

Original Research

Comparative Effects of Dietary Corn Oil, Safflower Oil, Fish Oil and Palm Oil on Metabolism of Ethanol and Carnitine in the Rat

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Objective: This study was launched to determine comparative effects of corn oil (CO), safflower oil (SO), fish oil (FO) and palm oil (PO) on carnitine status and ethanol metabolism in rats.

Methods: Twenty-four male Sprague-Dawley rats (300 g bw) were randomly divided into four groups (n = 6) and fed a semisynthetic diets containing fat as oils listed above. Blood and 24 hour urine samples were collected before and after dietary treatment and acute ethanol administration. Samples were analyzed for blood-ethanol concentration (BEC) and carnitine species.

Results: The diets containing FO and PO retarded ethanol metabolism compared to the diets containing CO and SO. The effect of these dietary fats on carnitine species in plasma and urine was varied before and after dietary treatment and following a single oral ethanol dose. The liver carnitine content was higher in the PO group after dietary and ethanol treatment.

Conclusion: It is concluded that attenuation of ethanol clearance was related to unique fatty acid makeup of the oils that in part may be attributed to the composite ratio of saturated to unsaturated fatty acids in the oils.

INTRODUCTION

Alcohol abuse has been related to a number of biochemical changes and diseases in humans and animals. Nutrition, nutritional status and the interaction of food nutrients with alcohol modulate alcohol toxicity. The amounts and types of dietary fat are equally important in affecting the metabolism and elimination of alcohol. It was demonstrated that the increasing amount of dietary fat produced a progressively severe liver damage [1]. It was further documented that chronic intra-gastric tube feeding of alcohol and high fat diet resulted in sustained high blood ethanol concentration (BEC) which was important in inducing liver fibrosis in rats [1]. In a similar study, while 5% dietary fat caused hepatic steatosis and focal fibrosis, a 25% fat diet extended the injury to fibrosis [2,3]. These and other similar studies lead to postulation that high fat diet is essential for ethanol to induce liver fibrosis in animals [4].

High saturated fat diets seem to be more protective against alcohol-induced liver injury than high polyunsaturated fat diets. An epidemiological study of 17 countries indicated that high saturated fat is correlated with a lower incidence of alcoholic

cirrhosis [5]. Beef fat [6] and coconut oil [7], but not lard [8], suppressed liver damage due to alcohol. Olive oil diet produced a more severe fatty liver than the corn oil diet, and the rats fed high levels of alcohol along with the unsaturated fat (corn and olive oil) diets maintained high BEC [9]. Fish oil induced greater alcoholic liver disease than corn oil in an alcoholic liver disease animal model [10]. Rats fed fish oil and ethanol had a higher degree of liver necrosis and inflammation than those fed corn oil and ethanol [11], associated with a greater activity of cytochrome P450-2E1 and enhanced lipid peroxidation in the rats fed ethanol and fish oil than ethanol and corn oil [12].

Almost all the studies related to fat and ethanol have been chronic studies, and outcome has been measured in terms of liver pathology. Some years ago we got interested in the effects of various dietary fats and oils on the clearance (pharmacokinetics) of ethanol after an acute oral dose of ethanol. It was found that animals fed a diet containing coconut oil maintained higher BEC and slower ethanol clearance than those fed corn oil diet regardless of duration (30–120 days) of dietary treatment [13]. Further it was noted that plasma carnitine concentrations were higher in the animals fed coconut oil than corn oil

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[13]. Higher plasma carnitine concentrations produced by L-carnitine supplementation have been shown to retard alcohol clearance and development of hepatic steatosis in rats [14–16] and broilers [17]. The explanation was that saturated fatty acids in coconut oil diet prevent alcohol-induced liver damage by slowing ethanol metabolism perhaps mediated by high carnitine and acetylcarnitine [18,19]. In light of these observations, this study was launched to determine the comparative effects of corn oil, safflower oil, fish oil and palm oil on ethanol clearance and carnitine status in rats.

MATERIALS AND METHODS

Experimental Protocol

This study was approved by the Animal Care and Use Committee at the University of Tennessee, Knoxville, TN, and follows the guidelines of the National Institutes of Health on the humane use of laboratory animals. Twenty-four male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing about 300 g were housed in an American Association for the Accreditation of Laboratory Animal Care (AAALAC) approved animal facility with temperature controlled at 22°–24°C, relative humidity at 50%, and 12 hour alternating periods of light and darkness. The animals were acclimatized to individual wire-mesh-bottom, suspended, stainless steel cages in a cubicle and had free access to water and diet (Agway R-M-H 3000, Agway Country Foods, Inc., Syracuse, N.Y.) for seven days. The rats were then randomly divided into four groups (n = 6) and fed with their respective diets.

Diets

Each group of animals were fed with modified AIN⁷⁶ diet (Table 1) containing 10% corn oil (CO), safflower oil (SO), menhaden fish oil (FO) or palm oil (PO) as the source of fat. The CO was purchased from a local Kroger store and SO and FO from Sigma Chemical (St. Louis, MO). PO (Knife brand) was supplied by the courtesy of Palm Oil Research Institute of

Malaysia, Embassy of Malaysia in Washington, D.C. No carnitine was detected in any of the oils used in this study. Diets were prepared twice weekly and stored in plastic bags at –80°C. Daily feed intake was determined by recording weigh-back that was discarded and fresh diet given in a new clean glass container. Body weight was recorded once every week.

Specimen Collections

Urine and blood samples were collected three times; on the day before the rats were placed on the different diets (baseline or pre-diet samples) and 56 days after being on the dietary treatment (post-diet samples). The third blood sample was collected after a dose of ethanol (post-diet and post-ethanol samples) on day 60 and a urine sample on day 62 after another acute dose of ethanol. Thus animals got only two doses of ethanol two days apart. At these dosages ethanol is completely cleared from the blood in six to eight hours [15]. For urine collection, the rats were placed in individual metabolic cages, and a 24-hour urine was collected in a tube containing 1 mL 0.1% HCl as preservative, removed twice daily, pooled, volume measured and an aliquot frozen at –80°C. At the end of the 24-hour urine collection, a lateral tail vein was catheterized (Instyline intravenous catheter placement unit, Deseret Medical, Inc., Sandy, UT). Tail vein blood was taken from the catheter using 60 µL heparinized microhematocrit capillary tubes (Scientific Products, Travenol Laboratories, Inc., McGaw Park, IL) and drawn into 1.5 mL microcentrifuge tubes containing 130 µL of 0.9% saline, centrifuged and stored at –80°C. On the 62nd day, another dose of ethanol (65.1 mmol/kg body weight) was given and 24-hour urine samples were collected. The animals were anesthetized with methoxyflurane (Pitman-Moore, Inc., Mundelein, IL). Cardiac blood was collected into heparin-containing tubes and centrifuged, and the plasma was stored at –80°C until analyzed. The rats were killed by exsanguinations, and the livers were perfused with 0.9% NaCl, removed, blotted, frozen in liquid nitrogen and stored at –80°C.

Ethanol Pharmacokinetics

On day 60, without prior starvation, a single dose of 13% ethanol solution (65.1 mmol/kg body weight) was administered orally via a stainless steel feeding cannula. Tail vein blood samples (20 µL) were collected at 15, 30, 45, 60, 120, 240, and 360 minutes post-ethanol administration for ethanol pharmacokinetic analysis. Blood samples were analyzed for ethanol concentration on the day of collection according to Bernt and Gutmann procedure [20]. The analysis of ethanol pharmacokinetic was performed on the BEC mean ± SEM value following Baggot [21].

Carnitine Assays

All urine, plasma and liver samples were measured for free or non-esterified carnitine (NEC), short-chain or acid-soluble

Table 1. Composition of Modified AIN⁷⁶ Diet

Ingredient	Percent
Casein	20.0
DL-Methionine	0.3
Cornstarch	15.0
Sucrose	45.0
Cellulose	5.0
Fat ¹	10.0
AIN-76 Mineral Mix	3.5
AIN-76 Vitamin Mix	1.0
Choline bitartrate	0.2

¹ Fat source is corn oil, safflower oil, Menhaden fish oil, or palm oil. Calories from proteins, carbohydrates, and fats amount to 19, 59 and 22 percent.

acylcarnitines (ASAC) and long-chain or acid-insoluble acylcarnitine (AIAC) according to radioisotopic method of Cedergren and Lindstedt [22] as modified by Sachan *et al.* [23]. Total carnitine (TC) was calculated by adding the NEC, ASAC and AIAC concentrations.

Statistics

All values are expressed as group mean \pm SEM. The data were analyzed by one-way ANOVA using GLM (General Linear Model) procedures of SAS (release 6.08, 1989, SAS Institute, Inc., Cary, NC), and when F test was significant, Duncan New Multiple Range Test was used to determine difference between group means. The minimum level of significance accepted was $p \leq 0.05$.

RESULTS

The weight gains of the rats were not significantly affected by the four different fat diets (Table 2). The liver weights, expressed in grams or percent body weight, were not significantly different. The changes in the blood ethanol concentrations (BEC) with time are shown in Table 3. The BEC did not differ statistically among the groups for the first 60 minutes post-ethanol administration. However, at 120 minutes and beyond both FO and PO fed animals maintained significantly higher BEC than the CO and SO fed animals. The pharmacokinetic parameters calculated from the mean BEC revealed higher BEC peak, longer peak time, slower absorption and elimination rates and longer half-life in the FO and PO groups compared to the CO and SO groups.

The effect of different fat diets and an ethanol dose on the plasma carnitine profiles is presented in Table 4. The post-experimental diet plasma NEC of the PO group was significantly higher than the other three groups that remained similar. The PO and FO animals had statistically lower AIAC as compared to CO and SO animals. The FO group had the lowest AIAC and total carnitine concentrations of the groups. The acylcarnitine to NEC ratio was significantly lower in the PO group than the other groups. After an ethanol dose, the only difference in the carnitine profiles among the groups was in the NEC fraction; that is, the SO group exhibited higher NEC than

the CO group. Acylcarnitine to NEC ratio was lowest in the SO and PO groups before and after dietary treatments, respectively, but there was no difference after the ethanol dose.

The urinary carnitine excretion for 24 hours is shown in Table 5. At the end of experimental diet period, the NEC and total carnitine concentrations were similar among the groups except the FO group where the values were lowest. After the ethanol dose, the NEC, AIAC and TC remained similar among the groups, but the ASAC and acylcarnitine to NEC ratios were significantly increased in the FO and PO groups. The liver NEC, AIAC and total carnitine levels were highest in the FO fed animals (Table 6). No significant differences were found in NEC, AIAC and total carnitine among the CO, SO, and PO groups. The hepatic ASAC concentrations were not statistically different among the groups. The hepatic acylcarnitine to NEC ratios were significantly lower in FO and PO groups as compared to the SO group.

DISCUSSION

The FO and PO diets retarded blood ethanol clearance in rats that was evident at 120 minutes following an acute dose of orally administered ethanol. The time course was similar to that found in our earlier studies where coconut oil (saturated FA) and CO (unsaturated FA) were used [13]. It is surprising that BEC of the FO and PO groups were not different when the former is considered more unsaturated (iodine value 140–180) and the latter more saturated (iodine value 44–54). The iodine values of CO and SO are 103–128 and 140–150, respectively. Thus the SO and FO are similar with regard to the unsaturated FA content. This raises the question about the validity of discussing BEC in the context of saturated and unsaturated fats or fatty acids. The examination of the fatty acid composition of these oils reveals that both CO and SO are rich in oleic and linoleic acids, but FO and PO are rich in palmitic and oleic acids. So it would seem that palmitic (in FO and PO) and linoleic acids (in CO and SO) are responsible for the opposing effects of these fats on ethanol metabolism. Linoleic acid is known to promote severity of liver damage in rats given chronic ethanol [6,8]. The relatively faster rate of ethanol clearance in CO vs. coconut oil fed rats has been equated with

Table 2. Body Weight, Weight Gain and Liver Weight of Rats Fed Diet Containing Corn Oil (CO), Safflower Oil (SO), Fish Oil (FO) or Palm Oil (PO) as Source of Fat for Eight Weeks¹

Groups	CO	SO	FO	PO
Initial body weight, g	312.8 \pm 3.5	309.0 \pm 3.4	307.5 \pm 4.3	305.3 \pm 5.4
Weight gain, g	139.0 \pm 3.7	141.3 \pm 9.2	163.4 \pm 13.0	143.5 \pm 8.8
Liver weight				
g	13.1 \pm 0.39	14.1 \pm 0.64	14.3 \pm 0.80	13.6 \pm 0.74
% body wt	2.90 \pm 0.07	3.12 \pm 0.14	3.05 \pm 0.19	3.03 \pm 0.10

¹ The values are mean \pm SEM for n = 6 except for weight gain and liver weight for SO and FO where n = 5. The differences of the respective weights between the groups are not significant ($p \geq 0.05$).

Table 3. Blood-Ethanol Concentrations (mmol/L) Following Single Oral Ethanol Gavage in Fats Fed Diet Containing Corn Oil (CO), Safflower Oil (SO), Fish Oil (FO) or Palm Oil (PO) as Source of Fat¹

Time (minutes)	CO	SO	FO	PO
15	63.4 ± 4.3	67.0 ± 1.8	56.2 ± 4.6	56.3 ± 3.2
30	77.8 ± 3.9	73.9 ± 3.4	77.5 ± 5.6	68.6 ± 3.5
45	80.9 ± 5.6	75.0 ± 3.2	80.8 ± 3.7	75.8 ± 3.3
60	88.2 ± 2.5	86.3 ± 3.9	88.0 ± 1.5	78.4 ± 2.0
120	82.4 ± 3.4 ^b	79.2 ± 4.8 ^b	104.6 ± 4.2 ^a	109.5 ± 4.7 ^a
240	70.7 ± 4.2 ^b	71.2 ± 3.1 ^b	95.4 ± 2.0 ^a	94.8 ± 0.7 ^a
360	61.6 ± 2.4 ^b	65.3 ± 5.6 ^b	91.1 ± 1.3 ^a	90.5 ± 2.6 ^a

¹ Values are means ± SEM (n = 6). The means with different superscript letters among the groups (rows) are significantly different (p < 0.05).

Table 4. Plasma Carnitine Concentrations (μmol/L) in Rats Fed Diet Containing Corn Oil (CO), Safflower Oil (SO), Fish Oil (FO) or Palm Oil (PO) as Source of Fat, and an Oral Dose of Ethanol¹

	CO	SO	FO	PO
Pre-Experimental Diet (Agway R-M-H 3000, 1 week)	(n = 6)	(n = 6)	(n = 6)	(n = 6)
NEC	40.0 ± 1.4	42.1 ± 1.4	41.3 ± 2.2	40.9 ± 0.6
ASAC	18.2 ± 1.0	15.1 ± 0.6	15.1 ± 1.4	18.6 ± 1.7
AIAC	5.1 ± 0.5	4.4 ± 0.4	4.8 ± 0.7	5.9 ± 0.5
Total	63.3 ± 1.1	60.7 ± 1.0	59.5 ± 3.7	64.1 ± 1.5
ASAC + AIAC:NEC	0.64 ± 0.07 ^a	0.44 ± 0.03 ^b	0.51 ± 0.03 ^{ab}	0.64 ± 0.06 ^a
Post-Experimental Diet (Modified AIN-diet, 8 weeks)				
NEC	42.4 ± 1.0 ^b	42.0 ± 0.9 ^b	38.1 ± 0.7 ^b	49.9 ± 2.2 ^a
ASAC	14.8 ± 1.1	14.5 ± 0.8	14.1 ± 0.8	12.7 ± 1.0
AIAC	4.2 ± 0.2 ^a	4.0 ± 0.2 ^a	2.0 ± 0.2 ^c	2.9 ± 0.1 ^b
Total	61.4 ± 1.0 ^{ab}	58.6 ± 1.7 ^{bc}	54.0 ± 0.7 ^c	65.4 ± 2.6 ^a
ASAC + AIAC:NEC	0.45 ± 0.03 ^a	0.47 ± 0.05 ^a	0.42 ± 0.03 ^a	0.31 ± 0.02 ^b
Post-Ethanol Dose (65.1 mmol/kg body weight)				
NEC	73.8 ± 3.8 ^b	84.4 ± 3.1 ^a	78.8 ± 3.6 ^{ab}	82.7 ± 1.4 ^{ab}
ASAC	15.0 ± 0.7	16.2 ± 1.8	13.6 ± 3.5	11.4 ± 1.8
AIAC	2.3 ± 0.2	2.3 ± 0.2	1.8 ± 0.4	1.9 ± 0.2
Total	91.5 ± 3.4	102.8 ± 4.6	91.7 ± 5.3	95.1 ± 2.6
ASAC + AIAC:NEC	0.24 ± 0.02	0.22 ± 0.02	0.17 ± 0.06	0.21 ± 0.07

¹ Values are means ± SEM. The means with different superscript letter among the groups (rows) are significantly different (p ≤ 0.05). Abbreviations: NEC = nonesterified carnitine, ASAC = acid soluble acylcarnitine, AIAC = acid insoluble acylcarnitine. ASAC + AIAC:NEC is indicative of fatty acid metabolism.

a higher load of acetaldehyde and consequential liver damage in earlier reports [13]. Coconut oil is rich in lauric and myristic acids. FO has relatively more myristic and palmitic acid (21% to 25%) than does the CO or SO (7% to 12%). PO has the highest percentage of lauric, myristic and palmitic acids (48%). So it seems that presence of lauric, myristic and palmitic acids (21% to 25%) in the PO and FO contributes to the retardation of blood-ethanol clearance in Sprague Dawley rats. Though palmitic acid is a common denominator in FO and PO (16–42), it is quite low in the coconut oil (9%). It is logical, therefore, to associate slower ethanol clearance to the combination of lauric, myristic and palmitic acids or the specific oil because of its unique FA composition. Relating the change in ethanol metabolism to a particular oil or fat type has practical significance since we use oils in food preparation and nonspecific beef tallow has been shown to prevent ethanol-induced liver disease compared to lard and corn oil [6]. Coconut oil retards ethanol metabolism [13] like FO and PO in the present study. The faster ethanol clearance indicates faster ethanol metabolism and greater liver damage because of oleic and linoleic acids. An

examination of the saturated/unsaturated (S/U) FA ratio of these oils [24] reveals that this value is lower for CO and SO (0.15 and 0.11) than for FO and PO (0.73 and 1). In this regard PO is similar to beef tallow (S/U = 1.04), which has been shown to retard ethanol-induced liver disease [6]. A distinguishing feature of menhaden FO is that it has significant amount of arachidonic acid (17%), which is not present in any other oils used in this or our previous studies. Dietary arachidonic acid (7.1% of fatty acids) has been shown to reduce fatty liver of alcoholism [25], perhaps by retarding ethanol metabolism.

A secondary objective of this study was to determine effects of these oils on carnitine profiles and how these profiles may get modulated by a single dose of ethanol. Following eight weeks of PO diet, NEC concentration was increased in plasma (Table 4), but not in urine (Table 5). The single dose of ethanol had no significant impact on the plasma carnitine profiles of the dietary groups (Table 4); however, urinary excretion of ASAC was increased in the PO and FO groups (Table 5). The hepatic NEC, AIAC and TC were higher in the FO group (Table 6).

Table 5. Urinary Carnitine Concentrations ($\mu\text{mol}/\text{day}$) in Rats Fed Diet Containing Corn Oil (CO), Safflower Oil (SO), Fish Oil (FO) or Palm Oil (PO) as Source of Fat, and an Oral Dose of Ethanol¹

	CO	SO	FO	PO
Pre-Experimental Diet (Agway R-M-H 3000, 1 week)				
NEC	ND	ND	0.33 ± 0.01	0.32 ± 0.05
ASAC	ND	ND	0.23 ± 0.01	0.25 ± 1.7
AIAC	ND	ND	0.08 ± 0.006	0.09 ± 0.015
Total	ND	ND	0.64 ± 0.03	0.66 ± 0.08
ASAC + AIAC:NEC	ND	ND	0.95 ± 0.04	1.11 ± 0.07
Post-Experimental Diet (Modified AIN-diet, 8 week)				
NEC	0.84 ± 0.09^a	0.95 ± 0.10^a	0.29 ± 0.02^b	0.76 ± 0.15^a
ASAC	0.03 ± 0.01^b	0.16 ± 0.05^a	0.03 ± 0.01^b	0.12 ± 0.03^{ab}
AIAC	0.024 ± 0.001	0.022 ± 0.002	0.012 ± 0.001	0.017 ± 0.005
Total	0.88 ± 0.09^a	1.12 ± 0.13^a	0.32 ± 0.03^b	0.87 ± 0.17^a
ASAC + AIAC:NEC	0.06 ± 0.01	0.19 ± 0.05	0.17 ± 0.05	0.20 ± 0.08
Post-Ethanol Dose (65.1 mmol/kg body weight)				
	(<i>n</i> = 6)	(<i>n</i> = 5)	(<i>n</i> = 5)	(<i>n</i> = 6)
NEC	1.49 ± 0.21	1.60 ± 0.32	1.40 ± 0.35	1.72 ± 0.29
ASAC	0.45 ± 0.11^b	0.32 ± 0.04^b	1.08 ± 0.27^a	1.33 ± 0.23^a
AIAC	0.05 ± 0.02	0.07 ± 0.02	0.04 ± 0.00	0.06 ± 0.02
Total	1.99 ± 0.03	1.99 ± 0.35	2.49 ± 0.62	3.11 ± 0.52
ASAC + AIAC:NEC	0.34 ± 0.04^b	0.27 ± 0.05^b	0.77 ± 0.00^a	0.82 ± 0.02^a

¹ Values are means \pm SEM (*n* = 6). The means with different superscript letter among the groups (rows) are significantly different ($p \leq 0.05$). Abbreviations: ND = not done, NEC = nonesterified carnitine, ASAC = acid soluble acylcarnitine, AIAC = acid insoluble acylcarnitine. ASAC + AIAC:NEC indicates activity of carnitine system i.e. higher ratio is associated with higher fatty acid mobilization and/or oxidation.

Table 6. Liver Carnitine Concentrations (nmol/g) in Rats Fed Diet Containing Corn Oil (CO), Safflower Oil (SO), Fish Oil (FO) or Palm Oil (PO) as Source of Fat, and an Oral Dose of Ethanol

	CO (<i>n</i> = 6)	SO (<i>n</i> = 5)	FO (<i>n</i> = 5)	PO (<i>n</i> = 6)
NEC	158.3 ± 12.7^b	142.7 ± 7.6^b	231.6 ± 22.1^a	180.3 ± 16.3^b
ASAC	34.7 ± 2.1	45.7 ± 9.3	43.8 ± 7.1	31.6 ± 3.2
AIAC	6.0 ± 0.8^b	4.0 ± 0.6^b	8.5 ± 1.1^a	4.8 ± 0.7^b
Total	198.9 ± 13.8^b	184.2 ± 7.8^b	283.9 ± 25.9^a	216.8 ± 16.4^b
ASAC + AIAC:NEC	0.26 ± 0.02^{ab}	0.37 ± 0.08^a	0.23 ± 0.03^b	0.21 ± 0.03^b

¹ Values are means \pm SEM. The means with different superscript letter among the groups (rows) are significantly different ($p \leq 0.05$). Abbreviations: NEC = nonesterified carnitine, ASAC = acid soluble acylcarnitine, AIAC = acid insoluble acylcarnitine.

The significance of higher carnitine concentrations is related to the fact that higher plasma carnitine in carnitine supplemented rats retarded ethanol metabolism [14–16] and acetylcarnitine was found to inhibit ethanol metabolism in hepatocytes [19] by competing with NAD^+ [18]. In our earlier study higher BEC were associated with higher plasma carnitine concentrations in the rats fed coconut oil diet and lower BEC were associated with lower carnitine concentrations in the rats fed corn oil diet [13]. Such a relationship is not very convincing in the current study.

In summary, diets containing FO and PO retarded ethanol metabolism compared to the diets containing CO and SO after eight weeks of treatment. The effect of the dietary fats on carnitine species in plasma, urine and liver was varied and did not relate to the change in ethanol clearance. It is concluded that attenuation of ethanol metabolism was related to the unique fatty acid makeup of the oil that in part may be attributed to its composite ratio of saturated to unsaturated fatty acid.

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