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Effects of Carnitine on the Lipid Metabolism in the Ethanol-Fed Rats

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The effect of dietary carnitine on ethanol-induced fatty liver and hypertriglyceridemia was examined in an animal model. Consistent with literature, ethanol fed at 5g/Kg of b.w. to rats produced a significant increase in hepatic concentrations of total lipid, triglyceride, phospholipid, free cholesterol and esterified cholesterol as well as elevated plasma concentrations of triglyceride.

It was when the ethanol diet was supplemented with D.L. carnitine that there was a singinicant reduction in the accumulation of lipids in the ethanol-compromised liver. Dietary carnitine was also effective in ameliorating ethanol-induced hypertriglyceridemia. Total protein contents in the plasma was not varied among the groups.

KEY WORDS: Lipid metabolism, Ethanol, Carnitine, Rat liver

Many studies have documented that the effect of ethanol in the aspect of nutrition (Lieber, 1980), lipid (Isselbach, 1977), carbohydrates (Krebs *et al.*, 1969), protein (Baraona *et al.*, 1980), intermediary metabolism (Nisimura *et al.*, 1980), and amine metabolism (Kim *et al.*, 1983) as well as pharmacological actions were focused not only on central organs but also on peripheral ones.

Especially, ethanol-induced alterations in lipid metabolism such as hyperlipidemia and fatty liver usually manifest saliently. Both dietary and endogenously synthesized fatty acids contribute to deposition of fat in the liver (Scheig, 1971). It has been reported that oxidation of fatty acids is decreased when the lipid load of an ethanol-compromised liver is further accentuated (Blomstrand *et al.*, 1973). Moreover, in chronic alcoholism, endogenous biosynthesis of fatty acids was reported additionally to cause the hepatic steatosis (Blomstrand and Kager, 1973).

On the other hand to prevent ethanol-induced fatty liver and hyperlipidemia, many investigators have screened the effect of clofibrate, asparagine and barbiturate (Koff *et al.*, 1970) and choline (Mally and Kane, 1984). Nevertheless, the results remain unsatisfactory. Recently, some reports have focused on the lipid-lowering effects of carnitine on ethanol-induced fatty liver and hyperlipidemia in the rats (Sachan *et al.*, 1984; Sachan and Berger, 1987).

Carnitine (3 - hydroxy - 4 - N - trimethylamino butyrate), a cofactor of long-chain fatty acid oxidation, plays an important role in lipid metabolism. Carnitine and its Co-A esters generated in the process of long chain fatty acid oxidation regulate the activity of multiple enzymes. According to Bahl and Bressler (1987), carnitine is involved as a cofactor in the following metabolic events; 1) delivery of activated long chain fatty acid into the inside of mitochondria for beta oxidation, 2) removal of intramitochondrial acyl groups, 3) regulation of the rate of extramitochondrial long chain fatty acid activation, and 4) removal of acyl groups that are inhibitory and toxic. These results have been based on the reports of carnitine-deficient

animals (Rudman *et al.*, 1970), and alcoholic liver cirrhosis (Hosein and Bexton, 1975).

However, the mechanism of action of carnitine in the lipid metabolism remains obscure. Furthermore, the relationship of carnitine with ethanol-induced hyperlipidemia and fatty liver is not clearly identified. Thus, in the present study, to elucidate the action of carnitine in the lipid metabolism, the author evaluated the changes in lipid variables in plasma and some organs in the following cases such as ethanol plus carnitine feeding in comparison to those of ethanol alone feeding.

Materials and Methods

Animals and diet

Twenty male Sprague-Dawley rats of weighing 160-210 g were divided into four groups of five animals each and houshed individually.

The animals were allowed to adapt to environment during five days before treatment. Animals were supplemented at 9:00 A.M. and 8:00 P.M. daily as follows; 1) Non-ethanol (NE) group were received saline (same volume received together other goups) by gastric intubation. 2) Non-ethanol-carnitine (NEC) group were treated as NE group, except that carnitine (0.4 mg/g of body weight) was added to the saline. 3) Éthanol (E) group were received 30 %, ethanol (5 g/Kg of body weight) in saline (w/v) by gastric intubation. 4) Ethanol-carnitine (EC) group treated as E group, except that carnitine (0.45 mg/Kg of body weight) were added to the solution. Rats were permitted to consume the chew diet and water ad libitum during experimental periods (21 days).

Sample collection

The animals were sacrified under sodium barbital anethesia about 12 hr after the last meal on day of 21 of the experimental period. Blood was collected in EDTA-treated tubes by heart puncture, and the plasma was separated by centrifugation at 1,500 xg for 10 minutes at 4°C. And liver was excised quickly, weighed, and frozen immediately in dry ice. Liver and plasma were stored at −60°C in a deep freezer until analyses.

Analyses

Plasma was used for measuring the levels of total protein and total lipid (Gornall *et al.*, 1949), triglyceride (Gottfried and Rosenberg, 1973), phospholipid (Connerty et al., 1961), free cholesterol and total cholesterol (Searcy and Berquist, 1960), and free fatty acid (Novak, 1965).

Extractions of tissue lipid were performed by the method of Folch *et al.* (1957). Tissue (0.3 g) was trasferred into a ground glass homogenizer containing 2.0 ml of chloroform-methanol mixture (1:1 v/v) and homogenized while the vessel was kept in the ice water. The homogenate was transferred into a small glass vial and the homogenate vessel was rinsed with two portions of 2.0 ml of chloroform-methanol mixture. The homogenate (6.0 ml) was sonicated for 10 seconds (three times) and centrifuged at 3,000 xg for 100 min at 4°C. The supermatant fraction was used for measuring total lipid (Gomall *et al.*, 1949), triglyceride (Gottfried and Rosenberg, 1973), phospholipid (Connerty *et al.*, 1961), total cholesterol and free cholesterol (Search and Berquist, 1960), and free fatty acid.

Statistics

All data were expressed as mean ± SEM and difference among group means were determined by analysis of variation and Newman-Keuls multiple range test ($p < 0.05$).

Results

The effects of carnitine on ethanol-induced hepatic steatosis (fatty liver) were examined in healthy rats for the experimental period of 21 days and were compared with either an unsupplemented ethanol group or an unsupplemented nonethanol groups.

Carnitine-unsupplemented nonethanol group (NE group; control group) of rats consumed more food significantly than carnitine-supplemented ethanol group (EC group) (Table 1). Supplementation with carnitine significantly decreased daily food consumption. The relative gain in body weight was NE > NEC > E > EC but showed no significant differences among the groups (Table

Table 1. Effects of carnitine on body weight, food consumption and weight of organs in the ethanol-fed rats.

Parameters	NE	E	EC	NEC
Food consumption (g/day)	19.42 ±1.03[a]	16.14 ± 0.97[a]	13.2 ± 1.11[a]	18.8 ± 0.26[a]
Weight gain (g)	15.12 ± 4.81[a]	12.3 ± 2.58[a]	7.73 ± 1.85[a]	15.06 ± 2.81[a]
Liver (g)	5.98 ± 0.39[a]	6.12 ± 0.26[a]	6.13 ± 0.17[a]	6.27 ± 0.28[a]

Note. The values are means ± SEM for five animals. Values without a common superscript letter are significantly different from each other ($p < 0.05$). NE; Non-Ethanol, E; Ethanol, CE; Ethanol-Carnitine, NEC; Non-Ethanol--Carnitine.

1). Liver weighed within the identical mean range among the groups (Table 1).

Plasma concentrations (mg/dl) of lipid classes in the each group animals was described in table 1 and Fig. 1. NEC group rats showed no difference in plasma total lipid from the NE group (Fig. 1). However plasma total lipid concentration of EC group rats were significantly lower than those of the E group rats (Fig. 1). The concentration in the E group significantly increased when compared with those in the NE group.

Plasma triglyceride contents (mg/dl) of carnitine-supplemented animals among ethanol-fed rats were markedly reduced (Fig. 1). Cholesterol levels (mg/dl) in plasma showed different pattern from those in total lipids and triglycerides. The highest plasma concentrastions (mg/dl) of both total cholesterol and esterified cholesterol were found in the NE group (Fig. 1). When the E group compared with the NE group, both total cholesterol and esterified cholesterol levels in the E group were lowered (Fig. 1). In the EC group, total cholesterol levels were not different from those in the E group; also, in the NEC group, total cholesterol levels were not different from those in the NE group. However, esterified cholesterol levels in the EC group and NEC group were higher than those of the E group and NE group, respectively (Table 1). The NEC group showed the highest levels of esterified cholesterol among the groups. Plasma free cholesterol levels (mg/dl) were lower in the carnitine-supplemented groups (EC group and NEC group) than those in the carnitine-unsupplemented groups (E group and NE group), and the levels in the E group were not statistically different from those in the NE group, although the E group were lower than those of the NE group. The concentrations (mg/dl) of plasma phospholipid were markedly elevated in the E

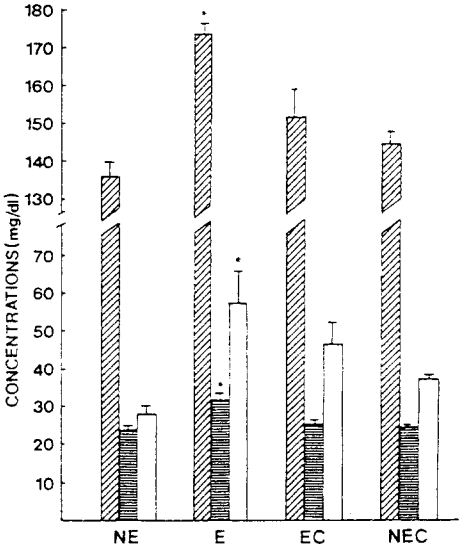

Fig. 1. Effects of D.L.-carnitine on the concentrations of plasma in the ethanol-fed rats.
▨; total lipid, ▤; triglycerides, and □; phospholipid
NE; non-ethanol E; ethanol NEC; non-ethanol--carnitine EC; ethanol-carnitine

and EC group but decreased in the EC group when compared with the E group animals (Fig. 1). Plasma free fatty acid contents showed a similar pattern to those of phospholipid, but free fatty acid levels (mg/dl) were lower in the NE group than those in the NEC group. Concentrations (mg/ml) of plasma total protein did not differ among the groups (Table 1).

Hepatic total lipid contents (mg/g of wet tissue weight) were increased in the E group when compared with those in the NE group (Fig. 2). The concentrations of heptic total lipid were significantly decreased in the EC group, and the relative

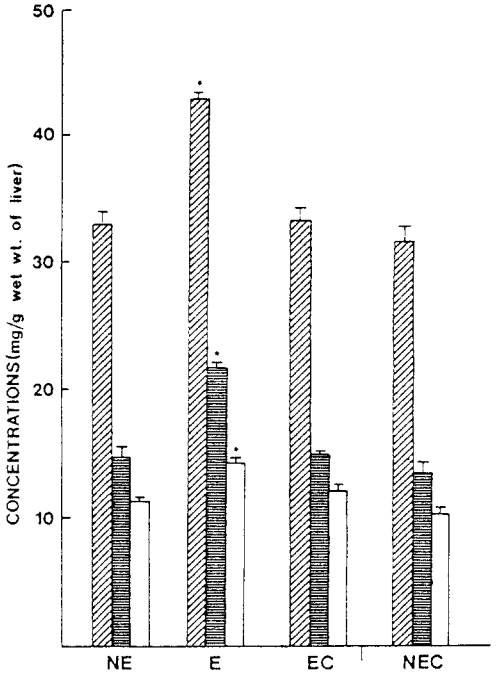

Fig. 2. Effects of D.L.-carnitine on the concentrations of liver in the ethanol-fed rats.
▨; total lipid, ▤; triglycerides, and □; phospholipid
NE; non-ethanol E; ethanol NEC; non-ethanol--carnitine EC; ethanol-carnitine

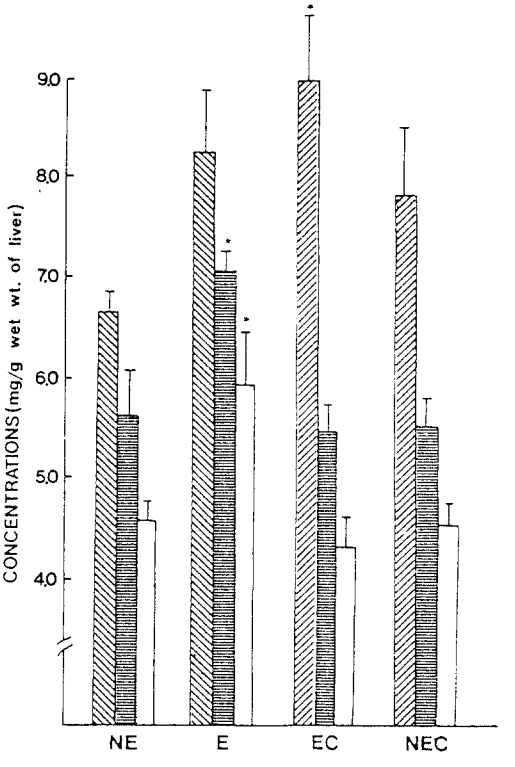

Fig. 3. Effects of D.L.-carnitine on the concentrations of liver in the ethanol-fed rats.
▨; free fatty acid, ▤; total cholesterol, □; esterified cholester.
NE; non-ethanol E; ethanol, EC; ethanol-carnitine NEC; non-ethanol-carnitine

values were NEC < NE < EC < E (Fig. 2). Values (mg/g of wet tissue weight) of hepatic triglycerides and phospholipid were also increased in the E group and decreased in the EC group when compared with the NE group (Fig. 2). Concentrations (mg/g) of esterified cholesterol, total cholesterol and free cholesterol showed a similar pattern of changes to those of total lipid and triglyceride (Figs. 2 and 3), e.g. elevated values in the E group and decreased values in the EC group and not significantly differed with the NEC group from the NE group. Unlike other lipid classes, hepatic contents (mg/g) of free fatty acid were significantly elevated in the EC group and the relative value were NE < NEC < E < EC.

Discussion

Hyperlipidemia and fatty liver are the most salient features of ethanol-induced alteration of lipid metabolism. Ethanol feeding to rats at a level of 5g/kg of body weight by gastric intubation produced fatty liver and accompanying hypertriglyceridemia (Figs. 1 and 2). These results are consistent with the observations reported in the large amount of literature available on the ethanol-iduced fatty liver (Zar, 1968; Menelson and Mello, 1973). The concentration of total lipid in ethanol--compromised liver were increased 28 % (42.24 ± 0.8 vs 32.97 ± 2.14 mg/g) over the normal liver in agreement with the 60 % increase (Rhew and Sachan, 1986). The ethanol-induced inceases in hepatic triglycerides of 42 % (21.16 ± 0.66 vs

14.89 ± 1.59 mg/g) and in phospholipid of 27 % (14.84 ± 0.38 vs 11.68 ± 0.37 mg/g) are also comparable with literature values of a 2 - fold increase in triglycerides and a 38 % increase in phospholipid (Rhew and Schan, 1986). Similarly, the 26 % increase in hepatic total cholesterol, 30 % increase in esterified cholesterol and 23 % increase in free fatty acid are well in agreement with literature values of a 45 % elevation in total cholesterol, and a 21 % in free fatty acid (Rhew and Sachan, 1986).

The ethanol-induced increase in plasma triglycerides of 32 % (31.88 ± 1.07 vs 24.21 ± 0.63 mg/dl) found in present study are supported by ethanol-induced increase reported in literature value such as a 2-fold elevation (Rhew and Sachan, 1986). The elevation of other lipid levels, e.g. 27.63 % of total lipid, 51.5 % of phospholipid and 37.25 % of free fatty acid in plasma are also comparable with literature values (Menelson and Mello, 1973). On the contrary to reference, the levels of total cholesterol and esterified cholesterol were increased during 21 days of experimental period.

Supplementation of ethanol diet with D.L.-carnitine 0.4 mg per g of body weight significantly reduced the concentration of lipid classes in livers (Fig. 2) of alcoholic rats. The lipotropic effects of dietary carnitine were difinitely evident in the changes in total lipid, triglycerides, free cholesterol, and phospholipid contents of the livers (Figs. 2 and 3). The results are consistent with the observations of Hosein and Bexton (1975). Lieber and DeCarli (1970) also demonstrated a lipotropic effect of dietary carnitine (1.0 mg/cal) in rats on total lipids (114.5 ± 17.9 vs 132.6 ± 24.3 mg/g) and triglycerides (34.0 ± 4.5 vs 39.5 ± 4.5 mg/g). In the present studies, the hyperlipidemia commonly associated with chronic ethanol consumption was also minimized by carnitine (Fig. 1); these results are comparable with the decrease in plasma triglycerides (from 144.0 ± 9.98 to 111.0 ± 5.2 mg/dl) observed by Hosein and Bexton (1975) following carnitine treatment of ethanol-fed rats by gastric intubation.

Some observations showed that lipotropic effect of carnitine is due to transformation of carnitine to acylcarnitine, thus, consuming the fatty acid pool which is knowen to increase in chronic ethanol consumption (Sachan *et al.*, 1984). However, such a simple explanation can be only a part of the total answer because the increases in acylcarnitines in the liver were too small to affect the large magnitude of decrease in hepatic lipid contents in the carnitine-supplemented ethanol-fed groups. Also, Sachan *et al.* (1987) suggested that supplementary carnitine is readily absorbed from the gastrointestinal tract at all levels of supplementation. Also, they showed 1 % supplementary D.L.-carnitine raised blood carnitine concentrations to a steady state after 3 days of feeding, the elevation of blood ethanol concentration in carnitine supplemented animals was sustained and does related, and carnitine inhibited ethanol clearance from the blood. They suggested that the latter action of carnitine may in part be reason fro the lower lipid contents in the livers of carnitine supplemented than in the those of carnitine-unsupplemented chronic alcolic rats.

Availability of carnitine may be affected by subcellular distribution, ionization, binding to macromolecules and activation of carnitine in manners yet be elucidated. Such a hypothesis best accomodates the observations of those who proposed possible carnitine deficiency in alcoholic cirrhosis (Menelson and Mello, 1973) or prolonged ethanol feeding (Hosein and Bexton, 1975) as well as those who may choose to disagree with it because of an increase in carnitine concentrations of the cirrhotic patients (Rubin and Lieber, 1968) and ethanol-fed rats (Baraona and Lieber, 1970).

In conclusion, dietary carnitine is effective in preventing lipid accumulation cause by chronic feeding in rats. This effect does not appear to be mediated primary through acylation of fatty acids to carnitine was an effective hypolipidemic agent that was closely related to the hypercarnitinemia. The observations are consistent with a deficiency of functional carnitine possibly due to impaired carnitine biosynthesis in chronic ethanol-fed rats. Because of Sachan *et al.* (1984) suggested that exogenous carnitine added to the ethanol diet significantly reduced lipid accumulation in livers which were otherwise laden with lipids, especially with triglycerides and endogenous carnitine was unable to prevent accumulation of fat in ethanol--compromised livers. Accordingly, in the present study, dietary carnitine added to the ethanol diet

effectively ameliorated the ethanol-induced fatty liver and hyperlipidemia in the rats.

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에탄올을 투여한 흰쥐에 있어서 지방대사에 미치는 D.L.-Carnitine의 영향

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Ethanol의 대사과정에 관여하는 영향중에 특징적인 것으로 과유지방혈증(hyperlipidemia)과 지방간(fatty liver)을 거쳐 간경변에 이르는 간에 관계하는 일련의 증상들을 들 수 있다. 본 실험에서는 만성적 ethanol의 지방대사 장해에 대한 D.L.-carnitine의 효과에 대해 고찰하였다.

실험용 흰쥐를 사용하여 실험군(ethanol group)에게 체중 kg당 5g의 ethanol(30 % in saline)을 투여하여 알콜유발성 지방간과 과유지방혈증을 일으키고, 그 실험군 흰쥐들에게 carnitine(0.4 mg/g of body weight)을 첨가하여 그 효과를 관찰하였다. 그 결과 carnitine을 첨가하여 투여한 흰쥐들에서 ethanol처리군과 비교하여 볼 때 간과 혈장에서 지방축적이 현저히 감소하는 것을 관찰할 수 있었다.