J Food Sci Nutr
Vol 9, p 58~64 (2004)

J Food Science and Nutrition

Gamma-Aminobutyric Acid and/or Carnitine Supplementation Alters Lipid and Some Immune Related Nutrient Levels in Mice

Ju-Ryoun Soh and Youn-Soo Cha[†]

Department of Food Science and Human Nutrition, Chonbuk National University, Chonju 561-756, Korea

Abstract

This study investigated the effects of carnitine and/or γ-aminobutyric acid (GABA) supplementation on lipid profiles and some immune related nutrient in mice. Balb/c male mice were orally treated with either an AIN-76 diet (Con), a control diet plus carnitine (CS, 0.5 g/kg bw), a control diet plus GABA (GS, 0.5 g/kg bw) or a control diet plus carnitine plus GABA (CGS, 0.25 g/kg bw, respectively) for 6 weeks. There were no significant differences in feed consumption, energy intake, body weight gain or feed efficiency ratio among the groups during the experimental period. However, abdominal fat deposits were smaller in CS, GS and CGS groups compared with the Con group. Serum and liver triglycerides also were lower in CS, GS and CGS and serum total cholesterol was significantly lower in the CGS group compared with the Con group. Serum LDL cholesterol was lower in the CGS group and liver HDL cholesterol was significantly higher in the CS group compared with Con group. In serum, stearic acid and selecholeic acid were lower, but arachidic acid was higher in the GS group. Liver stearic acid was higher but oleic acid lower in CGS group compared with Con group. In carnitine supplemented groups, serum and liver nonesterified carntine (NEC), acidsoluble acylcarnitine (ASAC), total carnitine (TCNE) concentrations were higher in only the CS group, not CGS group. Serum vitamin A and E concentrations were not different among the groups. These results may suggest that carnitine and/or GABA supplementation improves lipid profiles in mice, but did not affect the immune-related nutrients that we measured under the experimental conditions of this study.

Key words: carnitine, gamma-aminobutyric acid (GABA), immune

INTRODUCTION

Carnitine, is a quaternary amine (β-hydroxy-γ-N-trimethylammonium butyric acid), that can be found in almost all cells of higher animals (1). It is an essential co-factor in the transfer of long-chain fatty acyl groups (fatty acids with 10 or more carbon atoms) from the outer mitochondria membrane into the inner mitochondiral matrix for β-oxidation to acetyl coenzyme A (2). Ethanol 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 ethanol-induced increases in various lipid fractions in rat's liver in a dose-related manner (3-6).

γ-aminobutyric acid (GABA) is a ubiquitous nonprotein amino acid that is produced primarily by the α-decarboxylation of L-glutamic acid (Glu) catalyzed by the enzyme glutamate decarboxylase (GAD) (7). It is well known that GABA functions in animals as a major inhibitory neurotransmitter (8,9). GABA is involved in the regulation of cardiovascular functions, such as blood pressure and heart rate, and plays a role in the sensations of pain and anxiety (10). Many neurological disorders, such as seizures, Parkinson's disease, stiff-man syndrome, and schizophrenia are known to be related to alterations of the GABA and GAD levels in the brain (10,11). Several researchers have attempted to identify irregularities in GABA metabolism resulting from chronic alcohol consumption; they found that alcoholics have remarkably low plasma GABA concentrations and reduced expression of GABA receptors in the brain (10,12).

Accumulation of carnitine by rat brain slices and synaptosomes was found to be competitively inhibited by GABA. It was also demonstrated that the uptake of GABA by the high-affinity system is competitively inhibited by carnitine, while the low-affinity system was inhibited in a mixed way. All these observations suggested that the carnitine entry into neural cells could be affected by GABA and visa versa. Carnitine up-take in rat brain slices is inhibited by GABA as a result of competition at a common carrier site (13-15). These

[†]Corresponding author. E-mail: cha8@chonbuk.ac.kr
Phone: +82-63-270-3822, Fax: +82-63-270-3854

studies indicated that carnitine and GABA have a nutrient-nutrient interaction in *in vivo* systems.

Recently, there is increasing interest in the utilization of GABA and carnitine as functional substances. In fact, several lines of evidence have shown that supplementation of carnitine or GABA promotes improvements in lipid profiles (16), immune function (17,18), and alcohol related disease symptoms (16). However, no attempts have been made to investigate the effects of carnitine. GABA, or both combined on immunoregulatory action or nutrients which is associated with immune function.

The aim of this study was to investigate whether carnitine and/or GABA supplementation alters lipid profiles and some immune-related nutrients in mice. These results will provide useful basic data for future investigations of the effects of carnitine and/or GABA supplementation on immune function.

MATERIALS AND METHODS

Animal and diets

Male Balb/c mice, aged 4 weeks, were purchased from Damul Science. (Dajeon, Korea). They were fed a normal chow (Jeil-jedaing, Suwon, Korea) for adaptation during the first week, and were then randomly divided into four groups of 8 each: control group (Con), carnitine supplemented group (CS), GABA supplemented group (GS), and carnitine and GABA supplemented group (CGS). 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 diet and water were provided *ad libitum*. The mice were orally administered carnitine and GABA once a day for 6 weeks in the supplement groups as shown in Table 2, and given distilled water of the same volume in the control group. The mice were weighed once a week. Before the mice were sacrificed the diet was removed from the cages for 12 hr. Blood samples were collected form each mouse and incubated on ice water for 1 h. Serum was separated from the blood by cnetrifugation at 1,100×g for 15 min at 4°C and kept at -80°C until analysis. The liver and abdominal fat 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[1)]

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

[1)]All components are in units of g/100 g diet.

Table 2. Experimental design and sample treatments

Sample	Groups[1)]			
	Con	CS	GS	CGS
Carnitine (g/kg)	-	0.5	-	0.25
GABA (g/kg)	-	-	0.5	0.25

[1)]Con, control diet; CS, carnitine supplemented; GS, GABA supplemented; CGS, carnitine plus GABA supplemented.

Analysis of lipids

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. (Seoul, Korea), based on the cholesterol oxidase method (20). HDL-cholesterol was analyzed enzymatically using a commercial kit (Asan Pharm. Co., Seoul, Korea). The HDL-cholesterol fractions were prepared by the dextran sulfate-Mg^{++} method (21). LDL-cholesterol concentrations were calculated by the Friedwald method (22).

Analysis of total fatty acid and vitamins

Total fatty acid composition was determined by making methyl esters (23) that were analyzed by gas chromatography (Hewlett-packard Co., USA). Vitamin A and vitamin E were analyzed by HPLC. A five hundred mg sample was placed in a 15 mL test tube with 5 mL ethanol and 0.1 g ascorbic acid. The test tube was placed 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×g for 5 min 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 high-performance liquid chromatography (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, μ-Bondapak C18 (Waters, Milford, MA) 5 μm column, 30 cm 3.9 mm i.d. was used. The mobil 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-tocoperol, and 85% methanol in water with a flow rate of 1.0 mL/min for retinal.

Protein and carnitine assay

Liver was prepared for carnitine assay as follows: 50 mg of liver 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 (0.9 mL) 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 (24). Non-esterified carnitine (NEC), acid-soluble acylcarnitine (ASAC), and acid-insoluble acylcarnitine (AIAC) in serum and tissues, were determined by the radioenzymatic procedure of Cederblad and Lindstedt (25), as modified by Sachan et al. (26). 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 acetyltransferase (Sigma, UAS) to esterify the carnitine to a [^{14}C]acetate from [1-^{14}C]acetyl CoA (Amersham, UK). Radioactivity of the samples was determined in a Beckman LS3801 liquid scintillation counter (Beckman Instruments, Palo Alto, USA).

Statistical analysis

Data from individual experiments are expressed as the mean ± standard deviation. All statistical analyses were performed on SAS version 8 (SAS Institute, Cary, NC, USA). Significant differences between mean values were determined by Duncan's multiple range test (27); $p < 0.05$ was judged to be statistically significant.

RESULTS AND DISCUSSION

Food intake, body weight gain, and abdominal fat deposition

There were no significant differences in feed consumption, energy intake, body weight gain and feed efficiency ratio among the groups during the experiment period (Table 3). However, abdominal fat deposits were smaller in carnitine and/or GABA supplemented groups (Fig. 1). Adiposity located centrally in the abdominal region is distinctly associated with hyperlipidemia, compared with generalized distributions of body fat, and is also associated with lipoprotein abnormalities characterized by elevated VLDL and LDL concentrations. This suggested that supplementation of carnitine and/or GABA can decrease the risk for coronary heart disease. Leptin is an adipocyte-secreted hormone that regulates weight centrally. Several in vivo studies (28,29) have suggested a potential role for leptin in modulating the immune response. Leptin could be a link between nutritional

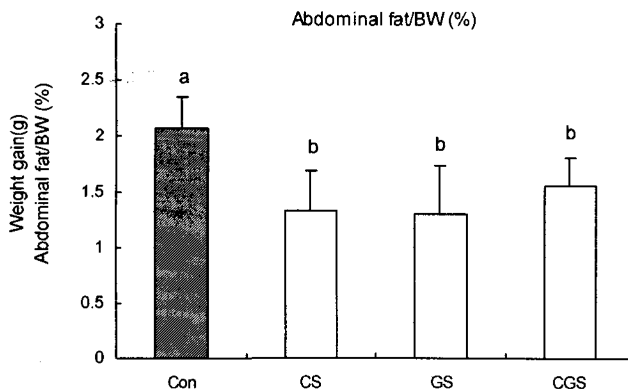

Fig. 1. Effects of Carnitine and/or GABA on abdominal fat rate. The error bars show the standard deviation of the mean for 8 rats. Letters above the bars are indicate significantly differences ($p < 0.05$) by Tukey's test. Con, control diet; CS, carnitine supplemented; GS, GABA supplemented; CGS, carnitine plus GABA supplemented.

Table 3. Effect of carnitine and/or GABA supplementation on feed consumption and body weight gain in mice

	Groups[1]			
	Con	CS	GS	CGS
Feed consumption (g/day)	4.37±0.39[2]	3.89±0.72	3.80±0.07	4.19±0.50
Energy intake (kcal/day)	16.83±1.50	14.96±2.76	14.62±0.27	16.12±1.93
Initial body weight (g)	18.16±0.62	18.88±1.05	18.41±0.77	18.15±0.57
Weight gain (g)	7.30±0.64	6.89±1.05	7.31±1.04	6.77±0.83
Feed efficiency ratio[3]	1.70±0.22	1.67±0.14	1.85±0.12	1.65±0.09

[1]See the legend of Table 2.
[2]All values are mean±SD (n=8).
[3]Feed efficiency ratio was calculated as weight gain (day) / dietary intake (day).

status and the immune system. Palacio et al. (28) reportd that low leptin levels found in malnourished infants have been associated with suppression of the lymphoproliferative response, and weight gain is followed by a significant increase in circulating leptin levels in parallel with a significant increase in Th1 activity (29). We did not assay serum leptin concentrations. We did not anticipate differences in leptin concentrations resulting from carnitine and/or GABA supplementation because our experimental conditions maintained a normal nutritional status for all animals. Further study is needed to investigate how carnitine and/or GABA supplementation modulates mice abdominal fat ratio without weight change in a normal state, and to elucidate the metabolic pathway involved.

Lipid levels and total fatty acid composition

It was observed that carnitine supplementation lowered ethanol-induced increases in various lipid fractions (4). We previously demonstrated that the supplementation of carnitine/GABA prevented ethanol induced increases in serum triglyceride concentrations (16). In this study, serum and liver triglycerides were decreased in all carnitine and/or GABA supplemented groups compared to the Con group. Serum total cholesterol was significantly decreased in the CGS group compared with Con group. Serum HDL cholesterol was significantly increased in the GS and CGS groups compared with Con group. Serum LDL-cholesterol concentrations are generally considered to be a good indicator of abnormal lipoprotein metabolism, and are directly correlated with risk for coronary heart disease and atherosclerosis (30,31). In this study, serum LDL cholesterol was only lower in the CGS group. In liver, HDL cholesterol was significantly increased in the CS group compared with the Con group (Table 4). Accordingly, we expect that carnitine and/or GABA supplementation may improve lipid parameters in mouse serum and liver.

In serum, stearic acid (C18:0) and selecholeic acid (C24:1) were lower but arachidic acid (C20:0) was higher in the GS group. Liver stearic acid (C18:0) was higher but oleic acid (C18:1) was lower in the CGS group (Table 5). There is little data on the effects of carnitine and/or GABA on free fatty acid concentrations. Therefore, further study is needed to determine how carnitine and/or GABA modulates the metabolism of free fatty acids.

Carnitine concentration

Carnitine supplementation has been previously shown to increase plasma carnitine concentrations (32-35). In this study, serum and liver carnitine (TCNE, NEC, and ASAC) concentrations were higher in only the group supplemented with only carnitine (Table 6). However serum and liver AIAC was the same in all groups. Supplementation with carnitine and antioxidants has been shown to increase serum AIAC only in long-term trained rats, which most likely reflects an increase in free fatty acid release from adipose tissue. Furthermore, this increase was significant, the AIAC increment in serum probably reflected an enhanced flux from serum to other organs through β-oxidation during exercise. Others have shown that maximal intensity exercise, but not submaximal exercise, causes a depletion of free carnitine accompanied by increased acylcarnitine (34-36). Previously, we showed that both exercise and a high fat diet can increase blood carnitine concentrations (37). We also showed that exercise alone increases carnitine concentrations (37,38). An interesting finding in the present study was the changes serum carnitine concentration by GABA supplementation (Table 6). Another study reported interactive effects of choline and carnitine in normal healthy humans and animals. Choline supplementation resulted in decreased urinary excretion of carnitine in young adult women. Guinea pigs were shown to be a suitable animal model for studying the effect

Table 4. Effects of carnitine and/or GABA administration on serum and liver lipid concentrations in mice

	Groups[1)]			
	Con	CS	GS	CGS
Serum (mg/dL)				
Triglyceride	209.46±20.70[2)a3)]	169.54±20.89[b]	157.51±27.73[b]	156.91±33.33[b]
Total cholesterol	177.59±10.75[a]	169.52±11.33[ab]	171.99±10.44[a]	151.36±27.53[b]
HDL-cholesterol	41.15±5.79[b]	50.88±11.34[ab]	64.16±9.78[a]	62.83±6.55 [a]
LDL-cholesterol	88.59±13.07[a]	79.97±8.54[a]	73.47±9.96[a]	54.78±13.57[b]
Liver (mg/g)				
Triglyceride	68.17±2.96[a]	47.69±5.12[b]	52.58±12.70[b]	55.23±6.21[b]
HDL-cholesterol	1.12±0.12[b]	1.90±0.30[a]	1.59±0.37[ab]	1.76±0.39[ab]

[1)]See the legend of Table 2.
[2)]All values are mean±SD (n=8).
[3)]Values with different superscripts in the same rows are significantly different ($p<0.05$).

Table 5. Effects of carnitine and/or GABA administration on serum and liver total fatty acid composition in mice

	Groups[1)]			
	Con	CS	GS	CGS
Serum (Con.%)				
C14:0	8.81±3.89[2)]	12.94±5.41	5.15±3.99	12.07±5.64
C16:0	10.51±2.14	11.18±2.97	13.41±3.75	11.29±3.04
C16:1	13.08±4.91	7.95±3.56	13.20±4.50	10.68±3.04
C17:0	3.12±1.59	4.63±1.99	4.30±1.72	5.18±2.58
C18:0	12.54±1.84[a3)]	11.44±1.78[a]	8.06±0.95[b]	11.00±1.40[a]
C18:1n9	5.43±2.51	5.54±2.09	5.43±2.88	3.91±1.79
C18:2n6	7.72±3.90	7.51±3.74	13.70±4.63	7.67±3.48
C20:0	14.38±2.95[b]	13.57±2.40[b]	18.62±1.59[a]	11.38±3.01[b]
C22:2	11.54±3.51	9.14±3.77	9.60±4.00	13.32±4.08
C24:0	8.37±3.17	11.51±4.08	7.92±3.88	7.90±3.79
C24:1	4.50±2.33[a]	4.59±1.56[a]	0.62±0.37[b]	5.62±2.90[a]
Liver (Con.%)				
C14:0	7.86±3.99	11.12±4.50	8.38±3.81	8.91±3.72
C16:0	6.85±1.76	6.38±1.90	5.29±1.45	5.66±1.88
C16:1	6.36±3.21	6.83±3.74	5.39±3.59	9.26±4.02
C17:0	7.77±2.04	4.88±2.51	8.50±3.96	4.25±2.99
C18:0	4.58±1.37[b]	7.54±2.34[ab]	7.73±2.17[ab]	11.87±1.92[a]
C18:1n9	15.96±3.41[a]	11.64±2.98[ab]	12.46±2.73[ab]	9.95±2.43[b]
C18:2n6	8.62±3.72	10.49±4.19	9.94±2.41	7.85±3.72
C18:3n6	8.51±3.92	5.36±2.84	6.96±2.95	6.11±3.01
C20:1	7.50±0.55	7.03±0.76	7.07±1.09	8.28±1.94
C20:3n3	9.92±2.96	10.60±3.72	12.08±4.17	12.44±3.99
C22:0	9.75±2.74	11.35±3.63	11.54±2.92	11.80±1.82
C23:0	6.32±3.91	6.79±2.84	4.66±1.79	3.61±2.09

[1)]See the legend of Table 2.
[2)]All values are mean±SD (n=8).
[3)]Values with different superscripts in the same rows are significantly different ($p<0.05$).

Table 6. Concentration of carnitine and ratio of acyl/free carnitine in serum

	Groups[1)]			
	Con	CS	GS	CGS
Serum (μmoL/L)				
NEC	18.03±4.37[2)b3)]	38.25±6.87[a]	33.67±11.84[a]	41.05±8.27[a]
ASAC	22.15±4.15[b]	34.10±6.12[a]	30.78±7.72[a]	21.05±6.43[b]
AIAC	0.32±0.27	0.65±0.48	0.56±0.69	0.66±0.26
TCNE	40.17±4.63[c]	69.98±5.23[a]	63.34±9.7[b]	59.59±7.85[b]
Acyl/Free	1.46±0.42[a]	0.84±0.19[b]	0.98±0.31[b]	0.58±0.33[b]
Liver (nmoL/mg NCP)				
NEC	3.14±0.8[b]	4.51±0.71[a]	3.62±0.80[ab]	4.22±0.94[ab]
ASAC	1.001±0.227[b]	1.243±0.001[a]	1.055±0.097[ab]	1.074±0.195[ab]
AIAC	0.52±0.13	0.56±0.05	0.54±0.09	0.55±0.10
TCNE	4.57±0.44[b]	5.98±1.67[a]	4.65±0.53[b]	5.13±0.72[ab]
Acyl/Free	0.48±0.12	0.39±0.13	0.45±0.13	0.38±0.17

[1)]Con, control diet; CS, carnitine supplemented; GS, GABA supplemented; CGS, carnitine plus GABA supplemented; NEC, Nonesterified carntine; ASAC, Acidsoluble acylcarnitine; AIAC, Acidinsoluble Acylcarnitine; TCNE, Total carnitine; Acyl/Free ratio, (ASAC+AIAC)/NEC; NCP, non-collagen protein.
[2)]All values are mean±SD (n=8).
[3)]Values with different superscripts in the same rows are significantly different ($p<0.05$).

of choline supplementation on carnitine status in humans, and that research demonstrated that choline results in a conservation of carnitine in guinea pigs and perhaps in humans (39). In our data, supplementation of GABA also increased serum carnitine concentration in mice (Table 6). We did not measure the urinary carnitine levels in this study, and further study is needed to elucidate the role of GABA in regulating carnitine metabolism.

Table 7. Effects of carnitine and/or GABA administration on serum vitamin A and E composition in mice (mg/dL)

	Groups[1]			
	Con	CS	GS	CGS
Vit. A	0.0035±0.0007[2]	0.0029±0.0004	0.0021±0.0005	0.0025±0.0005
Vit. E	0.0015±0.0002	0.0014±0.0001	0.0017±0.0003	0.0019±0.0003

[1]See the legend of Table 2.
[2]All values are mean±SD (n=8).

Vitamin A and E concentration

Serum vitamin A and E were not different among any of the groups (Table 7). Vitamin E is a major lipid-soluble antioxidant, which is concentrated in the hydrophobic interior cell membranes; it is widely known to trap free radicals and prevent oxidative damage of membranes. Early studies reported that high contents of vitamin E and vitamin A in diets reverses age-related declines in immune function (40), and that α-tocopherol in hippocampus, hypothalamus, striatum, cerebellum and cortex is increased by L-carnitine supplementation (300 mg/dw). The accumulation of lipofuscin was also found to be decreased after L-carnitine administration (41). The data suggests that decrement of lipofusctin accumulation by L-carnitine may be partially due to its antioxidant activity. However, in our study, serum vitamin A and E concentrations were unaffected by both carnitine and GABA supplementation. These data suggest that serum vitamin A and E concentrations may not be affected by supplementation of carnitine and/or GABA in the normal state in mice.

ACKNOWLEDGMENTS

This research was supported by a research grant (No. 04-2002-000-0113-0) from the Korea Science and Engineering Foundation (KOSEF).

REFERENCES

1. Cerretelli PC, Marconi L. 1990. L-carnitine supplementation in humans. The effects on physical performance. *Int J Sports Med* 11: 1-4.
2. Bremer J. 1983. Carnitine-metabolism and functions. *Physiol Rev* 63: 1420-1480.
3. McGarry JD, Foster DW. 1980. Regulation of hepatic fatty acid oxidation and ketone body production. *Ann Rev Biochem* 49: 395-420.
4. Rhew TH, Sachan DS. 1986. Dose-dependent lipotropic effect of carnitine in chronic alcoholic rats. *J Nutr* 116: 2263-2269.
5. Sachan DS, Cha YS. 1994. Acetylcarnitine inhibits alcohol dehydrogenase. *Biochem Biophys Res Comn* 203: 1496-1501.
6. Cha YS, Sachan DS. 1995. Acetyl carnitine-mediatd inhibition of ethanol oxidation in hepatocytes. *Alcohol* 12: 289-294.
7. Satyanarayan V, Nair PM. 1990. Metabolism enzymology and possible roles of 4-aminobutyrate in higher plants. *Phytochem* 29: 367-375.
8. Erlander MJ, Tobin AJ. 1991. The structural and functional heterogeneity of glutamic acid decarboxylase. *Neurochem Res* 16: 215-226.
9. Mody I, Dekoninck Y, Otis TS, Soltesz I. 1994. Bringing the cleft at GABA synapses in the brain. *Trends Neurosci* 17: 517-525.
10. Krogsgaard-Larsen P. 1989. GABA receptors. In *Receptor Pharmacology and Function*. Williams M, Glennon RA, Timmermans PMWM, eds. Marcel Dekker Inc, New York. p 349-383.
11. Bao J, Cheung WY, Wu JY. 1995. Brain L-glutamate decarboxylase. *J Biol Chem* 270: 6464-6467.
12. Morrow AL. 1997. Researchers study alcohol's channels to the brain. *Center Line* 8: 1-3.
13. Huth PJ, Schmidt MJ, Hall PV, Fariello RG, Shug AL. 1981. The uptake of carnitine by slices of rat cerebral cortex. *J Neurochem* 36: 715-723.
14. Fariello RG, Shug AL. 1981. Competitive inhibition by 3-amino propanesulfonic acid and γ-aminobutyric acid of carnitine transport in rat brain slices. *Biochem Pharm* 30: 1012-1013.
15. Wawrencyk A, Nalecz KA, Nalecz MJ. 1995. Effect of γ-aminobutric acid on the carnitine metabolism in neural cells. *Biochem Buiphys Res Commun* 213: 383-388.
16. Soh JR, Yamamoto TT, Cha YS. 2003. The effects of carnitine and/or gamma-aminobutyric acid (GABA) supplementation on the recovery of chronic ethanol administered rats. *Nutraceut Food* 8: 119-123.
17. Athanassakis I, Mouratidou M, Sakka P, Evangeliou A, Spilioti M, Vassiliadis S. 2001. L-carnitine modifies the humoral immune response in mice after *in vitro* or *in vivo* treatment. *International Immunopharmacology* 1: 1813-1822.
18. Oh SH, Oh CH. 2003. Brown rice extracts with enhanced levels of GABA stimulate immune cells. *Food Sci Biotechnol* 12: 248-252.
19. McGowan MW, Artiss JD, Strandbergh DR, Zak B. 1983. A peroxidase-couple method for the colormetric determination of serum triglycerides. *Clin Chem* 29: 538-542.
20. Allain CC, Poon LS, Chan CS, Richman W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470-475.
21. Warnick JB, Benderson J, Albers JJ. 1978. HDL precipitation by dextran sulfate-$MgCl_2$ method. *Clin Chem* 28: 1379-1383.
22. Friedwald WT, Levy RL, Fredrickso DS. 1972. Estimation of the concentration of low density lipoprotein cholesterol without use of the preparation ultracentrifuge. *Clin Chem* 18: 499-502.
23. Suzuki H, Wada S, Hayakawa S, Tamura S. 1985. Effects of oxygenabsorber and temperature on 3 polyunsaturated fatty acids of sardine oil during storage. *J Food Sci* 50: 358-360.

24. Bradford M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
25. Cederblad G, Linstedt SA. 1972. Method for determination of carnitine in picomole range. *Clin Chim Acta* 37: 335-343.
26. Sachan DS, Rhew TH, Ruark RA. 1984. Ameliorating effects of carnitine and its precursors on alcohol-induced fatty liver. *Am J Clin Nutr* 39: 738-744.
27. Duncan DB. 1993. Multiple range test for correlated and heteroscedastic means. *Biometerics* 13: 164-176.
28. Palacio A, Lopez M, Pere-Bravo F, Monkeberg F, Schlesinger L. 2002. Leptin levels are associated with immune response in malnourished infants. *J Clin Endocrinol Metab* 87: 3040-3046.
29. Faggioni R, Jones-Carson J, Reed DA, Dinarello CA, Feingold KR, Grunfeld C, Fantuzzi G. 2000. Leptin-deficient (ob/ob) mice are protected fro T cell-mediated hepatotoxicity. Role of tumor necrosis factor alpha and IL-18. *Proc Natl Acad Sci USA* 97: 2367-2372.
30. Goldstein JL, Brown MS. 1983. Lipoprotein receptors: Genetic defense against atherosclerosis. *Clin Res* 30: 417-423.
31. Steinberg D, Witztum JL. 1990. Lipoproteins and atherogenesis. *J Am A* 264: 3047-3052.
32. Marconi C, Sassi G, Carpinelli A, Cerretelli P. 1985. Effects of L-carnitine loading on the aerobic an anaerobic performance of endurance athletes. *Eur J Appl Physiol Occup Physiol* 53: 131-135.
33. Negrao CE, Ji LL, Schauer JE, Nagle Fj, Lardy HA. 1987. Carnitine supplemantation and depletion: tissue carnitine and enzymes in fatty acid oxidation. *The American Physiological Society* p 315-321.
34. Heinonen OJ, Takala J, Molt K. 1992. Effects of carnitine loading on long-chain fatty acid oxidation, maximal exercise capacity, and nitrogen balance. *Eur J Appl Physiol* 65: 13-17.
35. Sachan DS, Hongu N. 2000. Increase in VO_2max and metabolic markers of fat oxidation by caffeine, carnitine, and choline supplementation in rats. *J Nutr Biochem* 11: 521-526.
36. Harris RC, Foster CVL, Hultman E. 1987. Acetylcarnitine formation during intense muscular contraction in humans. *J Appl Physiol* 63: 440-442.
37. Cha YS, Sohn HS, Daily III JW, Oh SH. 1999. Effects of exercise training and/or high fat diet on lipid metabolism and carnitine concentration in rat. *Nutr Res* 19: 937-945.
38. Cha YS, Kim HY, Soh JR, Oh SH. 2001. Effects of regular endurance exercise of acute-exercise and rest on the levels of lipids, carnitine and carnitine palmitoyltransferase-I in rats. *J Biochem Mol Biol* 34: 434-439.
39. Daily III JW, Sachan DS. 1995. Choline supplementation alters carnitine homeostasis in humans and guinea pigs. *J Nutr* 125: 1938-1944
40. Moriguchi S, Muraga M. 2000. Vitamin E and immunity. *Vitam Horm* 59: 305-336
41. Juliet Arockia Rani P, Panneerselvam C. 2001. Carnitine as a free radical scavenger in aging. *Experimental Gernontology* 36: 1713-1726

(Received December 9, 2003; Accepted January 30, 2004)