CELL BIOCHEMISTRY AND FUNCTION
Cell Biochem Funct 2009; **27**: 344–350.
Published online 27 July 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/cbf.1579

Effects of β-carotene on antioxidant status in rats with chronic alcohol consumption

Wan-Teng Lin[1†], Chi-Chang Huang[2†], Tien-Jen Lin[3], Jiun-Rong Chen[4], Ming-Jer Shieh[4], Hsiang-Chi Peng[4], Suh-Ching Yang[4*] and Chih-Yang Huang[5,6**]

[1]*Department of Hospitality Management, College of Agriculture, Tunghai University, Taichung*
[2]*Agricultural Biotechnology Research Center, Academia Sinica, Taipei*
[3]*Division of Neurosurgery, Department of Surgery, Municipal Wan Fang Hospital; Graduate Institute of Injury Prevention, Taipei Medical University, Taipei*
[4]*School of Nutrition and Health Sciences, Taipei Medical University, Taipei*
[5]*Graduate Institute of Basic Medical Science, Graduate Institute of Chinese Medical Science; China Medical University, Taichung*
[6]*Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan, ROC*

This study examined the effects of β-carotene on antioxidant status in rats with chronic alcohol consumption. At the beginning of experiment (week 0), according to both the plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, rats ($n = 24$) were divided into 3 groups and fed with a standard diet (group C), a diet containing ethanol (group E), or a diet containing ethanol and β-carotene (group E+B). After 10 weeks, plasma AST and ALT, fat accumulation in the liver, antioxidant enzyme activities in erythrocytes and the liver, malondialdehyde (MDA), and α-tocopherol and retinol in plasma and hepatic samples were analyzed. The chronic alcohol diet significantly increased AST and ALT levels in plasma, and these changes were prevented by supplementing the diet with β-carotene. Glutathione (GSH) in erythrocytes and in the liver was significantly elevated in rats fed with a diet containing β-carotene. The results indicate that β-carotene supplementation can prevent ethanol-induced liver damage and increase GSH concentrations in erythrocytes and the liver.

KEY WORDS — alcoholic liver disease; antioxidant capacity; β-carotene; lipid peroxidation; oxidative stress

ABBREVIATIONS — ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GPX, glutathione peroxidase; GRD, glutathione reductase; GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; TAS, total antioxidant status

INTRODUCTION

Carotenoids are natural, fat-soluble pigments that provide bright coloration to plants and animals. β-Carotene is the most common carotenoid in food and is a nontoxic precursor of vitamin A. In addition, β-carotene is a strong antioxidant with the ability to scavenge free radicals and to physically quench singlet oxygen.[1] β-Carotene is primarily found in leafy green vegetables, carrots, sweet potatoes, red palm oil, mature squashes, pumpkins, and mangoes.[2] It has been reported that β-carotene functions as a radical-trapping antioxidant at low oxygen pressure to reduce the extent of nuclear damage and to inhibit lipid peroxidation.[3] Additionally, β-carotene is believed to inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone–induced lung carcinogenesis,[4] and improve cell viability.[5] However, β-carotene has not been to be an effective agent in the treatment of lung cancer in clinical trials and, perhaps, may even be harmfuls.[6] In addition to antioxidant activity, β-carotene might serve as a pro-oxidant, propagating free radical-induced reactions, depending on its intrinsic properties as well as on the redox potential of the biological environment in which it acts.[7]

The liver is the major site of ethanol oxidation, as well as the site of major damage (alcoholic liver disease, ALD) due to the prolonged consumption of excessive amounts of alcohol. Numerous studies indicated that excessive alcohol intake induces the mass production of free radicals in the body, which may play a role in ALD.[8] Moreover, the prolonged use of alcohol can lead to the depletion of vitamin A from the body.[9,10] It is hypothesized that β-carotene supplementation may lower the production of free radicals as well as prevent vitamin A depletion from excessive

* Correspondence to: S.-C. Yang, School of Nutrition and Health Sciences, Taipei Medical University, No. 250, Wu-Xin Street, Taipei 11031, Taiwan, ROC. E-mail: sokei@tmu.edu.tw
** Correspondence to: C. Huang, Graduate Institute of Basic Medical Science and Graduate Institute of Chinese Medical Science, China Medical University, No. 91, Hsueh-Shih Road, Taichung 40402, Taiwan, ROC. E-mail: cyhuang@mail.cmu.edu.tw
†Wan-Teng Lin and Chi-Chang Huang contributed equally to this work.

Received 24 January 2009
Revised 3 April 2009
Accepted 7 May 2009

alcohol intake. Based on this hypothesis, the objective of the present study was to investigate the effects of β-carotene on antioxidant capacity and vitamin A status in rats with chronic alcohol intake.

MATERIALS AND METHODS

Animals and diets

Six weeks old male Sprague-Dawley rats were purchased from the National Laboratory Animal Breeding and Research Center in Taiwan. Rats were fed with a standard laboratory diet and distilled water *ad libitum*, and housed in an air-conditioned room at 23 ± 2°C with 12 h of light per day. At the beginning of experiment (week 0), according to both the plasma AST and ALT activities, 24 rats were divided into three groups: the control (C), ethanol (E), and ethanol with β-carotene (E+B) groups. Rats in groups E and E+B were given semi-purified diet containing a powdered ethanol preparation (30% of calories in the diet), and rats in group C were fed an isocaloric, semi-purified diet without ethanol. The composition of the experimental diets was based on a previous study[11] with a slight modification as shown in the Table 1. One hundred grams of powdered alcohol containing 30.5 g of ethanol, 65.0 g of dextrose, and 4.5 g of water in beadlets (Vodka type, Alcock®, Sato Foods Industries Co., Ltd, Japan) were given daily to rats in groups E and E+B. β-Carotene was given as Solatene® (10% β-carotene in beadlets, Hoffmann-La Roche, USA) at dose of 5 mg per kg per day and administered admixed with powdered diet.

Sample preparation

After 10 weeks, all rats were anesthetized with ethyl ether and sacrificed. Blood was collected via the abdominal aorta, and the liver was carefully removed, rinsed in ice-cold normal saline (0.9% NaCl), blotted dry, and stored at −80°C for further analysis. Blood samples were centrifuged at 1500 × *g* at 4°C for 10 min. The supernatants (plasma) were used for the determination of plasma parameters. The pellets (erythrocytes) were washed three times with ice-cold saline. The erythrocytes were then suspended in four packed cell volumes of ice-cold distilled water to prepare hemolysates, as described in a previous report.[12] All chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless stated otherwise.

Table 1. Composition of experimental diets[1]

	Control	Ethanol
Ingredient[2]	(g/1000 kcal powdered diet)	
Casein	48.8	48.8
Soybean oil	14.6	14.6
Cellulose	19.5	19.5
AIN-76 Vitamins	4.9	4.9
AIN-76 Minerals	14.6	14.6
Dextrose	168.3	—
Powdered alcohol[3]	—	141.5

[1]Control diet: semi-purified diet; ethanol diet: semi-purified diet containing a powdered ethanol preparation (30% of calories in the diet).
[2]The components for the diets were purchased from ICN Biochemicals, Inc. (Costa Mesa, CA) with the exception of powdered alcohol.
[3]Powdered alcohol: 100 g of powdered alcohol contained 30.5 g of ethanol, 65.0 g of dextrose, and 4.5 g of water in beadlets that was obtained from Sato Foods Industries Co., Ltd, Japan.

Histological examination

All slices of liver tissue were fixed in 10% formaldehyde and embedded in paraffin for histological examination. Sections were stained with hematoxylin and eosin and examined under a light microscopy, as described in a previous report.[13]

Measurements of plasma AST and ALT activities

At the beginning of experiment, blood samples were collected into heparin-containing tubes via the tail vein and centrifuged. Plasma AST and ALT activities were measured by spectrophotometric methods with a latrozyme TA-LQ kit (Iatron Laboratories Inc., Tokyo, Japan).

Measurements of hepatic cholesterol and triacylglycerol levels

The extraction of liver lipids and determination of cholesterol and triacylglyceride concentrations were carried out according to Yang *et al.*[14]

Measurement of total protein concentration

In order to express the antioxidant enzymes activities per g protein, total protein concentration of the samples was estimated using a Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) and a spectrophotometer.

Measurement of superoxide dismutase (SOD) activity

SOD activities of erythrocyte hemolysates and liver samples were measured with a commercial kit (Randox Laboratories, Antrim). Fifty μL of diluted sample was added to 1.7 mL of mixed substrate (50 μmol L^{-1} xanthine and 25 μmol L^{-1} 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride). Two hundred and fifty μL of xanthine oxidase was added to the mixture and SOD activity was measured at 37°C on a Hitachi U-2000 Spectrophotometer at 505 nm for 3 min. The activity was expressed in U/mg protein in the samples.

Measurement of glutathione peroxidase (GPX) activity

GPX activities of erythrocyte hemolysates and liver were determined with a commercial kit (Randox Laboratories, Antrim). Twenty μL of diluted sample was added to 1 mL of mixed substrate (4 mmol L^{-1} glutathione, 0.5 U L^{-1} glutathione reductase and 0.34 mmol L^{-1} NADPH dissolved in 50 mmol L^{-1} phosphate buffer, pH 7.2, 4.3 mmol L^{-1}

DOI: 10.1002/cbf

EDTA). Forty μL of cumene hydroperoxide (diluted in deionized water) was added to the mixture and GPX activity was measured at 37°C on a Hitachi U-2000 Spectrophotometer at 340 nm for 3 min. The activity was expressed in $U\,mg^{-1}$ protein in the samples.

Measurement of glutathione reductase (GRD) activity

GRD activities of erythrocyte hemolysates and liver samples were measured with a commercial kit (Calbiochem-Novabiochem, CA, USA). Two hundred μL of diluted sample was added to 400 μL of 2.4 mmol L^{-1} GSSG buffer (dissolved in 125 mmol L^{-1} potassium phosphate buffer, pH 7.5, 2.5 mmol L^{-1} EDTA). Four hundred μL of 0.55 mmol L^{-1} NADPH (dissolved in deionized water) was added to the mixture and GRD activity was measured at 340 nm for 5 min on a Hitachi U-2000 Spectrophotometer. The activity was expressed in $mU\,mg^{-1}$ protein in the samples.

Measurement of catalase (CAT) activity

CAT activities of erythrocyte hemolysates and liver samples were determined at 25°C with Hitachi U-2000 Spectrophotometer UV–VIS spectrophotometer by the method of previous study.[15] Diluted sample was added to 59 mmol L^{-1} hydrogen peroxide (dissolved in 50 mmol L^{-1} potassium phosphate buffer, pH 7.0) and CAT activity was measured at 240 nm for 3 min. One unit of CAT activity was defined as the mmol of H_2O_2 degraded $min^{-1}\,mg^{-1}$ protein. The activity was expressed in $U\,mg^{-1}$ protein in the samples.

Measurement of glutathione (GSH) concentration

The concentrations of GSH in erythrocyte hemolysates and liver samples were assayed spectrophotometrically at 400 nm using a commercial kit (Calbiochem-Novabiochem, CA, USA).

Measurement of total antioxidant status (TAS) concentration

The concentration of TAS in plasma was assayed spectrophotometrically at 600 nm using a commercial kit (Randox Laboratories, Antrim).

Measurement of malondialdehyde (MDA) concentration

The concentrations of MDA in plasma and liver samples were assessed colorimetrically at 586 nm using a commercial kit (Calbiochem-Novabiochem, CA, USA).

Measurement of uric acid level

The uric acid level in plasma was assayed spectrophotometrically at 520 nm using a commercial kit (Randox Laboratories, Antrim).

Measurements of plasma and hepatic α-tocopherol, retinal, and β-carotene levels

For α-tocopherol, retinal, and β-carotene analysis, sample extraction and HPLC analysis were carried out with a slight modification of the method used in a previous study.[16] Approximately 1 g of liver was homogenized in 4 mL of absolute ethanol (containing 1 g L^{-1} of butylated hydroxytoluene) with a tissue homogenizer (Polytron PT3 100; Brinkmann Instruments, Littau, Switzerland). Homogenate was saponified by addition of 1 mL of saturated KOH. The mixture was saponified at 70°C in a water bath for 30 min. After the sample was cooled to room temperature, the extract was washed with 2 mL of deionized water and extracted three times with 6 mL of n-hexane. The n-hexane layer (1 mL) was evaporated to dryness under a vacuum. The residue was dissolved in 200 μL of methanol, and 50 μL of sample was subjected to HPLC. The analytical HPLC system was a Shimadzu LC-10A system (Shimadzu, Tokyo, Japan), including Shimadzu LC-10A HPLC Pump and Shimadzu SPD-10AV UV–Vis Detector. Separations were performed using a C_{18}, 5 μm, 4.6 mm i.d. × 250 mm reversed-phase column (Vydac 201TP54, Hesperia, CA, USA) in a column oven (Super Co-150,. Enshine, Taipei, Taiwan) maintained at 40°C. The concentrations of α-tocopherol and retinol were measured at 295 and 325 nm, respectively, using methanol as the mobile phase at a flow rate of 1 mL min^{-1}. The retention times for α-tocopherol and retinol were 4.4 and 3.2 min, respectively. The concentration of β-carotene was measured at 452 nm using a mixture of methanol, acetonitrile, and deionized water (88:9:3, v/v/v) as the mobile phase. The retention time for the β-carotene was 15.5 min. Data were analyzed by SISC-LAB chromatographic analysis software (Scientific Information Service Corp., Taipei, Taiwan).

Statistical analysis

Values are expressed as the mean ± SD. To evaluate differences between the three groups, one-way analysis of variance (ANOVA) with Fisher's *post hoc* test was used. The SAS software (version 6.12, SAS Institute Inc., Cary, NC, USA) was used to analyze all data. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Animals and diets

The energy intake and initial body weight in groups C, E, and E+B showed no differences. In addition, the alcohol intake in groups E and E+B was similar. However, the final body weight in group E was significantly lower by 9% compared with that in group C ($p < 0.05$). The relative liver weight in group E was significantly increased by 13% compared with that in group C ($p < 0.05$). In contrast, the relative liver weight in group E+B was nearly same as that in group C and significantly reduced by 16% compared with that in group E ($p < 0.05$) (Table 2).

DOI: 10.1002/cbf

Table 2. Energy intake, alcohol intake, body weight, and relative liver weight in each group[1–3]

Groups	Energy intake (kJ day^{-1})	Alcohol intake (g day^{-1})	Body weight (g)		Relative liver weight[4] (%)
			Initial	Final	
C	257 ± 0	0	148 ± 8	413 ± 7[b]	3.42 ± 0.39[a]
E	254 ± 0	2.62 ± 0.03	151 ± 5	377 ± 31[a]	3.86 ± 0.43[b]
E+B	255 ± 1	2.62 ± 0.02	155 ± 7	394 ± 12[a]	3.25 ± 0.43[a]

[1]Group C: control diet; Group E: ethanol diet; Group E+B: ethanol diet with β-carotene.
Data are mean ± SD ($n = 8$).
[2]Only the values in each column with a different superscript are significantly different ($p < 0.05$).
[3]Relative liver weight = liver weight/body weight × 100%.

Liver damage and histopathology examination

Plasma AST and ALT activities in group E were significantly elevated by 25 and 27%, respectively, compared with those in group C ($p < 0.05$). In contrast, the activities of AST and ALT in group E+B were significantly reduced by 12 and 15%, respectively, compared with those in group E ($p < 0.05$) (Table 3). Based on light microscopy of the liver, fat accumulation was observed in the group E. However, ethanol-induced fat accumulation was prevented by β-carotene supplementation (Figure 1). In addition, hepatic cholesterol in group E (31 ± 7 mg g^{-1}) was 33% higher than that in group C (23 ± 3 mg g^{-1}), and triacylglycerol in group E (61 ± 15 mg g^{-1}) was 73% higher than that in group C (35 ± 6 mg g^{-1}). However, hepatic cholesterol and triacylglycerol in group E+B (25 ± 3 and 38 ± 13 mg g^{-1} liver, respectively) was decreased by 20 and 38%, respectively, compared with those in group E.

Antioxidant enzymes activities

The activities of the four antioxidant enzymes in erythrocytes were not different among the three groups (Table 4). In addition, the activities of GRD and SOD in liver were not significantly different among the three groups. However, hepatic GPX activity in group E was significantly decreased by 21% compared with that in group C ($p < 0.05$). In contrast, hepatic CAT activity in group E was 27% higher than that in group C and 20% higher than that in group E+B ($p < 0.05$ for both) (Table 5).

Table 3. Plasma AST and ALT activities in each group[1–3]

Groups	AST (Karmen unit/L)		ALT (Karmen unit/L)	
	Week 0	Week 10	Week 0	Week 10
C	45.10 ± 6.32	44.73 ± 3.38[a]	10.20 ± 1.91	12.77 ± 0.49[a]
E	44.10 ± 6.13	56.02 ± 5.39[b]	10.15 ± 2.44	16.24 ± 1.34[b]
E+B	44.43 ± 4.67	49.95 ± 5.21[a]	9.93 ± 2.41	13.84 ± 1.15[a]

[1]Group C: control diet; Group E: ethanol diet; Group E+B: ethanol diet with β-carotene.
[2]Data are mean ± SD ($n = 8$).
[3]Only the values in each column with a different superscript are significantly different ($p < 0.05$).

Antioxidant concentrations and total antioxidant status

GSH concentrations of both erythrocytes and liver samples in group E were significantly decreased by 15 and 23%, respectively, compared with those in the group C ($p < 0.05$). However, GSH concentrations of erythrocytes and liver in the group E+B were significantly increased by 43 and 27%, respectively, compared with those in group E ($p < 0.05$) (Tables 6 and 7). Moreover, there were no significant differences in either plasma or liver vitamin E levels among the three groups (Tables 6 and 7). In contrast, plasma TAS in group E was significantly elevated by 19% compared with that in group C and by 23% compared with that in group E+B ($p < 0.05$ for both) (Table 6).

Lipid peroxidation (MDA concentrations)

MDA levels were not significantly different in either plasma or liver among the three groups (Tables 6 and 7).

Uric acid concentrations

The level of plasma uric acid in group E was significantly elevated by 34% compared with that in group C and by 22% compared with that in group E+B ($p < 0.05$ for both) (Table 6).

Vitamin A and β-carotene concentrations

β-Carotene concentration was not detected in plasma of the three groups. However, β-carotene was detected in liver samples from group E+B, but not from groups E or C. Furthermore, hepatic vitamin A content in group E+B was significantly increased by 33 and 51%, respectively, compared with that in groups C and E ($p < 0.05$) (Tables 6 and 7).

DISCUSSION

In this study, the average energy intake in groups C, E, and E+B, and the average alcohol intake in groups E and E+B were similar to that reported previouslys.[17] However, the final body weight was significantly lower and the relative liver weight significantly higher in group E than those in

DOI: 10.1002/cbf

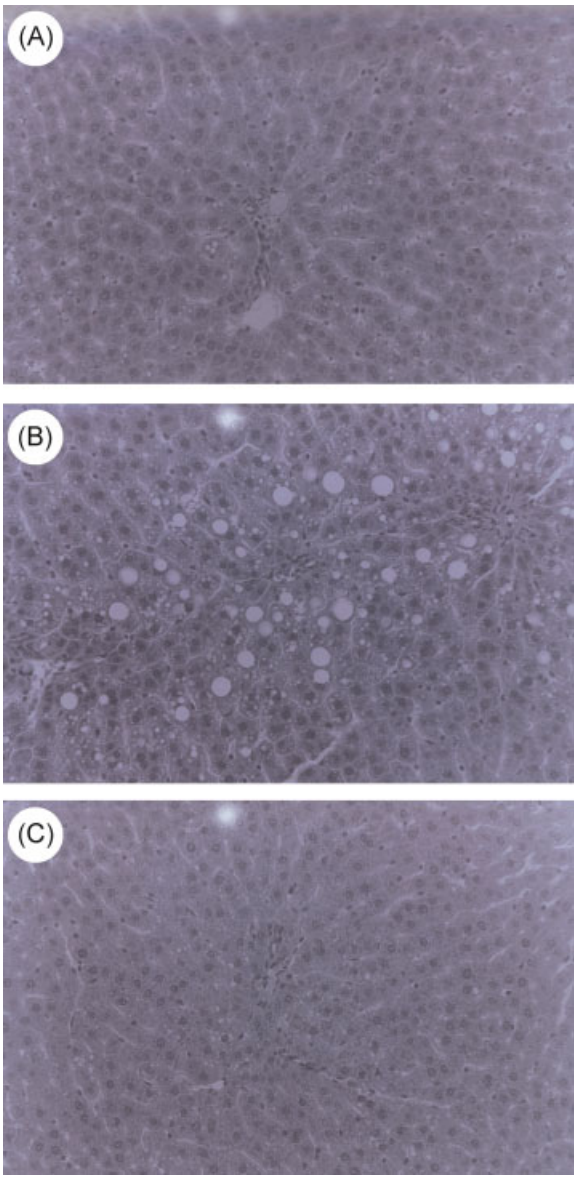

Figure 1. (**A**) Section of the liver from a rat fed the control diet for 10 weeks shows normal histology with no evidence of pathologic changes. (**B**) Section of the liver from a rat fed the ethanol diet shows the presence of fat accumulation. (**C**) Section of the liver from a rat fed ethanol with β-carotene diet shows normal histology with no evidence of pathologic changes. (H&E, magnification: $\times$ 200)

group C. A previous study reported that isocaloric substitution of carbohydrates by ethanol resulted in significantly lower weight gain and higher relative liver weight in spite of similar energy intakes.[18]

Both plasma AST and ALT are markers of liver damage as opposed to alcohol misuse.[19] The plasma AST and ALT activities in group E were significantly higher than those in group C, which is consistent with the long-term effects of ethanol administration on liver injury.[20] In contrast, the elevation of these two enzyme activities in group E+B was significantly less than that in group E, which indicate a reduction in ethanol-induced liver damage. Similarly, from the light micrograph of liver, fat accumulation was observed in group E, which might have been due to enhanced mobilization of free fatty acids from adipose tissue and increased hepatic biosynthesis of lipids, as previously suggested.[21] However, ethanol-induced fat accumulation was prevented by β-carotene supplementation. Thus, by eliminating fat accumulation in the liver, β-carotene supplementation may reduce ethanol−induced liver injury.

Since liver is the target tissue for ethanol, it is not surprising that four antioxidant enzymes activities in erythrocytes were no different among the three groups. Although our results also showed that both GRD and SOD activities in liver were not significantly different among the three groups, chronic ethanol feeding did result in significantly lower hepatic GPX activity in group E than that in group C. Furthermore, hepatic CAT activity in the group E was significantly increased compared with that in the other two groups. Both GPX and CAT can react with H_2O_2. Moreover, GPX is thought to be more active in the removal of H_2O_2, because of its dual location (mitochondria and cytosol).[22] However, a previous study demonstrated that superoxide is able to inactivate GPX activity, and this inactivation could be reversed by GSH.[22] Indeed, our results showed that long-term ethanol feeding caused a significant reduction in hepatic GSH levels. Therefore, GPX should be more sensitive and more easily alterable than CAT in response to chronic ethanol intake.[22] In contrast, β-carotene supplementation could inhibit the decrease of hepatic GPX activity via increasing hepatic GSH levels.

It has been reported that chronic ethanol feeding significantly decreases rates of GSH synthesis.[23] It is surprising that β-carotene supplementation was able to prevent the ethanol–induced decline in GSH in the present

Table 4. Antioxidant enzymes, SOD, GPX, GRD,and CAT, activities of erythrocytes in each group[1–3]

	SOD	GPX	GRD	CAT
Groups	(U mg^{-1} protein)	(mU mg^{-1} protein)	(mU mg^{-1} protein)	(U mg^{-1} protein)
C	9.31 ± 1.70	1599 ± 140	1.36 ± 0.53	295 ± 41
E	10.24 ± 1.48	1550 ± 192	1.72 ± 0.64	361 ± 102
E+B	10.39 ± 1.04	1486 ± 117	1.94 ± 0.69	295 ± 049

[1]Group C: control diet; Group E: ethanol diet; Group E+B: ethanol diet with β-carotene.
[2]Data are mean $\pm$ SD ($n = 8$).
[3]Only the values in each column with a different superscript are significantly different ($p < 0.05$).

DOI: 10.1002/cbf

Table 5. Hepatic antioxidant enzymes, SOD, GPX, GRD,and CAT, activities in each group[1–3]

	SOD	GPX	GRD	CAT
Groups	(U mg^{-1} protein)	(mU mg^{-1} protein)	(mU mg^{-1} protein)	(U mg^{-1} protein)
C	11.24 ± 2.09	1050 ± 146^{b}	32.64 ± 5.80	1234 ± 135^{a}
E	11.76 ± 2.29	833 ± 130^{a}	38.57 ± 8.86	1563 ± 267^{b}
E+B	11.13 ± 2.22	$930 \pm 124^{a,b}$	35.34 ± 4.81	1246 ± 171^{a}

[1]Group C: control diet; Group E: ethanol diet; Group E+B: ethanol diet with β-carotene.
[2]Data are mean ± SD ($n = 8$).
[3]Values in each column sharing the different superscripts are significantly different ($p < 0.05$).

Table 6. GSH concentration of erythrocytes, and plasma TAS, MDA, vitamin E, vitamin A, and uric acid levels in each group[1–3]

	GSH	TAS	MDA	α-Tocopherol	Retinol	Uric acid
Groups	(μmol L^{-1})	(mmol L^{-1})	(μmol L^{-1})	(μmol L^{-1})	(μmol L^{-1})	(μmol/L)
C	123 ± 6^{b}	0.59 ± 0.06^{a}	10.86 ± 0.96	14.78 ± 1.59	1.59 ± 0.15	70 ± 8^{a}
E	105 ± 20^{a}	0.70 ± 0.05^{b}	10.78 ± 3.23	15.42 ± 1.46	1.60 ± 0.19	94 ± 8^{b}
E+B	150 ± 7^{b}	0.57 ± 0.04^{a}	9.14 ± 1.85	14.51 ± 1.65	1.67 ± 0.40	77 ± 18^{a}

[1]Group C: control diet; Group E: ethanol diet; Group E+B: ethanol diet with β-carotene.
[2]Data are mean ± SD ($n = 8$).
[3]Values in each column sharing the different superscripts are significantly different ($p < 0.05$).

Table 7. Hepatic GSH, MDA, vitamin E, vitamin A, and β-carotene concentrations in each group[1–3]

	GSH	MDA	α-Tocopherol	Retinol	β-Carotene
Groups	(μmol L^{-1})	(μmol L^{-1})	(μg g^{-1} liver)	(mg g^{-1} liver)	(μg g^{-1} liver)
C	132 ± 19^{a}	3.17 ± 1.40	81.03 ± 25.85	1.57 ± 0.39^{a}	0
E	101 ± 19^{a}	3.40 ± 0.89	78.78 ± 15.29	1.38 ± 0.25^{a}	0
E+B	129 ± 15^{b}	2.98 ± 0.54	82.16 ± 16.67	2.09 ± 0.57^{b}	0.82 ± 0.22

[1]Group C: control diet; Group E: ethanol diet; Group E+B: ethanol diet with β-carotene.
[2]Data are mean ± SD ($n = 8$).
[3]Only values in each column with a different superscript are significantly different ($p < 0.05$).

study. However, there is no evidence that β-carotene supplementation increases GSH concentration via stimulating GSH synthetase activity. Therefore, GSH synthetase activity must be determined in future studies.

Plasma TAS in group E was significantly elevated compared with that in the other two groups. The assay kit for plasma TAS concentration measures the capacity of all of the antioxidants present in plasma samples, including GSH, vitamin C, vitamin E, β-carotene, bilirubin, uric acid and other antioxidants. However, it has been reported that uric acid is a strong determinant of plasma antioxidant capacity.[24] Furthermore it is known that chronic ethanol consumption results in hyperuricemia. In this study, we also detected a significantly higher plasma uric acid concentration in group E compared with that in groups C and E+B. Therefore, plasma TAS was greatly influenced by uric acid, and this suggests that TAS is not a suitable indicator for alcoholic liver disease.

Lipid peroxidation induced by long-term ethanol intake results not only from increased ROS production but also the combination of ethanol with a low vitamin E intake (2 IU/1000 kcal).[25] However, there were no significant differences in either plasma or liver MDA levels among the three groups. In our study, dietary fat intake only accounted for 13% of total calories, whereas it accounted for up to 36% of total calories in other studies.[26] Furthermore, our results showed that vitamin E concentration in both plasma and liver were not affected by ethanol. Thus, we speculate that less dietary fat and a sufficient vitamin E intake (30 IU/1000 kcal) were responsible for the lack of an increase in MDA in group E.

A previous study showed that an oral dose of β-carotene in oil increases the concentrations of retinoids and β-carotene at 3 h in the serum.[27] However, we did not detect β-carotene in plasma after 12 h of fasting. In contrast, β-carotene was detected in the liver of group E+B, and the hepatic vitamin A content was significantly higher than that in groups C and E. Our results are consistent with the view that β-carotene is converted to vitamin A by a central cleavage pathway in rats *in vivo*.[27]

In conclusion, our data show that β-carotene supplementation can prevent ethanol-induced liver damage and increase GSH concentrations in rats with chronic alcohol consumption.

DOI: 10.1002/cbf

ACKNOWLEDGEMENTS

This study is supported by the National Science Council of Taiwan (NSC89-2320-B-038-034).

REFERENCES

1. Diplock AT. Antioxidant nutrients and disease prevention: an overview. *Am J Clin Nutr* 1991; **53**: 189S–193S.
2. Pan MH, Ho CT. Chemopreventive effects of natural dietary compounds on cancer development. *Chem Soc Rev* 2008; **37**: 2558–2574.
3. Palozza P, Krinsky NI. The inhibition of radical-initiated peroxidation of microsomal lipids by both alpha-tocopherol and beta-carotene. *Free Radic Biol Med* 1991; **11**: 407–414.
4. Kim Y, Chongviriyaphan N, Liu C, Russell RM, Wang XD. Combined antioxidant (beta-carotene, alpha-tocopherol and ascorbic acid) supplementation increases the levels of lung retinoic acid and inhibits the activation of mitogen-activated protein kinase in the ferret lung cancer model. *Carcinogenesis* 2006; **27**: 1410–1419.
5. Yang SC, Huang CC, Chu JS, Chen JR. Effects of beta-carotene on cell viability and antioxidant status of hepatocytes from chronically ethanol-fed rats. *Br J Nutr* 2004; **92**: 209–215.
6. Albanes D, Heinonen OP, Taylor PR, *et al*. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst* 1996; **88**: 1560–1570.
7. Palozza P. Can beta-carotene regulate cell growth by a redox mechanism? An answer from cultured cells. *Biochim Biophys Acta* 2005; **1740**: 215–221.
8. Lieber CS. Roles of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver disease. *Adv Pharmacol* 1997; **38**: 601–628.
9. Leo MA, Rosman AS, Lieber CS. Differential depletion of carotenoids and tocopherol in liver disease. *Hepatology* 1993; **17**: 977–986.
10. Ahmed S, Leo MA, Lieber CS. Interaction between alcohol and beta-carotene in patients with alcoholic liver disease. *Am J Clin Nutr* 1994; **60**: 430–436.
11. Mochizuki S, Yoshida A. Effects of dietary ethanol on ascorbic acid and lipid metabolism, and liver drug-metabolizing enzymes in rats. *J Nutr Sci Vitaminol* 1989; **35**: 431–440.
12. Chang CY, Chen YL, Yang SC, *et al*. Effect of schisandrin B and sesamin mixture on CCl(4)-induced hepatic oxidative stress in rats. *Phytother Res* 2009; **23**: 251–256.
13. Shyur LF, Huang CC, Lo CP, *et al*. Hepatoprotective phytocompounds from *Cryptomeria japonica* are potent modulators of inflammatory mediators. *Phytochemistry* 2008; **69**: 1348–1358.
14. Yang SC, Liu SM, Yang HY, *et al*. Soybean protein hydrolysate improves plasma and liver lipid profiles in rats fed high-cholesterol diet. *J Am Coll Nutr* 2007; **26**: 416–423.
15. Beers R, Sizer I. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952; **195**: 133.
16. Lederman JD, Overton KM, Hofmann NE, Moore BJ, Thornton J, Erdman JW. Ferrets (*Mustela putoius furo*) inefficiently convert β-carotene to vitamin A. *J Nutr* 1998; **128**: 271–279.
17. Lieber CS, DeCarli LM. Animal models of chronic ethanol toxicity. *Methods Enzymol* 1994; **233**: 585–594.
18. Pirola RC, Lieber CS. Energy wastage in rats given drugs that induce microsomal enzyme. *J Nutr* 1975; **105**: 1544–1548.
19. Huang CC, Chen JR, Liu CC, Chen KT, Shieh MJ, Yang SC. Effects of long-term ethanol consumption on jejunal lipase and disaccharidase activities in male and female rats. *World J Gastroenterol* 2005; **11**: 2603–2608.
20. Navder KP, Baraona E, Lieber CS. Polyenylphosphatidylcholine attenuates alcohol-induced fatty liver and hyperlipidemia in rats. *J Nutr* 1997; **127**: 1800–1806.
21. Lieber CS. Hepatic, metabolic and toxic effect of ethanol: 1991 update. *Alcohol Clin Exp Res* 1991; **15**: 573–592.
22. Bailey SM, Patel VB, Young TA, Asayama K, Cunningham CC. Chronic ethanol consumption alters the glutathione/glutathione peroxidase-1 system and protein oxidation status in rat liver. *Alcohol Clin Exp Res* 2001; **25**: 726–733.
23. Loguercio C, Clot P, Albano E, *et al*. Free radicals and not acetaldehyde influence the circulating levels of glutathione after acute or chronic alcohol abuse: *in vivo* and *in vitro* studies. *Ital J Gastroenterol Hepatol* 1997; **29**: 168–173.
24. Rosell M, Regnstrom J, Kallner A, Hellenius ML. Serum urate determines antioxidant capacity in middle-aged men-a controlled, randomized diet and exercise intervention study. *J Intern Med* 1999; **246**: 219–226.
25. Kawase T, Kato S, Lieber CS. Lipid peroxidation and antioxidant defense system in rat liver after chronic ethanol feeding. *Hepatology* 1989; **10**: 815–821.
26. Polavarapu R, Spitz DR, Sim JE, *et al*. Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil and fish oil. *Hepatology* 1998; **27**: 1317–1323.
27. Barua AB, Olson JA. Beta-Carotene is converted primarily to retinoids in rats *in vivo*. *J Nutr* 2000; **130**: 1996–2001.

DOI: 10.1002/cbf