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Carnitine and/or GABA Supplementation Increases Immune Function and Changes Lipid Profiles and Some Lipid Soluble Vitamins in Mice Chronically Administered Alcohol

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Abstract

This study evaluated the effects of carnitine and/or GABA supplementation on immune function, lipid profiles and some vitamins in mice chronically administered alcohol. BALB/c mice were fed with either AIN-76 diet (N), control diet plus alcohol (4 g/kg bw, E), E plus 0.5 g/kg bw carnitine (EC), E plus 0.5 g/kg bw GABA (EG), or E plus 0.5 g/kg bw carnitine plus 0.5 g/kg bw GABA (ECG) for 6 weeks. Administrations of the carnitine and/or GABA prevented alcohol-induced increases in triglyceride concentrations in serum and liver. However, there was no difference among the supplemented groups. Serum vitamin E concentration was higher in mice supplemented with EC and EG, but not in mice given ECG. Phagocytic activity of peritoneal macrophages was increased in EG group compared with E group. The subpopulations of murine splenocyte's T_H cells were increased significantly in EC and ECG groups. These data suggest that immune function, lipid profiles and some immune-related lipid soluble vitamins were positively changed by supplementation of carnitine or GABA, but do not show any synergistic effect of mixed supplementation.

Key words: carnitine, GABA, ethanol, immune function, immune related lipid soluble vitamins

INTRODUCTION

Nutritional status is one major extrinsic factor determining the efficiency of the immune and host defence systems (1,2). Chronic alcoholism is considered to be the most common cause of malnutrition in affluent societies of the western world, and is associated with a high risk of nutritional immunosuppression in chronic alcoholics. Alcohol supresses the immune response on at least two different levels; both specific and non-specific host defenses. As a nutrient, alcohol has a direct toxic effect on cells and organs of the host defense system. Alcohol further affects nutrient intakes and impairs absorption, utilization, storage and excretion of nutrients, and thereby may induce malnutrition, which affects immunocompetence (3). Alcohol-induced malnutrition together with the direct immuno-toxic effects of alcohol may reduce the effectiveness of the host defense against infections. Epideminological data have shown that infectious and contagious illnesses are the most common cause of death among alcoholics (4). Human and animal studies have found decreased thymus weight as well as decreased lymphocyte proliferation in response to mitogens in vitamin A deficiency (5,6). Carotenoids also have important immunoregulatory functions involving both T and B lymphocytes, NK cells and macrophages (7). Vitamin E is primarily known for its ability to function as an antioxidant but it may also play an important role in the immune response. Vitamin E interacts with free radicals in order to prevent the peroxidation of fatty acids and proteins. Vitamin E may therefore protect arachidonic acid from oxidation thereby influencing the production of pro-inflammatory prostaglandins. Animal studies have demonstrated impaired cell-mediated immunity and decreased antibody synthesis in severe vitamin E deficiency (7). In addition, neutrophil and macrophage migration and phagocytosis are impaired in vitamin E deficient rats (8).

Carnitine is a naturally occurring vitamin-like compound that acts as a carrier to transport long-chain fatty acids into mitochondria for β-oxidation (9). Animals acquire carnitine from dietary sources and by *in vivo* biosynthesis. Alcohol administration, both in humans and laboratory animals, results in hyperlipidemia, fatty liver and ultimately the most severe stage of alcoholic liver disease. Many previous studies have shown that carnitine supplementation lowers alcohol-induced increase in various lipid fractions in rat's liver in a dose-re-

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lated manner (10-13).

γ-aminobutyric acid (GABA) is a ubiquitous non-protein amino acid that is produced primarily from an α-decarboxylation of L-glutamic acid (Glu) catalyzed by the enzyme glutamate decarboxylase (GAD) (14). It is well known that GABA functions in animals as a major inhibitory neurotransmitter in the central nervous system (15). GABA is involved in the regulation of cardiovascular functions, such as blood pressure and heart rate, and plays a role in the sensation of pain and anxiety (16). Many neurological disorders, such as seizures, Parkinson's disease, stiff-man syndrome, and schizophrenia are related to alterations in GABA and GAD levels in the brain (16,17). In alcoholics, remarkably low plasma GABA levels and a low expression of the GABA receptor in the brain, have been measured (16,18). However, no attempts have been made to investigate the effects of carnitine. GABA, or both combined on immunoregulatory action or nutrients associated with immune function.

In this present work, we analyzed the level of immune-related lipid soluble vitamins, and tested the effect of supplementation of carnitine and/or GABA on immuneregulatory action in mice chronically administered with alcohol. We also analyzed the effects of carnitine and/or GABA supplementation on blood and hepatic lipid levels, and enzyme activities of mice chronically administered with alcohol. Finally, the possible roles of carnitine and GABA on the effects were discussed in relation to the present findings.

MATERIALS AND METHODS

Animal and diets

Male BALB/c mice (4 weeks) were purchased from Damul Science (Daejeon, Korea) and fed normal chow (Jeil-jedang, Suwon, Korea) for adaptation during the first week. They were then randomly divided into five groups of 8 each: control group (N), alcohol group (E), E plus carnitine supplemented group (EC), E plus GABA supplemented group (EG) and E plus carnitine and GABA supplemented group (ECG). Each group was fed a normal AIN-76 purified diet, as shown in Table 1. Each mouse was housed in a polycarbonate cage and kept in a controlled environment, temperature (23±1°C), humidity (53±2%), and a 12 hr/12 hr light-dark cycle. The experimental design and sample treatment are shown in Table 2. Experimental diets and water were provided *ad libitum*. The mice were orally administered 20% (v/v) alcohol (4 g/kg body weight) once a day for 6 weeks in the alcohol-treated groups and distilled water of same volume in the control groups. The mice were weighed once a week. Before the mice were sacrificed the diet was revolved from the cages for 12 hr. Blood samples were collected from each mouse and incubated on ice for 1 hr. Serum was separated from the blood by centrifugation at 1,100×*g* for 15 min at 4°C and kept at -80°C until analysis. The liver and abdominal fats were removed, rinsed with a phosphate buffered saline solution, wiped with a paper towel, weighed, quickly frozen in liquid nitrogen, and stored at -80°C until assayed.

Table 1. Composition of experimental diets

Ingredient	Percent (%)
Casein	20.0
Sucrose	50.0
Starch	15.0
Corn oil	5.0
Cellulose	5.0
Mineral mix	3.5
Vitamin mix	1.0
Choline bitartrate	0.2
DL-methionine	0.3
Total	100.0

Table 2. Experimental design and sample treatments

Sample	Groups				
	N	E	EC	EG	ECG
Carnitine (g/kg)	−	−	0.5	−	0.5
GABA (g/kg)	−	−	−	0.5	0.5
Alcohol (g/kg)	−	4	4	4	4

N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA.

Analysis of lipids and enzyme activities

Triglyceride concentrations in serum and liver were determined by the lipase-glycerol phosphate method (19) using a commercial kit (Asan Pharm. Co., Seoul, Korea). Serum and liver total cholesterol was determined using a commercial kit from Asan Pharm. Co., based on the cholesterol oxidase method (20). HDL-cholesterol was analyzed enzymatically using a commercial kit (Asan Pharm. Co.). The HDL-cholesterol fractions were prepared by the dextran sulfate-Mg^{++} method (21). LDL-cholesterol concentration were calculated by the Friedewald method (22).

Serum glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) levels were determined using commercial kits (Asan Pharm. Co.).

Analysis of vitamin A and E

Vitamin A and vitamin E were analyzed by HPLC. 0.5 g sample was placed in a 15 mL test tube with 5 mL ethanol and 0.1 g ascorbic acid. The test tube was kept in a water bath and 50% KOH was added. The sample was saponified for 15 min at 80°C. After saponification, the flask was placed in an ice bath, and 3 mL water

and 5 mL hexane were added. The mixture was transferred to centrifuge bottle and centrifuged at 600 $\times g$ for 5 min after which the upper layer was transferred to a 125 mL separatory funnel. Extraction of the sample with 5 mL hexane was repeated twice. The pooled hexane layers were washed three times with 5 mL water, filtered through Na_2SO_4 and then evaporated under a stream of nitrogen. The sample was diluted with 1 mL mobile phase. The HPLC system consisted of a Shimadzu (Tokyo, Japan) pump, Sil-10A injector, RF-10A fluorescence detector with excitation at 290 nm and emission at 330 nm for α-tocopherol; and for retinal, excitation at 330 nm and emission at 420 nm. The mobile phases were 95% methanol in water with a flow rate 1.3 mL/min for 1 hr a flow rate of 1.0 mL/min for α-tocopherol, and 85% methanol in water with a flow rate of 1.0 mL/min for retinal.

Protein and carnitine assay

Liver tissues were prepared for carnitine assay as follows: 50 mg of liver tissue was homogenized in 1.5 mL of cold distilled water using a sonicator (Fisher Scientific Co., USA), One volume (0.1 mL) of tissue extract was added to 9 volumes of 50 mmol/L KOH and allowed to sit overnight at room temperature. Non-collagen protein (NCP) was determined using a Bio-Rad protein assay (BIO-RAD Co., USA), based on the method of Bradford (23). Non esterified carnitine (NEC), acid-soluble acylcarnitine (ASAC), and acid-insoluble acylcarnitine (AIAC) in serum and tissues, were determined by the radio-enzymatic procedure of Cederblad and Lindstedt (24), as modified by Sachan et al. (25). In this method, AIAC was precipitated with perchloric acid and centrifugation leaving the ASAC and NEC in the supernatant. An aliquot of the supernatant was assayed to determine the NEC and another aliquot hydrolyzed with 0.5 N KOH to assay all acid-soluble carnitines (ASAC+ NEC). ASAC was calculated as the difference between the NEC and the total acid-soluble carnitines. The pellets containing the AIAC were drained, washed, and hydrolyzed in 0.5 N KOH for 60 min in a hot water bath at 60°C. In each case carnitine was assayed by using carnitine acetyl-transferase (Sigma, USA) to esterify the carnitine to a [^{14}C] acetate from [1-^{14}C] acetyl CoA (Amersham, UK). Radioacitivity of the samples was determined in a Bechman LS3801 liquid scintillation counter (Beckman Instruments, Palo Alto, USA).

Assay of lymphocyte subpopulations in splenocytes and thymocytes

BALB/c mice were sacrificed and their splenocytes and thymocytes were collected, thereafter the suspensions of cells were prepared at 1×10^6 cells/well and subpopulations were measured by a laser flow cytometer (excitation: 488 nm, emission: 525 nm/FITC, 575 nm/PE) staining with PE/FITC conjugated anti mouse B220/ Thy1 or CD4/CD8 monoclonal antibody (26).

Lymphokine (IFN-γ, IL-4) assay

The levels of cytokines IFN-γ and IL-4 in the serum were assayed by a two-site sandwich enzyme-linked immunosorbent assay (27). ELISA plates were coated with 100 μL (4 μg/mL) of the anti-mouse IFN-γ antibody or anti-mouse IL-4 in 0.1 M phospate buffer (pH 9.0) and incubated for 24 hr at 4°C. The wells were then washed with phosphate-buffered saline (PBS) and blocked with 150 μL of 1% BSA-PBS for 1 hr at room temperature. After washing, diluted serum and 100 μL of recombinant mouse IFN-γ were added to each well. After 1 hr of incubation at room temperature, the wells were washed and incubated with 100 μL (2 μg/mL) of the biotinylate conjugated anti-murine IFN-γ or IL-4 antibody as second antibodies for 1 hr at room temperature. After washing, diluted streptavidin-alkaline phosphatase (2 μg/mL) of 100 μL were added to each well and incubation for 1 hr at room temperature. After the final wash, the reaction was developed by the addition of p-nitrophenyl phosphate to each well. Optical densities were measured at 405 nm in a microplate reader.

Phagocytic activity of peritoneal macrophage assay

BALB/c mice were sacrificed and their pertioneal cavity was injected with 5 mL of cold-PBS. The cells were collected and washed by centrifugation at 290 $\times g$ for 5 min. The collected cells were suspended in RPMI 1640 medium in a petri dish. After incubation at 37°C for 2 hr in humidified 5% CO_2, the plates were then washed with RPMI 1640 medium to remove nonadherent cells and the adherent cells were considered macrophages. The cells collected by scraping were prepared at 1×10^7 cells/ mL in DEM (0.34 g/L $NaHCO_3$, 2.6 g/L Hepes, pH 7.2). The cell suspension (100 μL) and lucigenin of 50 μL were added to each well in microplate. After incubation at 37°C for 5 min, zymosan was added in each well. Lucigenin chemiluminescence was measured with a luminometer at 5 min intervals for 60 min (28).

Statistical analysis

Data from individual experiments are expressed as the mean ± standard deviation. All statistical analyses were performed on SAS software (SAS Institute, Cary, NC, USA). Significant differences between mean values were determined by Duncan's multiple range test (29); $p < 0.05$ was judged to be statistically significant.

Table 3. Weight gain, liver and abdominal fat weight of mice

	Groups				
	N	E	EC	EG	ECG
Feed consumption (g/day)	4.37±0.39[a]	3.87±0.23[b]	3.99±0.43[b]	4.29±0.22[a]	4.36±0.47[a]
Energy intake (kcal/day)	16.83±1.50[a]	14.90±0.89[b]	15.36±1.66[b]	16.52±0.85[a]	16.79±1.81[a]
Initial body weight (g)	18.16±0.62	18.23±0.75	18.71±1.01	18.75±0.68	18.45±0.91
Weight gain (g)	5.76±0.93[a]	4.78±0.35[b]	5.72±0.78[a]	5.89±0.49[a]	5.96±0.26[a]
Feed efficiency	1.32±0.22[ab]	1.24±0.32[b]	1.43±0.48[a]	1.37±0.29[ab]	1.37±0.46[ab]
Liver/BW (%)	4.36±0.33[c]	4.69±0.11[bc]	4.96±0.01[a]	5.49±0.43[a]	4.91±0.46[b]
Abdominal fat/BW (%)	2.07±0.28[a]	1.59±0.12[b]	1.42±0.11[bc]	0.96±0.22[d]	1.20±0.22[cd]

All values are mean±SD (n=8). Values with different letters within the same row are significantly different at $p<0.05$ by Duncan's multiple range test. N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA.

RESULTS

Food intake, body weight gain, and abdominal fat deposition

There were no significant differences in initial body weight among the groups. Feed consumption and energy intake were lower in E and EC group compared with N, EG and ECG group. Also, weight gain was significantly lower in E group compared with the other groups. However, carnitine and/or GABA supplementation prevented the decreased weight gain resulting from the alcohol consumption (Table 3).

Lipid levels and enzyme activities

The lipid profiles of serum and liver are shown in Fig. 1. Triglycerides in serum and liver were decreased in carnitine and/or GABA supplemented groups (EC, EG and ECG) compared to the E group. Serum total cholesterol was significantly decreased in the E, EC, EG and ECG groups compared with N group. Serum HDL cholesterol was lower in the E group than in the N group, however, it was increased in EG and ECG groups. Serum LDL cholesterol was lower in the EC, EG and ECG group compared with N and E groups. There was no significant differences in total liver cholesterol among the groups. The carnitine and/or GABA supplementation significantly decreased the serum GOT and GPT level that was increased due to the alcohol administration (Fig. 2).

Carnitine concentration

Serum carnitine (TCNE, NEC) concentrations were higher in only the groups (EC and ECG) supplemented with carnitine. Levels of serum ASAC and liver TCNE were higher in EC group than E group. AIAC in serum and NEC, ASAC, AIAC in liver carnitine concentration were higher in EC, EG and ECG groups than E group. Serum acyl/free carnitine was higher in the N group than in the other groups. However, there were no significant differences in liver acyl/free carnitine among the groups (Table 4).

Vitamin A and vitamin E concentration

Serum vitamin A concentration was decreased in E group compared with the N group, but increased in the EC, EG and ECG group by carnitine and/or GABA supplementation. Serum vitamin E concentration in the EC, EG and ECG group was higher than E group (Fig. 3).

Lymphocytes subpopulation change in mice splenocytes and thymocytes

There were no significant differences in B cell, T cell and Tc/Ts in splenocytes and T_H and Tc/Ts in thymocytes among the groups. In splenocytes, T_H was higher in only the groups supplemented with carnitine (EC and ECG) than E group (Fig. 4).

Lymphokin (IFN-γ and IL-4) production in mice serum

Serum IFN-γ and IL-4 concentration was significantly decreased in E group compared with the N group, but increased in the EC, EG and ECG group by carnitine and/or GABA supplementation (Fig. 5).

Phagocytic activity of peritoneal macrophage

Phagocytic activity of peritoneal macrophage in mice are shown in Fig. 6. Phagocytic acitivity of peritoneal macrophage was increased only in the EG group by GABA supplementation.

DISCUSSION

In this study we evaluated whether carnitine and/or GABA supplementation alters immune function and immune-related nutrients in mice chronically administerd with alcohol. Body weight gain was decreased significantly in the E group by alcohol administration compared with the N group. Previously, it was shown that over consumption of alcohol leads to decreased body weight (30,31). This is because alcohol lacks essential nutrients, other than calories, which results in primary malnutrition by displacing other nutrients in the diet (32). Therefore, the combination of repeated treatment of alcohol with decreased feed consumption may induce

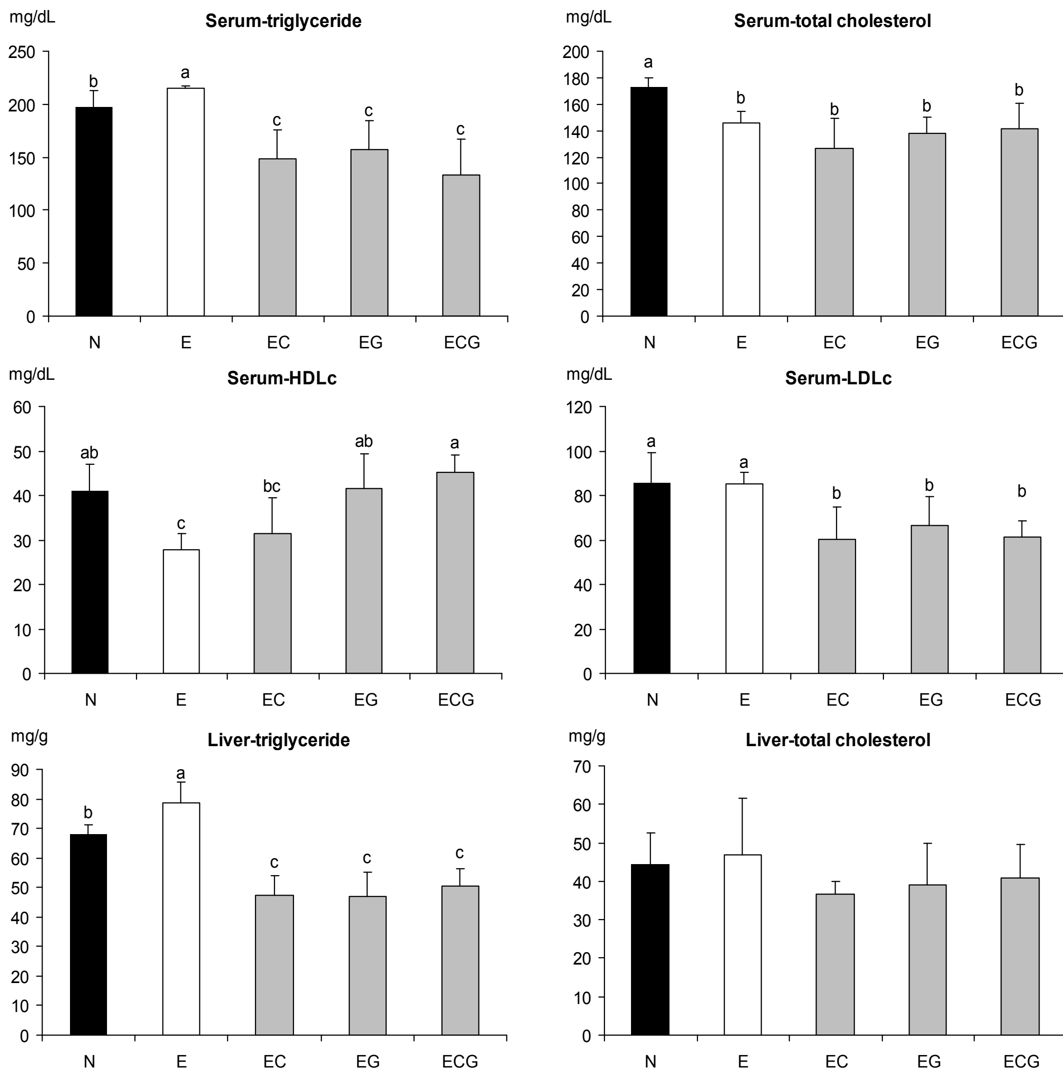

Fig. 1. Effects of carnitine and/or GABA adminstration on serum and liver lipid concentrations in mice. Different letters incidate significant differences at $p<0.05$ by Duncan's multiple range test. N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA.

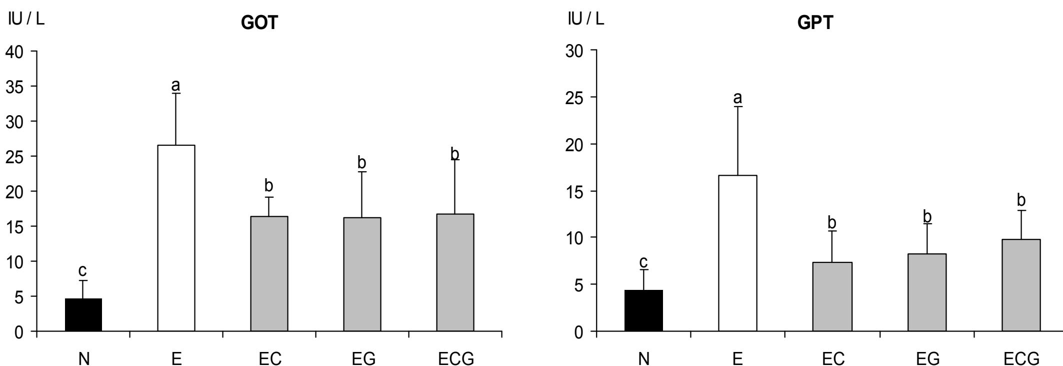

Fig. 2. Serum enzyme activities. Different letters incidate significant differences at $p<0.05$ by Duncan's multiple range test. N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase.

malnutrition in a relatively-short period. In this study, feed consumption was decreased by alcohol administration in E group, but remained normal in EG and ECG group.

Alcohol and fat metabolism have a close relationship. The increase of NADH in the liver by alcohol metabo-

Table 4. Concentration of carnitine and ration of acyl/free carnitine in serum and liver

	N	E	EC	EG	ECG
Serum (μmol/L)					
NEC	18.03 ± 4.37^{b}	15.79 ± 6.01^{b}	24.37 ± 8.95^{a}	18.75 ± 6.5^{b}	32.52 ± 12.69^{a}
ASAC	22.15 ± 4.15^{a}	7.84 ± 3.71^{b}	18.5 ± 4.46^{a}	10.97 ± 5.57^{b}	15.86 ± 7.51^{ab}
AIAC	0.32 ± 0.27^{b}	0.19 ± 0.08^{c}	0.57 ± 0.39^{ab}	0.48 ± 0.24^{ab}	0.83 ± 0.44^{a}
TCNE	40.17 ± 4.63^{a}	23.84 ± 6.35^{b}	40.5 ± 6.88^{a}	27.22 ± 7.65^{b}	40.14 ± 4.33^{a}
Acyl/Free	1.46 ± 0.42^{a}	0.48 ± 0.21^{b}	0.88 ± 0.35^{b}	0.62 ± 0.08^{b}	0.52 ± 0.23^{b}
Liver (nmol/mg NCP)					
NEC	3.14 ± 0.09^{a}	2.78 ± 0.06^{b}	4.11 ± 0.10^{a}	3.28 ± 0.06^{a}	3.80 ± 0.68^{a}
ASAC	1.00 ± 0.22^{a}	0.71 ± 0.02^{b}	1.03 ± 0.01^{a}	1.01 ± 0.20^{a}	1.00 ± 0.11^{a}
AIAC	0.52 ± 0.13^{a}	0.2 ± 0.01^{b}	0.46 ± 0.05^{a}	0.51 ± 0.10^{a}	0.56 ± 0.10^{a}
TCNE	4.57 ± 0.44^{ab}	3.69 ± 0.90^{b}	5.21 ± 1.33^{a}	4.03 ± 0.78^{ab}	4.50 ± 0.69^{ab}
Acyl/Free	0.48 ± 0.12	0.31 ± 0.16	0.36 ± 0.15	0.45 ± 0.20	0.40 ± 0.18

All values are mean±SD (n=8). Values with different letters within the same row are significantly different at $p<0.05$ by Duncan's multiple range test. N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA; NEC, non-esterified carnitine; ASAC, acid-soluble acylcarnitine; AIAC, acid-insoluble acylcarnitine; TCNE, total carnitine; acyl/free, (ASAC+AIAC)/NEC; NCP, non-collagen protein.

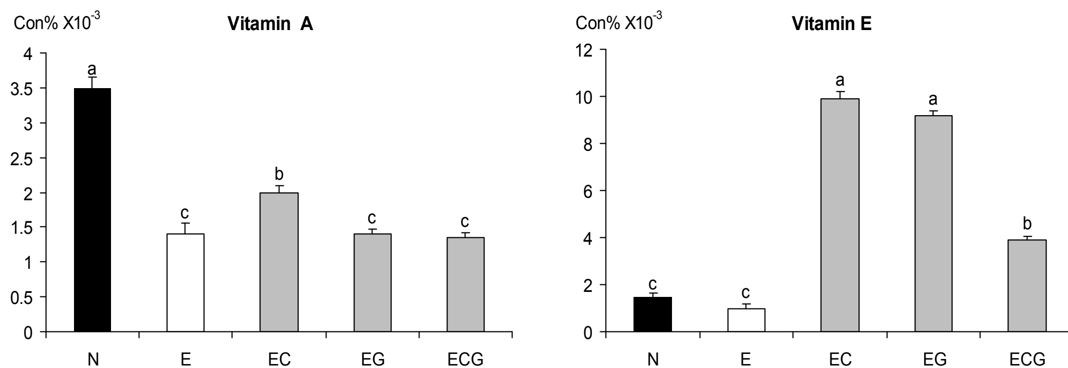

Fig. 3. Concentration of vitamin A and vitamin E in serum (con%$\times 10^{-3}$). Different letters incidate significant differences at $p<0.05$ by Duncan's multiple range test. N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA.

lism affects fat metabolism and results in the triglyceride accumulation with the liver and blood. It was observed that carnitine supplementation lowered alcohol-induced increases of various lipid fractions (11). We previously reported that the supplementation of carnitine/GABA prevented alcohol induced increases in serum triglyceride concentrations (33). The extreme alcohol drinking could injure internal organs such as liver, heart and pancreas. In this present study, the activities of GOT and GPT were increased by alcohol administration in E group, but decreased in carnitine and/or GABA supplemented group. The GOT and GPT are essential enzymes determining te status of liver diseases. In general, impaired liver function is signaled by the increased GOT and GPT amount in blood (34,35). It has been reported that the activities of GOT and GPT increase as a result of problems with liver metabolism and loss of liver cells by alcohol intake (35). Carnitine is an essential co-factor in the transfer of long-chain fatty acyl groups from the outer mitochondria membrane into the inner mitochondrial matrix for β-oxidation to acetyl coenzyme A (9). Other studies reported that carnitine levels and CPT-I activity were decreased by alcohol may induce deterioration in liver function. However, carnitine supplementation has been previously shown to increase plasma carnitine concentrations (36,37). In the present study, carnitine levels in serum and liver were decreased by alcohol administration, but were replaced in groups with carnitine supplementation (EC and ECG). Early studies reported that α-tocopherol in hippocampus, hypothalamus, striatum, cerebellum and cortex is increased by L-carnitine supplementation (300 mg/bw). The accumulation of lipofuscin was also found to be decreased after L-carnitine administration (38). The data suggests that decrement of lipofuscin accumulation by L-carnitine may be partially due to its antioxidant activity.

Vitamins (vitamin A and E) have been associated with improved immune response. Several studies have shown that the serum antioxidant status (α-tocopherol, retinol, β-carotene, selenium, zinc) in alcoholics is significantly

Fig. 4. Lymphocytes subpopulation change in splenocytes and thymocytes. The cells were collected and lymphocytes subpopulation were measured by a flow cytometer staining with PE or FITC conjugated anti-B220/Thy1 or CD4/CD8 monoclonal antibody. Different letters incidate significant differences at $p<0.05$ by Duncan's multiple range test. N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA.

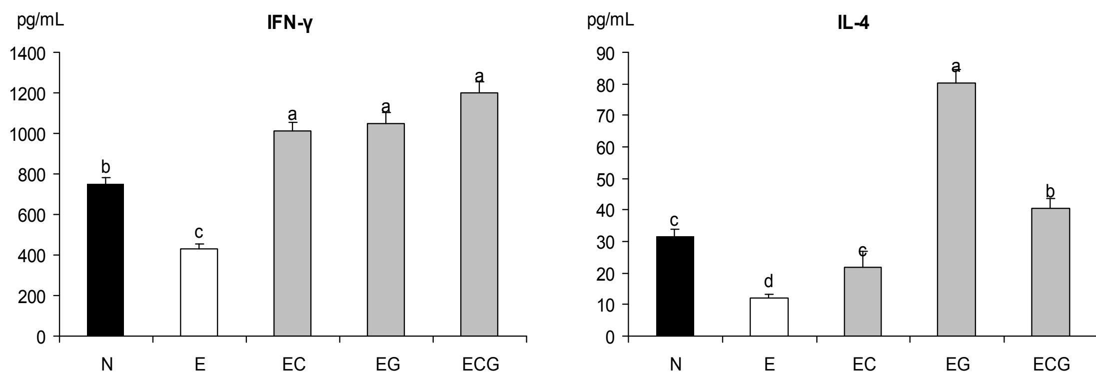

Fig. 5. IFN-γ and IL-4 production in carnitine and GABA-administered mice serum. Different letters incidate significant differences at $p<0.05$ by Duncan's multiple range test. N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA; IFN-γ, γ-interferon; IL-4, interleukin 4.

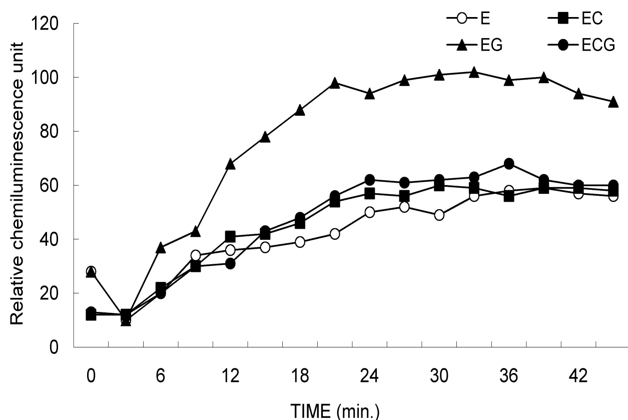

Fig. 6. Phagocytic activity of peritoneal macrophage in mice. The peritoneal macrophages were collected and cultured in RPMI1640 media mixed with opsonized zymosan. Lucigenin chemiluminescence was measured 5 minute intervals for 50 minutes. The EG group was significantly higher than the other groups at $p<0.05$ by Duncan's multiple range test. N, AIN 76 diet; E, N plus alcohol; EC, E plus carnitine; EG, E plus GABA; ECG, E plus carnitine plus GABA.

decreased compared with controls (39,40). Vitamins A and E have important immunoregulatory functions involving both T and B lymphocytes, NK cells and macrophages (6-7,41). In this study, vitamin A levels that were decreased by alcohol were increased in carnitine supplemented group (EC). Plasma vitamin E was 9-fold higher with carnitine supplementation (EC) and GABA supplementation (EG) compared with the alcohol adminstered group (E).

Lymphocytes are the central cells of the immune system, responsible for acquired immunity and the immunologic attributes of diversity, specificity, memory, and self/nonself recognition. These cells can be broadly subdivided on the basis of function and cell-membrane components into T, B and NK cells. T lymphocyes are divided into CD4+(T_H) and CD8+(Tc) cells. T_H cells secrete various cytokines, which play a central role in the activation of B cells, Tc cells and a variety of other cells that participate in the immune response. Changes in the pattern of cytokines produced by T_H cells can result in qualitative change in T_H1 response, which results in a cytokine profile that activates mainly cytotoxic T cells and macrophages, whereas the T_H2 response activates mainly B cells. It is known that T_H1 cells mainly produce γ-interferon and IL-2, and T_H2 cells produce the IL-4, IL-5, IL-6 and IL-10 (42). In a study by Poddar et al. (43), the effect of dietary protein on hypothalamic GABAergic activity and immune resonse of rats in relation to age was studied. The age-induced (due to increase of age from 3 to 18 months) decrease in hypothalamic GABAergic activity and immune responses were potentiated with the supplementation of protein rich diet under both short- and long-term conditions. Longterm consumption of protein-poor diet, in contrast, activated hypothalamic GABAergic activity with an immunopotentiation as age increased from 3 to 18 months; whereas, short-term supplementation of low protein diet did not show any effect (43).

To determine the effects of the cell proliferative response *in vivo*, carnitine and GABA were administered to mice, and then the subpopulations of B and T_H cells were measured. The subpopulations of T_H cells were increased significantly by the carnitine and carnitine with GABA treatments. These results suggest that carnitine or GABA has an immunoregulatory effect. Carnitine might be important for energy substrate's transport and the energy charge required for immune cell regeneration (44). It is known that macrophages have important roles in phagocytic activity, antigen presentation, apoptotic activity, and lymphocyte activation *in vivo* (45). Most macrophage functions are performed after that cell acquires various capacities (e.g., the ability to produce nitric oxide). Those capacities are often attained through interaction with the microenvironment. Signals from a variety of sources, including the extracellular matrix, foreign macromolecules, and other cells, can determine the activational state and thus the role that macrophages will play in homeostasis or in pathologic processes. Those functions, *in vivo* and *in vitro* are exquisitely sensitive to alterations of several dietary components. Other studies have confirmed that micronutrients (e.g., vitamins and trace elements) can significantly alter macrophage function and thus alter their role in innate immunity, inflammation, and several disease processes (46-51). In the present study, to determine the changes in phagocytic activity of peritoneal macrophages, the peritoneal macrophages were collected from the carnitine and/or GABA-fed mice and cultured with opsonized zymosan, and lucigenin chemiluminescence was measured. Phagocytic activity increased only in GABA-administered mice compared with E group, and the supplementation of GABA increased levels of vitamin A and E in serum. In a recent study, GABA-enriched plant extracts stimulated phagocytic activity of peritoneal macrophage and nitric oxide production from peritoneal macrophage (52-54). GABA occurs naturally in many kinds of foods at low levels. These studies suggested that the supplementation of extracts with enhanced GABA levels can stimulate apoptotic activity via nitric oxide production (55,56).

T_H1 cells produce IL-2 and IFN-γ, which increase cell-mediated immune responses, while T_H2 cells produce IL-4, IL-5 and IL-10, which stimulate antibody production by B cells and up-regulate humoral immune re-

sponses (57). INF-γ and IL-4 were also decreased in E group compared with N group, but increased by all supplemented groups. This data suggest that both of T_H1 cells and T_H2 cells were stimulated by the treatment of carnitine and/or GABA. Overall, these results suggest that the carnitine and/or GABA effectively improved immunoregulatory action, possibly due to the enhanced vitamin A and vitamin E concentration in blood and replaced feed consumption and weight gain, but not exhibit any synergistic effect of mixed supplementation.

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