by measurement of MDA, an end-product of peroxidized fatty acids, and TBA reaction product. The sample of 20% liver homogenate was mixed with 1.0 mL of 0.4% TBA in 0.2 N HCl and 0.15 mL of 0.2% BHT in 95% ethanol. The samples were incubated in a 90°C water-bath for 45 min. After incubation, the TBAMDA adduct was extracted with isobutanol. The isobutanol extract was mixed with methanol (2:1) prior to injection into the system of HPLC. The supernatant was examined by using the HPLC system at an excitation 515 nm and an emission 550 nm on a Hitachi Fluorescence Detector (Japan)⁽²³⁾.

VII. GSH Measurement

GSH reacts non-enzymatically with DTNB to yield GSSG and TNB. GSSG is then reduced enzymatically by NADPH and GR to regenerate GSH. Concentrations of DTNB, NADPH and GR are chosen such that the rate of the overall reaction is linearly proportional to the concentration of total GSH. The rate of formation of TNB is followed spectrophotometrically, and assay is calibrated using standards. GSH is derivatized if the sample is reacted with 2-vinylpyridine. Only GSSG is detected during subsequent assay⁽²⁴⁾.

Table 1. Composition of the experimental diet for animal test of taurine, oxidized fish oil and oxidized cholesterol

Ingredient (%)	Diets ^a				
	Control	Oxidized fish oil +Cholesterol	Oxidized fish oil +Cholesterol +Taurine	Oxidized fish oil +Oxidized cholesterol	Oxidized fish oil +Oxidized cholesterol +Taurine
Casein	20	20	20	20	20
Methionine	0.3	0.3	0.3	0.3	0.3
Cellulose	10	10	10	10	10
Corn oil	3	0	0	0	0
Oxidized fish oil	0	3	3	3	3
Cholesterol	2	2	2	0	0
Oxidized cholesterol	0	0	0	2	2
Choline	0.2	0.2	0.2	0.2	0.2
AIN mineral	3.5	3.5	3.5	3.5	3.5
AIN vitamin	1	1	1	1	1
Taurine	0	0	5	0	5
Corn starch	25	25	20	25	20
Surcose	35	35	35	35	35

^aOxidized fish oil + cholesterol: 3% oxidized fish oil and 2% cholesterol in diet; Oxidized fish oil + cholesterol + taurine: 3% oxidized fish oil and 2% cholesterol and 5% taurine in diet; Oxidized fish oil + oxidized cholesterol: 3% oxidized fish oil and 2% oxidized cholesterol in diet; Oxidized fish oil + oxidized cholesterol + taurine: 3% oxidized fish oil and 2% oxidized cholesterol and 5% taurine in diet.

VIII. Histopathology

Liver samples were fixed in 10% formalin phosphate-buffer, dehydrated, paraffin-embedded, and archived. Sections of 2-4 μ M of all zones of hepatic lobule and median part of kidney were sagittally cut and mounted on aminopropyltriethoxysilane-coated slides (APTS, A-3648, Sigma). Following deparaffinization in xylene, sections were rehydrated, stained with hematoxylin and eosin (H&E) and examine for light microscopy⁽²⁵⁾.

IX. Statistical Analysis

Statistical analysis for differences among rats in the experimental groups was performed by the 2-way analysis of variance procedure and Duncan's new multiple range tests⁽²⁶⁾. A *P* value < 0.05 was considered statistically significant.

RESULTS

In clinical plasma examination, the indicators concerning blood characters, liver function and kidney function have been determined in the toxicity. Activities of AST, ALT and ALP in plasma are generally tested as indicators for liver functions, and the levels of creatinine,

plasma urea nitrogen are tested as indicators for kidney functions⁽²⁷⁻²⁹⁾.

The effects of taurine, oxidized cholesterol and oxidized fish oil in rats after 6 weeks of feeding, and the effect of RBC, Hgb and Hct on the blood of rats are shown in Figure 1. The RBC, Hgb and Hct in the 6 weeks of rats fed, group B, group C, group D and group E were significantly higher than of basal diet (group A) (*P* < 0.05), but WBC were not significantly different on the blood (*P* > 0.05) (data not shown).

The effects of taurine, oxidized cholesterol and oxidized fish oil on the relative of liver weight to the body weight in rats fed diet after 6 weeks are shown in Figure 2. After 6 weeks of treatment, group B and D were significantly higher than group A, C, and E on the relative liver weight, but five groups were not significantly different on the relative kidney weight (*P* > 0.05) (data not shown).

The activities of taurine, oxidized cholesterol and oxidized fish oil on AST, ALT, ALP of rats during 6 weeks of treatment are shown in Figure 3. The activity of AST in the 4th week of treatment Group B, C, D and E were significantly higher than basal diet (*P* < 0.05), but in the 6th week of treatment Group C and E were not significantly different than basal diet (*P* > 0.05). In terms of the ALT activity in the first 2 weeks, Group B was significantly higher than the other groups (*P* < 0.05), but

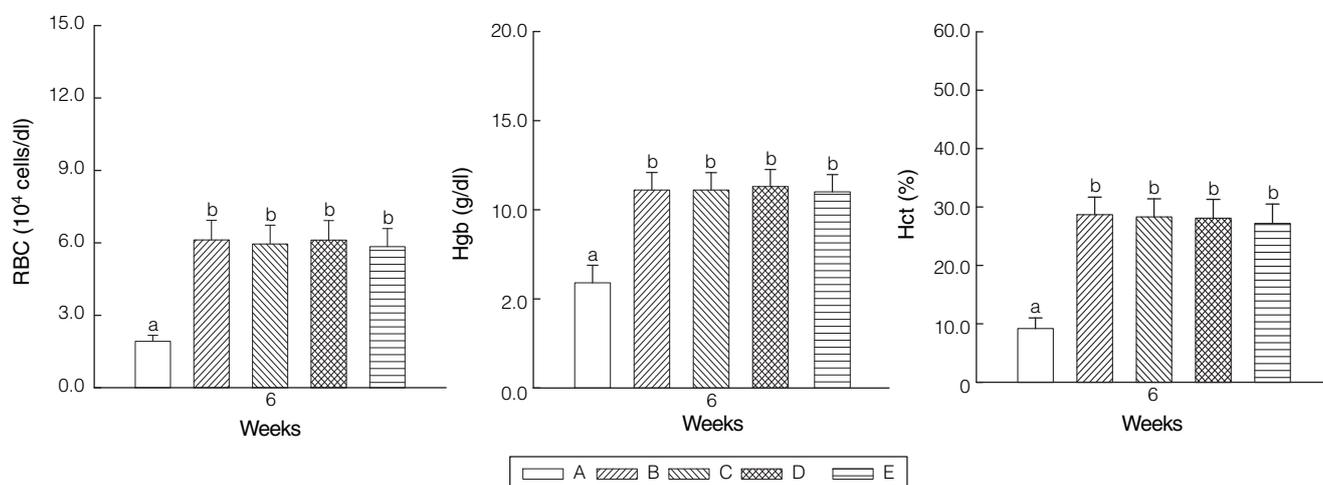


Figure 1. Effects of taurine, oxidized fish oil and oxidized cholesterol on the RBC, Hgb and Hct of rats fed diet after 6 groups. a-b: values in the same week with different superscript are significantly different ($P < 0.05$). Group A = received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B = oxidized fish oil + cholesterol diet (3% oxidized fish oil + 2% cholesterol), Group C = oxidized fish oil + cholesterol + taurine diet (3% oxidized fish oil + 2% cholesterol + 5% taurine), Group D = oxidized fish oil + oxidized cholesterol diet (3% oxidized fish oil + 2% oxidized cholesterol), Group E = oxidized fish oil + oxidized cholesterol + taurine diet (3% oxidized fish oil + 2% oxidized cholesterol + 5% taurine).

in the first 4 and 6 weeks, Group B and D were significantly higher than Group C, E and A. As for the order of ALP activity in the first 4 and 6 weeks, Group D was significantly higher than Group B, E, C and A ($P < 0.05$). The effects of taurine, oxidized cholesterol and oxidized fish oil on the concentration of GSH in the plasma of rats are shown in Figure 3. After 6 weeks of treatment, Group A, C and E were significantly higher than Group B and D ($P < 0.05$). The effects of BUN and creatinine in the plasma by rats fed diet for 6 weeks are shown in Figure 3. Throughout the 6-week treatment, the BUN and creatinine in the 2, 4 and 6 weeks were not significantly different ($P > 0.05$).

The effects of the triglyceride in the plasma by rats fed diet for 6 weeks are shown in Figure 4. Group B and D had significantly higher triglyceride than Group A, C and E in the 2, 4 and 6-week periods ($P < 0.05$).

The effects of the total cholesterol, HDL-C and LDL-C+VLDL-C in the plasma by rats fed diet for 6 weeks are shown in Figure 4. In the 2nd, 4th and 6th week, Group D had the highest total cholesterol effect than group B, E, C and A ($P < 0.05$). After 6 weeks of treatment, Group A was significantly higher than the other groups on the HDL-C values ($P < 0.05$). On the total LDL-C +VLDL-C, Group D and B were significantly higher than Group A, C and E ($P < 0.05$).

The effects of TBARS in the liver of rats fed diet after 6 weeks are shown in Figure 5. The level of TBARS in the kidney is not significantly different. In terms of the TBARS level in the liver in the 6th week, Group D was significantly higher than the other groups ($P < 0.05$).

We have not observed the synergetic toxicity of oxidized fish oil and oxidized cholesterol in rats, suggest-

ing that individual component has maximal toxic effects in rats.

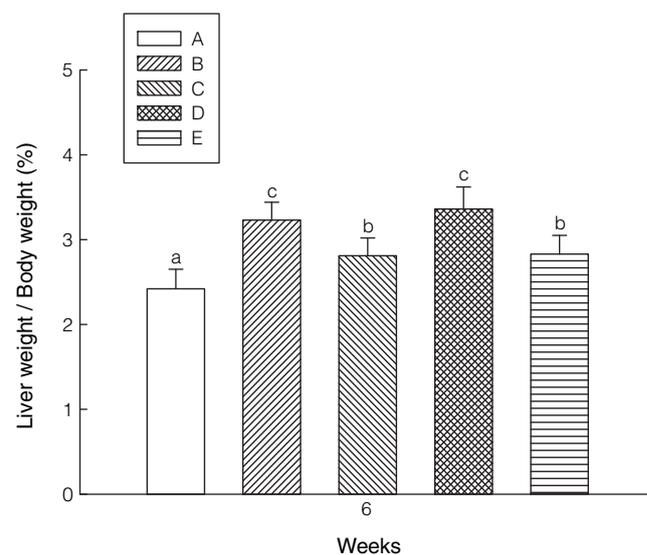


Figure 2. Effects of taurine, oxidized fish oil and oxidized cholesterol on relative liver weight in rats fed diet after 6 groups. a-c: values in the same week with different superscript are significantly different ($P < 0.05$). Group A = received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B = oxidized fish oil + cholesterol diet (3% oxidized fish oil + 2% cholesterol), Group C = oxidized fish oil + cholesterol + taurine diet (3% oxidized fish oil + 2% cholesterol + 5% taurine), Group D = oxidized fish oil + oxidized cholesterol diet (3% oxidized fish oil + 2% oxidized cholesterol), Group E = oxidized fish oil + oxidized cholesterol + taurine diet (3% oxidized fish oil + 2% oxidized cholesterol + 5% taurine).

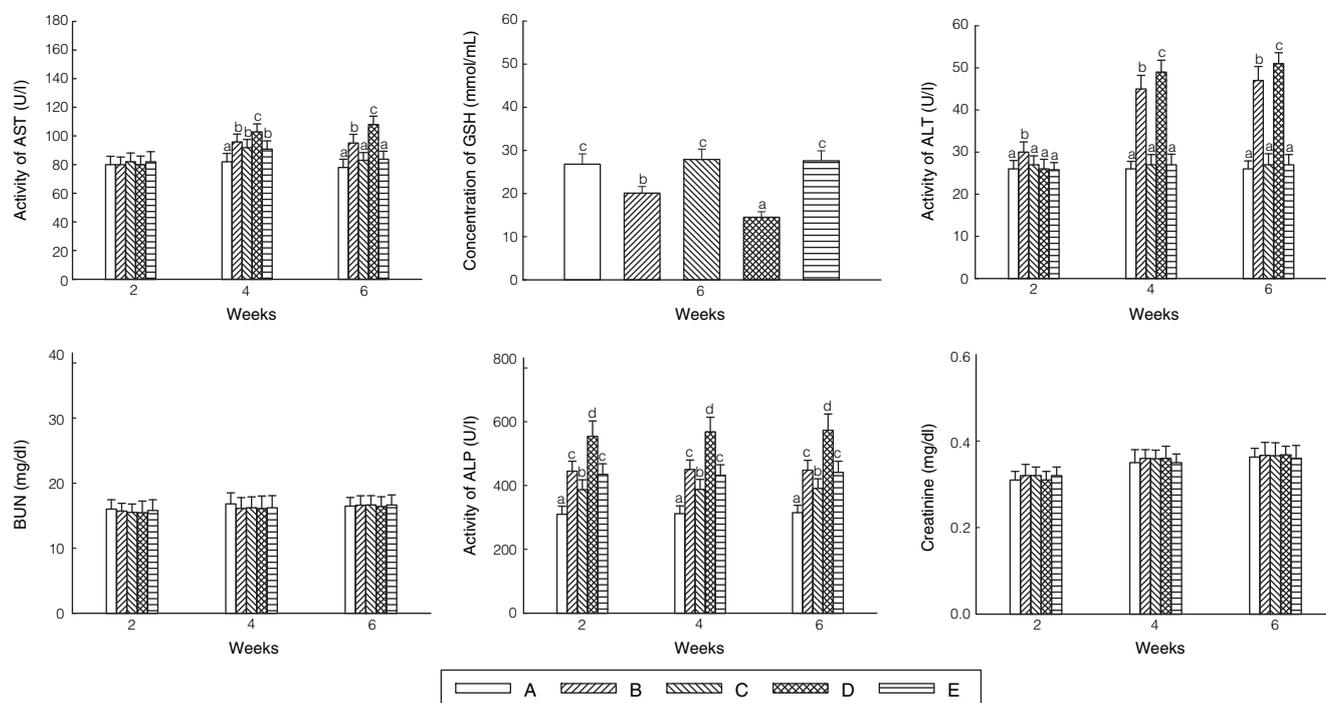


Figure 3. Effects of taurine, oxidized fish oil and oxidized cholesterol on the activity of AST, ALT, ALP, BUN, creatinine and concentration of GSH in the plasma of rats fed diet for 2, 4 and 6 groups. a-d values in the same week with different superscript are significantly different ($P < 0.05$). Group A = received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B = oxidized fish oil + cholesterol diet (3% oxidized fish oil + 2% cholesterol), Group C = oxidized fish oil + cholesterol + taurine diet (3% oxidized fish oil + 2% cholesterol + 5% taurine), Group D = oxidized fish oil + oxidized cholesterol diet (3% oxidized fish oil + 2% oxidized cholesterol), Group E = oxidized fish oil + oxidized cholesterol + taurine diet (3% oxidized fish oil + 2% oxidized cholesterol + 5% taurine).

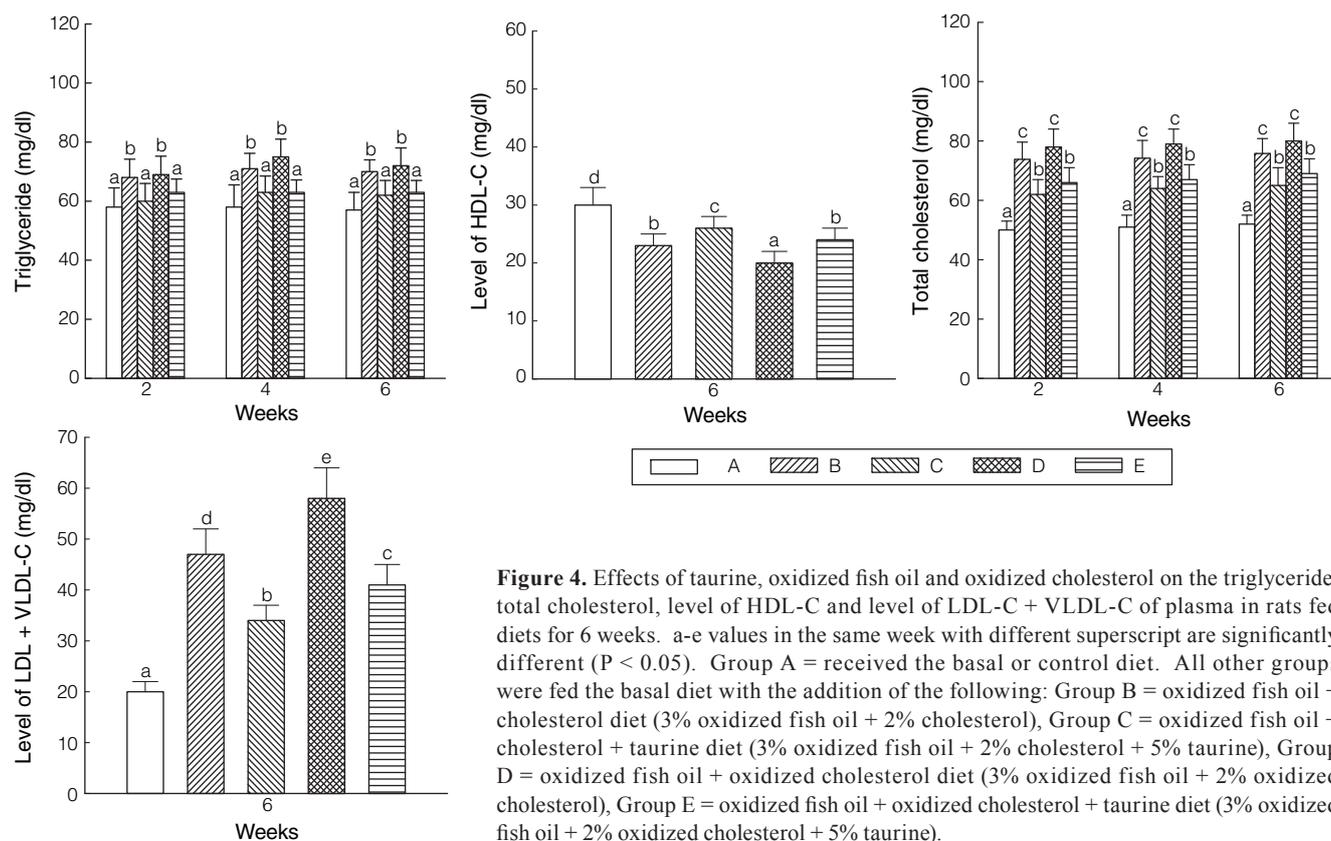


Figure 4. Effects of taurine, oxidized fish oil and oxidized cholesterol on the triglyceride, total cholesterol, level of HDL-C and level of LDL-C + VLDL-C of plasma in rats fed diets for 6 weeks. a-e values in the same week with different superscript are significantly different ($P < 0.05$). Group A = received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B = oxidized fish oil + cholesterol diet (3% oxidized fish oil + 2% cholesterol), Group C = oxidized fish oil + cholesterol + taurine diet (3% oxidized fish oil + 2% cholesterol + 5% taurine), Group D = oxidized fish oil + oxidized cholesterol diet (3% oxidized fish oil + 2% oxidized cholesterol), Group E = oxidized fish oil + oxidized cholesterol + taurine diet (3% oxidized fish oil + 2% oxidized cholesterol + 5% taurine).

Histopathological changes were assessed by observing liver section for necrotic and swollen hepatocytes. Referring to the histological finding is shown in Figure 6, swollen cells are identified by enlargement and ruptured plasma membrane. Morphological alterations involving all zones of hepatic lobule were observed in the Group B and D treatment revealed necrosis or degen-

eration and enlargement of the tubular or peritubular tissues.

DISCUSSION

The activities of AST and ALT in the plasma of rats were significantly elevated by cholesterol, oxidized fish oil and oxidized cholesterol injury to the liver⁽³⁰⁾. Wright *et al.* pointed out that taurine was presented by the high content of taurine in cell membrane to preserve liver cells⁽³¹⁾. In this study, the activities of AST, ALT and ALP in the plasma of rats showed to be significantly affected by oxidized cholesterol and oxidized fish oil. In the experimental period, the food consumption of rats significantly decreased in the rats groups fed with oxidized fish oil diet. The palatability of the diet might also be affected. Therefore, the oxidized cholesterol and oxidized fish oil diet may induce liver injury and reduce diet palatability in rats.

A study suggested taurine may be involved in lipid metabolism from daily urinary taurine excretion and serum HDL-C⁽³²⁾. The ratio between HDL-C and LDL-C +VLDL-C is known as the atherogenic index. In animals fed a normal diet, the serum total cholesterol, LDL-C, triglyceride, and hepatic cholesterol, triglyceride, and free fatty acid were effectively decreased by taurine⁽³³⁻³⁵⁾. HDL-C, the antiatherogenic form of serum cholesterol, was found to be reduced by taurine⁽³⁴⁾. The significance of these effects has yet to be explained and given practical and scientific meaning. This study showed that the total cholesterol or the total LDL-C+VLDL-C in the plasma of rats are significantly affected by taurine. However, the data strongly suggest that taurine modulates serum lipid levels in rats.

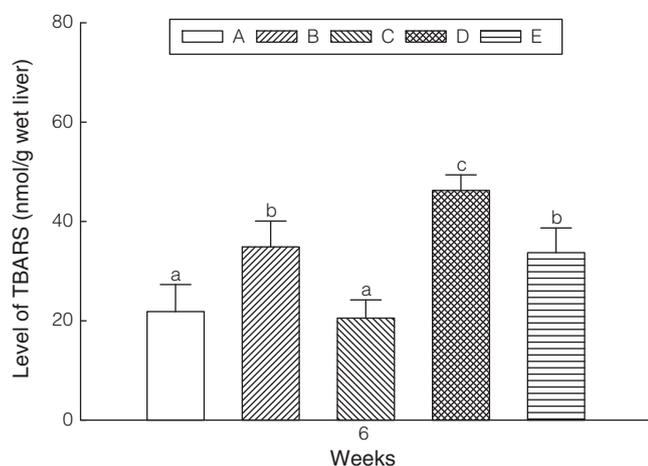


Figure 5. Effects of taurine, oxidized fish oil and oxidized cholesterol on the level of TBARS in the liver of rats fed diets after 6 weeks. a-c values in the same week with different superscript are significantly different ($P < 0.05$). Group A = received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B = oxidized fish oil + cholesterol diet (3% oxidized fish oil + 2% cholesterol), Group C = oxidized fish oil + cholesterol + taurine diet (3% oxidized fish oil + 2% cholesterol + 5% taurine), Group D = oxidized fish oil + oxidized cholesterol diet (3% oxidized fish oil + 2% oxidized cholesterol), Group E = oxidized fish oil + oxidized cholesterol + taurine diet (3% oxidized fish oil + 2% oxidized cholesterol + 5% taurine).

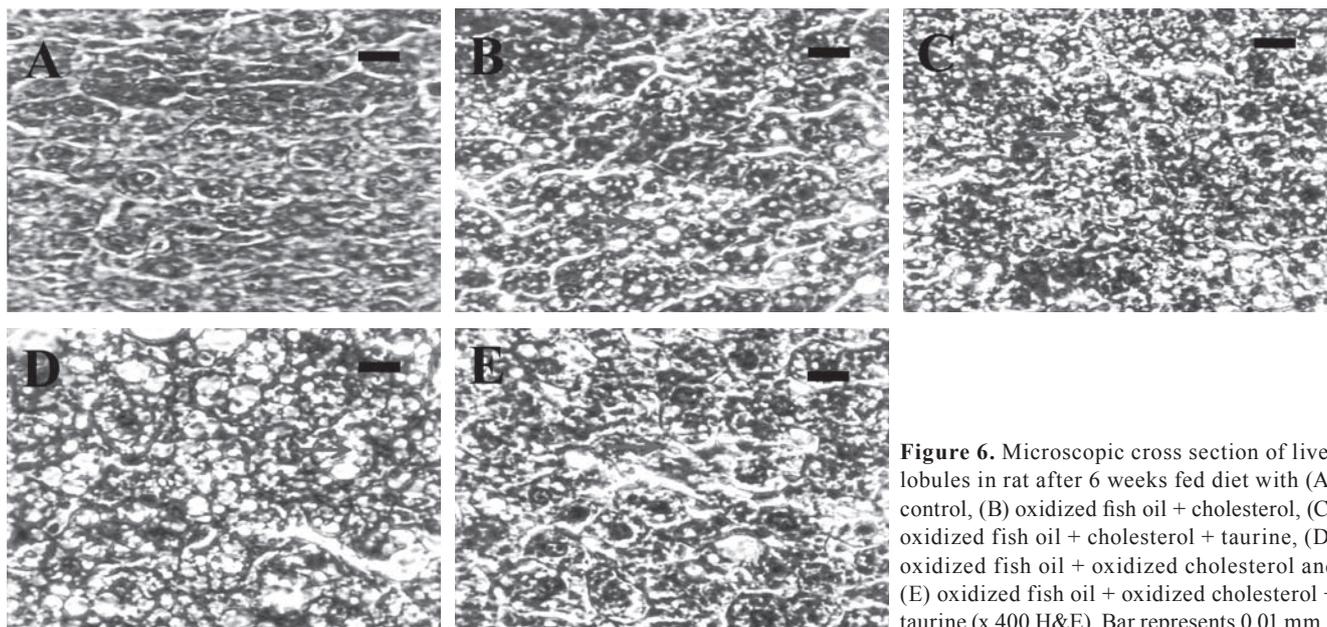


Figure 6. Microscopic cross section of liver lobules in rat after 6 weeks fed diet with (A) control, (B) oxidized fish oil + cholesterol, (C) oxidized fish oil + cholesterol + taurine, (D) oxidized fish oil + oxidized cholesterol and (E) oxidized fish oil + oxidized cholesterol + taurine (x 400 H&E). Bar represents 0.01 mm.

Dietary intake of long chain PUFAs is known to be associated with a decrease in the incidence of cardiovascular disease⁽³⁶⁻³⁹⁾. Fish oils have a high content of n-3 long-chain PUFAs. The most reproducible effect of dietary fish oil on serum lipids is a decrease in triglyceride levels^(35,40). Fish is also the richest dietary source of taurine than poultry and beef, which likewise have a platelet-stabilizing effect^(41,42). Although both fish oil and taurine can lower elevated the blood pressure^(30,43-45), the stroke protection associated with fish ingestion persists after statistical adjustment for blood pressure. Those not wishing to eat fish may likely obtain comparable benefit by taking fish oil and taurine supplements. It is conceivable that the reduction of stroke risk associated with high protein intakes in Asian epidemiology reflects, in part, the fact that sulfhydryl amino acids are the biosynthetic precursors for taurine; moreover, the only dietary sources of pre-formed taurine are flesh foods. Plasma taurine levels tend to be lower in vegans than in omnivores and urinary taurine levels are dramatically lower⁽⁴⁶⁾.

The TBARS and GSH levels in the liver are additional indicators of liver injury. TBARS is an end-product of lipid peroxidation. The level of TBARS in rat liver was significantly reduced when the rats were fed with the supplement of taurine. This result is the same as that reported previously^(47,48). Therefore, it is reasonable to assume that taurine may act as a good scavenger in reducing the production of lipid peroxidation induced by drugs⁽⁴⁹⁾, vitamin A⁽⁵⁰⁾ and heavy metal⁽⁵¹⁾. The level of GSH in rat liver was raised significantly when the rats were fed with the supplement of taurine. It means that taurine may play an important role in the metabolism of GSH, but this related mechanism should be studied further. On the other hand, taurine can also function as a regulator of intracellular calcium homeostasis⁽⁵²⁾ that can be disturbed due to cholesterol, oxidized fish oil and oxidized cholesterol toxicity⁽⁵³⁾. Taurine has been shown to protect against endothelial cell death by modulating intracellular calcium fluxes⁽¹²⁾. Finally, taurine may ameliorate oxidized cholesterol and oxidized fish oil induced hepatic injury by enhancing the activities of endogenous antioxidants. Support for this concept comes from our results, which show that taurine produced a remarkable significant increase in hepatic GSH level. This could be attributed to the role of taurine in maintaining a normal GSH level⁽⁵⁴⁾ and its antioxidant action against lipid peroxidation, thus conserving the internal antioxidants system. The stimulatory effect of taurine on endogenous antioxidants was reported by others^(55,56). However, the TBARS and GSH levels in the liver are additional indicators of liver injury. The data did not prove the mechanism of oxidized cholesterol and oxidized fish oil injury by lipid peroxidation but it is strongly suggestive that it plays an important role. The level of TBARS in rat plasma and liver was significantly reduced when the rats were fed diet with the supplement of taurine^(57,58). While it was raise significantly when

the rats were fed diet with the supplement of taurine. It means that taurine may play an important role in the metabolism of GSH and in preventing lipid peroxidation but these related mechanism should be studied further.

Taurine supplementation in our study significantly mitigated oxidized cholesterol and oxidized fish oil induced oxidative stress and hepatotoxicity. This was clearly manifested by the improvement in all the biochemical variables determining oxidized cholesterol hepatotoxicity (Figures 3 and 5). In addition, taurine inhibited lipid peroxidation, diminished the decrease in catalase and GSH-Px activities, and abrogated GSH depletion induced by oxidized cholesterol.

Consistent with our finding, taurine has been demonstrated to protect against hepatotoxicity induced by several free radicals generating insults including lipopolysaccharide⁽⁵⁹⁾, acetaminophen⁽⁶⁰⁾, thioacetamide⁽⁶¹⁾, and ischemia/reperfusion⁽⁶²⁾. Moreover, the antioxidant effect of taurine was shown in other organs including the lung⁽⁶³⁾, kidney⁽⁶⁴⁾ and heart⁽⁶⁵⁾. In addition, other antioxidants have been shown to reduce oxidized cholesterol induced hepatotoxicity.

Taurine has been demonstrated to function as a direct antioxidant that scavenges or quenches oxygen free radicals, thus inhibiting lipid peroxidation, and as an indirect antioxidant that prevents the increase in membrane permeability resulting from oxidant injury in many tissues including liver⁽⁶²⁾. Taurine might stimulate *s*-nitrosylation of GSH producing *s*-nitrosoglutathione, which is approximately 100 times more potent than the classical GSH. In addition, *s*-nitrosylation of cysteine residues by nitrosoglutathione can inactivate caspase-3, thus preventing hepatic cell apoptosis⁽⁶⁶⁾. Moreover, taurine might lessen oxidized cholesterol induced oxidative injury either by forming chloramines, known to be more stable and less reactive molecules, with hypochlorous (HOCl) and HOCl-metalloproteins, or by binding free metal ions such as Fe²⁺ to its sulfonic acid group^(67,68).

As an indirect antioxidant, taurine has been proposed as a membrane stabilizer that can maintain membrane organization, prevent ion leakage and water influx, and subsequently, avoid cell swelling^(62,65). The stabilizing effect of taurine on cellular membrane has been suggested to be associated with the interaction between taurine and polyunsaturated fatty acids in the membrane, which results in increasing in the affinity of taurine for its carrier transport and the interaction between taurine and the sites related to anion transport and water influx. This property of taurine may also partly account for its protection against oxidized cholesterol induced hepatocyte necrosis.

On the other hand, taurine can also function as a regulator of intracellular calcium homeostasis⁽²⁾ that can be disturbed by oxidized cholesterol toxicity. Taurine has been shown to protect against endothelial cell death by modulating intracellular calcium fluxes⁽⁶⁹⁾. Finally, taurine may ameliorate oxidized cholesterol and oxidized

fish oil induced hepatic injury by enhancing the activities of endogenous antioxidants. Support for this concept comes from our results, which show that taurine promoted a remarkable significant increase in hepatic GSH level and GSH-Px and catalase activities. This could be attributed to the role of taurine in maintaining a normal IGF-I level⁽⁷⁰⁾ and its antioxidant action against lipid peroxidation, thus conserving the internal antioxidants system. The stimulatory effect of taurine on endogenous antioxidants was reported by others^(64,71). Together, the results of the present study demonstrate that administration of taurine has a therapeutic role in preventing cyclosporine-induced hepatotoxicity, possibly through its unique cytoprotective properties such as antioxidant activity.

In conclusion, we suggest that: (a) the imbalance between production of oxygen free radicals and the endogenous antioxidant defense system, as a result of the effect of oxidized cholesterol and oxidized fish oil, is the main mechanism responsible for peroxide accumulation and hepatotoxicity; and (b) taurine reduces the oxidative stress through the inhibition of lipid peroxidation (a widely known mechanism).

REFERENCES

1. Parcell, S. 2002. Sulfur in human nutrition and applications in medicine. *Altern. Med. Rev.* 7: 22-44.
2. Huxtable, R. J. 1992. Physiological actions of taurine. *Physiol. Rev.* 72: 101-163.
3. Militante, J. D., Lombardini, J. B. and Schaffer, S. W. 2000. The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus. *Cardiovasc. Res.* 46: 393-402.
4. Militante, J. D. and Lombardini, J. B. 2001. Increased cardiac levels of taurine in cardiomyopathy: the paradoxical benefits of oral taurine treatment. *Nutr. Res.* 21: 93-102.
5. Hayes, D. 1995. Taurolidine, an antilipoplysaccharide agent, has immunoregulatory properties that are mediated by the amino acid taurine. *J. Leukoc. Biol.* 58: 299-306.
6. Yan, P. S. and White, P. J. 1990. Cholesterol oxidation in heated lard enriched with two levels of cholesterol. *J. Am. Oil Chem. Soc.* 67: 927-931.
7. Zunin, P., Evangelisti, F., Caboni, M. F., Penazzi, G., Lercker, G. and Tiscorna, E. 1995. Cholesterol oxidation in baked foods containing fresh and powdered eggs. *J. Food Sci.* 60: 913-915.
8. Osada, K., Kodama, T., Cui, L., Yamada, K. and Sugano, M. 1993. Levels and formation of oxidized cholesterols in processed marine foods. *J. Agric. Food Chem.* 41: 1893-1898.
9. Paniangvait, P., King, A. J., Jone, A. D. and German, B. G. 1995. Cholesterol oxides in foods of animal origin. *J. Food Sci.* 52: 57-62.
10. Addis, P. B. 1986. Occurrence of lipid oxidation products in foods. *Food Chem Toxicol.* 24: 1021-1030.
11. Vine, D. F., Mamo, C. L., Beilin, L. J., Mori, T. A. and Croft, K. D. 1995. Dietary oxysterols are incorporated in plasma triglyceride rich lipoproteins, increase their susceptibility to oxidation and increase aortic cholesterol concentration of rabbits. *J. Lipid Res.* 39: 1995-2004.
12. Lyons, M. A., Samman, S., Gatto, L. and Brown, A. J. 1999. Rapid hepatic metabolism of 7-ketocholesterol in vivo: implications for dietary oxysterols. *J. Lipid Res.* 40: 1846-1857.
13. Takahashi, T. 1985. Molecular species of phospholipids. *Muscle Lipids of Aquatic Animal.* pp. 24-37. Kayama, M. ed. Koseisha-Koseigaku. Tokyo.
14. Hwang, D. F., Lin, M. F., Jeng, S. S. and Cheng, H. M. 1997. Fish oil inhibits the acute induction of hypertriglyceridemia and liver enlargement by a single mega dose of retinyl palmitate in rats. *Nutr. Sci. J.* 22: 131-143.
15. Frankel, E. N. 1980. Lipid oxidation. *Pro. Lipid Res.* 19: 1-8.
16. Negishi, H., Fujimoto, K. and Kaneda, T. 1980. Effect of autoxidized methyl linoleate on glutathione peroxidase. *J. Nutr. Sci. Vitaminol.* 26: 309-317.
17. Liu, J. F. and Huang, C. J. 1995. Tissue-tocopherol retention in male rats is compromised by feeding diets containing oxidized frying oil. *J. Nutr.* 125: 3071-3081.
18. Hwang, D. F., Hour, J. L. and Cheng, H. M. 2000. Effect of taurine on toxicity of oxidized fish oil in rats. *Food Chem. Toxicol.* 38: 585-591.
19. Kaunitz, H. and Johnson, R. E. 1972. Exacerbation of heart and lesions in rats by feeding of various mildly oxidized fats. *Lipids* 8: 329-336.
20. Chien, J. T., Wang, H. C. and Chen, B. H. 1998. Kinetic model of the cholesterol oxidation during heating. *J. Agric. Food Chem.* 46: 2571-2577.
21. American Institute of Nutrition. 1977. Report of the American Institute and hoc committee on standards for nutritional studies. *J. Nutr.* 107: 1340-1348.
22. Konosu, S. and Yamaguchi, K. 1982. The flavor components in fish and shellfish. In *Chemistry and Biochemistry of Marine Food Products.* pp.367-385. Martin, I. and Roy, E. ed. Avi: Westport. CT.
23. Tatum, V. L., Changchit, C. and Chow, C. K. 1990. Measurement of malondialdehyde by high performance liquid chromatography with fluorescence detection. *Lipids* 25: 226-229.
24. Griffith, O. W. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* 106: 207-212.
25. Yeh, Y. H., Wang, D. Y., Deng, J. F., Chen, S. K. and Hwang, D. F. 2003. Effect of chenodeoxycholic acid on the toxicity of 5 α -cyprinol sulfate in rats. *Toxicology* 179: 21-28.
26. Puri, S. C. and Mullen, K. 1980. Multiple comparisons. In *Applied statistics for food and agricultural scientists.* pp.146-162. Hall, G. K. ed. Medical Publishers. Boston.

27. Guyton, C. 1991. Textbook of Medical Physiology. pp. 344-347. Saunders, New York.
28. Ronald, L. and Koretz, M. D. 1992. Chronic hepatitis: science and superstition. Current Hepatology. Gitnick, G. ed. Mosby-Year. Chicago.
29. Pagana, K. D. and Pagana, T. J. 1998. Manual of Diagnostic and Laboratory Tests. pp. 826-853. Mosby. New York.
30. Morin, R. J., Hu, B. and Peng, S. K. 1992. Sevanian A. Cholesterol oxidation and cancer. In Biological Effects of Cholesterol Oxides. pp.191-202. Peng, S. K. and Morin, R. J. ed. CRC Press: Ann Arbor.
31. Wright, C., Tallan, H. and Lin, H. 1986. Taurine: biological update. Annu. Rev. Biochem. 55: 427-453.
32. Kohashi, N., Okabayashi, T., Hama, J. and Katori, R. 1983. Decreased urinary taurine in essential hypertension. Prog. Clin. Biol. Res. 125: 73-87.
33. Yokogoshi, H., Mochizuki, H., Nanami, K., Hida, Y., Miyachi, F. and Oda, H. 1999. Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. J. Nutr. 129: 1705-1712.
34. Park, T. and Lee, K. 1998. Dietary taurine supplementation reduces plasma and liver cholesterol and triglyceride levels in rats fed a high-cholesterol or a cholesterol-free diet. Adv. Exp. Med. Biol. 442: 319-325.
35. Yany, Y., Xiao, R., Qiu, F. and Li, X. 2002. Effect of taurine on blood and liver lipids in rats. Wei Sheng Yan Jiu 31: 63-65.
36. Daviglius, M., Stamler, J., Orenca, A. J., Dyer, A. R., Kiang, L. and Greenland, P. D. 1997. Fish consumption and 30-year risk of fatal myocardial infraction. New Eng. J. Med. 336: 1046-1053.
37. Eritsland, J., Arnesen, H., Gronseth, K., Fjeld, N. B. and Abdelnoor, M. 1996. Effect of dietary supplementation with n-3 fatty acids on coronary artery bypass graft patency. Am. J. Cardiol. 77: 31-36.
38. Schindler, O. S. and Rost, R. 1996. Effect of low dose omega-3 fatty acid supplementation on plasma lipids and lipoproteins in coronary patients with dyslipoproteinemia. Ernahrungswiss 35: 191-198.
39. Simopoulos, A. P. 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 54: 438-446.
40. Murakami, S., Kondo-Ohta, Y. and Tomisawa, K. 1999. Improvement in cholesterol metabolism in mice given chronic treatment of taurine and fed a high-fat diet. Life Sci. 64: 83-91.
41. Harris, W. S. 1989. Fish oils and plasma lipid and lipoprotein metabolism in human: a critical review. J. Lipid Res. 30: 785-807.
42. Nestei, P. J., Connor, J. W., Reardon, M. F., Connor, S., Wong, S. and Boston, R. 1984. Suppression by diets rich in fish oil of very low density lipoprotein production in man. Clin. Invest. 74: 82-89.
43. Appel, L. J., Miller, E. R., Seidler, A. J. and Whelton, P. K. 1993. A meta-analysis of controlled clinical trials. Arch. Inter. Med. 153: 1429-1438.
44. Fujita, T., Ando, K., Noda, H., Ito, Y. and Sato, Y. 1987. Effects of increased adrenomedullary activity and taurine in young patients with borderline hypertension. Circulation 75: 525-532.
45. Yamori, Y., Nara, Y., Ikeda, K. and Mizushima, S. 1996. Is taurine a preventive nutritional factor of cardiovascular diseases or just a biological marker of nutrition. Adv. Exp. Med. Biol. 403: 623-629.
46. Rana, S. K. and Sanders, T. A. 1986. Taurine concentrations in the diet, plasma, urine and breast milk of vegans compared with omnivores. Br. J. Nutr. 56: 17-27.
47. Tadolini, B., Gianfrance, P., Gavino, G. P., Federico, B. and Flavia, F. 1995. Effect of taurine and hypotaurine on lipid peroxidation. Biochem. Biophys. Res. Commun. 213: 820-826.
48. Wang, L. C., Hwang, D. F., Jeng, S. S. and Cheng, H. M. 1998. Effect of high dose of dietary taurine on toxicity of lead in rats. J. Chin. Agri. Chem. Soc. 35: 612-620.
49. Alvarez, J. G. and Storey, B. T. 1983. Taurine, hypotaurine, epinephrine and albumin inhibit lipid peroxidation in rabbit spermatozoa and protect against loss of motility. Biol. Reports 29: 548-555.
50. Yeh, Y. H., Lee, Y. T., Hsieh, H. S. and Hwang, D. F. 2008. Effect of taurine on toxicity of vitamin A in rats. Food Chem. 106: 260-268.
51. Hwang, D. F., Wang, L. C. and Cheng, H. M. 1998. Effect of taurine on toxicity of copper in rats. Food Chem. Toxicol. 36: 239-244.
52. Yamori, Y., Liu, L., Ikeda, K., Miura, A., Mizushima, S. and Miki, T. 2001. Distribution of twenty-four hour urinary taurine excretion and association with ischemic heart disease mortality in 24 populations of 16 countries: results from the WHO-CARDIAC study. Hypertens Res. 24: 453-357.
53. Schroeppfer, G. J. J. 2000. Oxysterols: modulators of cholesterol metabolism and other processes. Phys. Rev. 80: 361-554.
54. Kannan, R., Tang, D., Mackic, J. B., Zlokovic, B. V. and Fernandez-Checa, J. C. 1992. A simple technique to determine glutathione (GSH) levels and synthesis in ocular tissues as GSH-bimane adduct: application to normal and galactosemic guinea-pigs. Exp. Eye Res. 56: 45-50.
55. Cazevielle, C. and Osborne, N. N. 1997. Retinal neurons containing kainate receptors are influenced by exogenous kainate and ischaemia while neurones lacking these receptors are not melatonin counteracts the effects of ischaemia and kainate. Brain Res. 755: 91-100.
56. Schütte, M. and Werner, P. 1998. Redistribution of glutathione in the ischemic rat retina. Neurosci. Lett. 246: 53-56.
57. Muller, L., Craig, G. and Stacey, N. H. 1988. Dose response of rat liver to low level cadmium. Bull.

- Environ. Contam. Toxicol. 40: 301-308.
58. Kang, Y. J. and Enger, M. D. 1988. Glutathione is involved in the early cadmium cytotoxic response in human lung carcinoman cells. *Toxicology* 48: 93-97.
59. Kim, S. K. and Kim, Y. C. 2002. Attenuation of bacterial lipopolysaccharide-induced hepatotoxicity by betaine or taurine in rats. *Food Chem. Toxicol.* 40: 545-549.
60. Waters, E., Wang, J. H., Redmond, H. P., Wu, Q. D., Kay, E. and Bouchier-Hayes, D. 2001. Role of taurine in preventing acetaminophen-induced hepatic injury in the rat. *Am. J. Physiol. Gastrointest. Liver Physiol.* 280: G1274-G1279.
61. Dogru-Abbasoglu, S., Kanbagli, O., Balkan, J., Cevikbas, U., Aykac-Toker, G. and Uysal, M. 2001. The protective effect of taurine against thioacetamide hepatotoxicity of rats. *Hum. Exp. Toxicol.* 20: 23-27.
62. Chen, Y. X. 1993. Protective action of taurine on ischemia reperfusion liver injury in rats and its mechanisms. *Chin. Med. J. Engl.* 73: 276-279.
63. Banks, M. A., Porter, W. D., Martin, W. G. and Castranova, V. 1992. Taurine protects against oxidant injury to rat alveolar pneumocytes. *Adv. Exp. Med. Biol.* 315: 341-354.
64. Saad, S. Y. and Al-Rikabi, A. C. 2002. Protection effects of taurine supplementation against cisplatin-induced nephrotoxicity in rats. *Chemotherapy* 48: 42-48.
65. Milei, J., Ferreira, R., Llesuy, S., Forcada, P., Covarrubias, J. and Boveris, A. 1992. Reduction of reperfusion injury with preoperative rapid intravenous infusion of taurine during myocardial revascularization. *Am. Heart J.* 123: 339-345.
66. Chiueh, C. C. and Rauhala, P. 1999. The redox pathway of s-nitrosoglutathione. *Free Radic. Res.* 31: 641-650.
67. Trachtman, H., Del Pizza, R. and Futterweit, S. 1992. Taurine attenuates renal disease in chronic aminonucleoside nephropathy. *Am. J. Physiol.* 262: F117-F123.
68. Schuller-Levis, G., Quinn, M. R., Wright, C. and Park, E. 1994. Taurine protects against oxidant-induced lung injury: possible mechanisms of action. *Adv. Exp. Med. Biol.* 359: 31-39.
69. Wang, J. H., Redmond, H. P., Watson, R. W. G., Condron, C. and Bouchier-Hayes, D. 1996. The beneficial effect of taurine on the prevention of human endothelial cell death. *Shock* 6: 331-338.
70. Dawson, Jr. R., Liu, S., Eppler, B. and Patterson, T. 1999. Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats. *Mech. Ageing Dev.* 107: 73-91.
71. Erdem, A., Gundogan, N. U., Usubutun, A., Kiine, K., Erdem, R. S., Kara, A. and Bozkurt, A. 2000. The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. *Nephrol. Dial. Transplant.* 15: 1175-1182.