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L-Carnitine increases liver α-tocopherol and lowers liver and plasma triglycerides in aging ovariectomized rats

Richard M. Clark[a,*], Anju Balakrishnan[a], David Waters[a], Dimple Aggarwal[a], Kevin Q. Owen[b], Sung I. Koo[a]

[a]*Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269-4017, USA*
[b]*Lonza, Inc., Allendale, NJ 07401, USA*

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Abstract

The objective of this study was to determine whether dietary L-carnitine can influence the status of α-tocopherol, retinol and selected lipid parameters in aging ovariectomized rats, an animal model for the menopausal state. Fourteen Fisher-344 female rats 18 months old were acclimated for 4 weeks and ovarectomized. Seven rats per treatment were assigned to either a control group fed ad libitum AIN-93M diet or a carnitine group fed the same diet supplemented with L-carnitine. After an 8-week feeding period, blood and selected tissues were taken for analyses. No differences were noted in food intake, body weight, or organ weights due to L-carnitine. Dietary carnitine significantly increased liver α-tocopherol and tended to increase plasma α-tocopherol ($P<.09$). No changes in α-tocopherol were observed in other tissues including the brain, lungs and retroperitoneal fat. Retinol levels in plasma and tissues were not affected by supplemental L-carnitine. Significant decreases in liver and plasma triglyceride (TG) levels were noted, suggesting increased utilization of fatty acids. No differences were observed in the fatty acid profile of tissues. The results provide evidence that dietary supplementation of L-carnitine enhances the α-tocopherol status and improves the utilization of fat leading to lowering of the liver and plasma levels of TG in aging ovariectomized rats. Whether supplemental L-carnitine may be of benefit to postmenopausal women in lowering plasma TG and improving the antioxidant status remains to be studied.

Keywords: Carnitine; Menopause; α-Tocopherol; Retinol; Triglyceride; Fatty acids

1. Introduction

Menopause is a period in life associated with increased risk for heart disease, diabetes and other age related diseases [1–3]. Altered lipid metabolism contributes to the development of many of these diseases. The balance between lipid synthesis and breakdown pathways within a cell is, in part, controlled by the transfer of fatty acids across mitochondrial, peroxisomal and endoplasmic reticulum membranes. Fatty acids cross these membranes as acyl-carnitine derivatives to enter pathways for oxidation, acylation, chain shortening or chain elongation-desaturation [4]. Therefore, carnitine-dependent fatty acid transfer is central to lipid metabolism. During menopause, the body's ability to efficiently utilize nutrients begins to deteriorate, affecting carnitine status and subsequent lipid homeostasis. In this study, we used an aging ovariectomized rat model to simulate physiological changes associated with menopause to determine the effect of L-carnitine supplementation on tissue status of fat-soluble vitamins and profile of fatty acids.

L-Carnitine is a conditionally essential nutrient synthesized endogenously from lysine and methionine in the liver, kidney and brain [5,6]. In the young healthy adult, the requirement for L-carnitine can be met by endogenous synthesis. However, with aging, tissue levels of carnitine decline in both humans and animals [7–9]. This adversely affects carnitine-dependent pathways. In aged rats, dietary carnitine stimulates the carnitine-dependent fatty acid transport system in the liver [10] and improves mitochondrial function and increased oxidative metabolism [11], whereas dietary carnitine has little effect on young animals. These observations suggest an increased need for dietary carnitine in the older rats. A decrease in capacity to oxidize fatty acids in the body due to less than optimal levels of

* Corresponding author. Tel.: +1 860 486 0121; fax: +1 860 486 3674.
E-mail address: richard.m.clark@uconn.edu (R.M. Clark).

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carnitine could contribute to increased TG accumulation and storage in tissues. In addition, there is a growing body of evidence that the compromised status of fat-soluble vitamins including α-tocopherol and retinol may contribute to age-related diseases. A recent study with aged rats showed that carnitine supplementation improves the intestinal absorption of lipid and α-tocopherol [12]. At present, no information is available on whether supplemental carnitine improves the absorption and status of other fat-soluble vitamins in aging animals.

The brain, retina, liver and heart contain significant levels of docosahexaenoic acid (DHA, 22:6n-3) phospholipids, as required for their optimal function. DHA is synthesized from α-linolenic acid (18:3n-3) via a series of elongation and desaturation steps in the endoplasmic reticulum, followed by a chain-shortening step in the peroxisomes [13]. Carnitine has a role in the export of chain-shortened fatty acids from peroxisomes [14] and for transport of fatty acids across the endoplasmic reticulum [15]. If carnitine is limiting in tissues of aged animals, it is possible that carnitine supplementation may improve de novo synthesis of long-chain polyunsaturated fatty acids, particularly DHA, and alter the fatty acid profile of tissues.

The specific objectives of this study were to determine if dietary L-carnitine affects total body weight, accretion of lipid, vitamins A and E and tissue fatty acid profiles in aging ovariectomized rats. To our knowledge, this is the first report utilizing this animal model to study the effect of dietary carnitine.

2. Materials and methods

2.1. Animals

Eighteen-month old female Fisher 344 rats were purchased from Harlan Sprague–Dawley (Harlan Sprague Dawley, Indianapolis, IN, USA). They were received by the animal care facility of the University of Connecticut and housed individually in stainless steel cages with a 12-h dark/light cycle. Upon arrival, the animals were given a 4-week acclimatization period, during which they were fed a standard rat chow for 2 weeks ad libitum, followed by powdered AIN-93M rodent diet [16] purchased from Dyets (Bethlehem, PA, USA), for another 2 weeks. Animals were cared for in accordance with the animal care and use guidelines approved by the University of Connecticut Institutional Animal Care and Use Committee (Protocol #AHL 0201).

2.2. Diets and experimental protocol

After the acclimatization period, a baseline blood sample was taken, and the animals were ovariectomized. Seven rats per treatment were randomly assigned to either a control group fed AIN-93M diet containing no supplemental carnitine or to the L-carnitine group fed the same diet containing 150 mg L-carnitine L-tartrate (L-Carnipure tartrate; Lonza, Allendale, NJ, USA) per kilogram of diet. This level of L-carnitine when standardized for daily energy intake provides an amount that is within the range typically consumed by an adult human [17]. Food and water were provided ad libitum throughout the 8-week feeding period. Food consumption was monitored daily, and body weight was determined three times per week. At the end of the 8 weeks, animals were fasted overnight, and a blood sample was taken via the orbital sinus. The animals then were euthanized by an isoflurane overdose followed by pneumothorax. Immediately after euthanasia, tissues including liver, lung, heart, kidney, brain and adipose tissues were removed, weighed and stored at −80°C.

2.3. Plasma retinol and α-tocopherol analysis

To prepare plasma samples for retinol and α-tocopherol analysis, an aliquot (100 μl) of plasma was mixed with 100 μl of ethanol to denature protein, and 50 μl of the internal standard (α-tocopherol acetate) were added. The sample then was extracted three times with hexane. Combined hexane extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 200 μl of 2-propanol for high-performance liquid chromatography (HPLC) analysis.

Simultaneous analysis of retinol and α-tocopherol was performed by using reverse phase isocratic HPLC, as described by Barua et al. [18]. Vitamins were separated on a Microsorb-MV 100-3 C18 column (Varian, Palo Alto, CA, USA) with a mobile phase of methanol/water (98:2 vol/vol). Retinol was detected at 325 nm and α-tocopherol at 290 nm. A standard curve for each analyte was constructed by plotting the peak area vs. injected mass of α-tocopherol and retinol standards. A regression equation for each standard curve was used to quantitate the concentration of retinol and α-tocopherol in the samples.

2.4. Tissue α-tocopherol and retinol analysis

Total lipids were extracted from finely minced individual tissue samples by the method of Folch et al. [19]. Solvents were removed with nitrogen, and the extract was reconstituted with chloroform/methanol (5:1 vol/vol) mixture with butylated hydroxyl toluene (151 μmol/L) and stored under nitrogen at −20°C. To prepare samples for vitamin analysis, an aliquot of the lipid extract was saponified for 30 m at 70°C with 10 volumes of 10% potassium hydroxide. The mixture was cooled in ice water and extracted three times with hexane. The hexane layer was transferred to another tube, and δ-tocopherol (Sigma-Aldrich, Saint Louis, MO, USA) added as the internal standard. The lipid extract was dried under nitrogen and reconstituted with methanol/chloroform (1:3 vol/vol) prior to HPLC analysis, as described above.

2.5. Tissue fatty acid analysis

Total fatty acids were extracted from tissues and transmethylated in methanol/hexane (4:1, vol/vol) in the presence of acetyl chloride by the method of Lapage and Roy [20]. Fatty acid methyl esters were separated by gas-liquid

chromatography on a Supelcowax 10 fused silica capillary column (30 m×0.53 mm internal diameter) (Supelco, Bellefonte, PA, USA). Identification of fatty acids was based on retention time of known fatty acid standards (Nu-Chek-Prep, Elysian, MN, USA). The fatty acid profile was expressed as a percentage of the peak area of an individual fatty acid relative to the sum of total fatty acids.

2.6. Plasma and tissue lipids

Total cholesterol and TG in the plasma were determined enzymatically utilizing Roche Diagnostics standards and kits [21,22]. Liver TG was determined using an aliquot of the total lipid extract used for vitamin analysis.

2.7. Statistical analysis.

Data were analyzed using Student's *t* test to compare the group means. Values were expressed as mean±S.D. Differences were considered significant at $P<.05$.

3. Results

3.1. General observation

At the end of the 8-week experimental period, animals were 21 months old. During the study, 1 animal from the control and 1 from the carnitine group were found sick and euthanized as advised by the university veterinarian. At the end of the study, one of the animals in the carnitine group was eliminated from the experiment because of reduced food intake and weight gain during the last 2 weeks of the feeding period. The final number of animals in this study was five animals in control group and six animals in carnitine treatment group.

Dietary treatment did not affect food intake or body weight gain. The mean daily food intake over the 8-week feeding period was similar for both groups (control=14.9±3.3 g, carnitine=15.7±3.2 g). No significant differences between groups were noted in the initial body weight (282.2±18 g, control, vs. 282.0±10 g, carnitine) or final body weight (326.0±22.2 g, control, vs. 325.0±16 g, carnitine). Weights of different tissues (brain, liver, kidney, heart, spleen, retroperitoneal fat, perirenal fat and epididymal fat) are shown in Table 1. There were no differences in tissue weights in response to carnitine treatment.

Table 1
Body and selected organ weights after an 8-week feeding period

	Control	Carnitine	Significance
Total body	326.0±22.2	325.0±16.5	NS
Liver	8.33±1.28	7.81±0.85	NS
Brain	1.37±0.14	1.32±0.06	NS
Kidney	1.84±0.19	1.85±0.14	NS
Heart	0.78±0.03	0.75±0.05	NS
Spleen	0.60±0.04	0.58±0.06	NS
Retroperitonial fat	10.06±1.44	11.12±2.51	NS
Perirenal fat	11.14±3.12	9.07±2.04	NS
Epidiymal fat	12.33±4.52	12.92±1.51	NS

Values are expressed as mean±S.D. (g); control, $n=5$; carnitine, $n=6$.
NS, no statistical significance ($P>.05$).

Table 2
Change in plasma TG, cholesterol, α-tocopherol and retinol

	Control	Carnitine
TG (mmol/L)		
Initial	1.57±0.77	1.49±0.53
Final	1.17±1.11	0.73±0.38
Paired *t* test	NS	$P<.05$
Cholesterol (mmol/L)		
Initial	5.50±0.75	5.45±0.43
Final	5.81±0.99	6.57±0.99
Paired *t* test	NS	NS
α-Tocopherol (μmol/L)		
Initial	30.0±5.6	24.1±3.1
Final	28.4±5.6	27.9±3.2
Paired *t* test	NS	$P<.09$
Retinol (μmol/L)		
Initial	1.14±0.17	1.00±0.27
Final	0.89±0.11	0.84±0.09
Paired *t* test	NS	NS

Values are expressed as mean±S.D.; control, $n=5$; carnitine, $n=6$.

3.2. Tissue and plasma lipids

The liver concentration of TG was reduced by 33%, whereas the liver concentration of α-tocopherol increased by 72%, in response to carnitine feeding. No change was noted in liver retinol levels. No differences were observed in α-tocopherol and retinol levels in retroperitoneal fat or lungs because of carnitine (data not shown). The concentrations of α-tocopherol in the brain remained unaffected. Retinol in the brain was less than 1 nmol/g and was not quantitated. Plasma was collected from animals after a 12-h fast and analyzed for TG, cholesterol, retinol and α-tocopherol (Table 2). The plasma level of TG was significantly lower in response to carnitine feeding ($P<.01$). However, no significant differences were noted in plasma cholesterol and retinol due to supplemental carnitine. The plasma level of α-tocopherol tended to increase ($P<.09$) after 8 weeks of carnitine feeding when compared to the baseline level (Table 3).

3.3. Tissue fatty acids

Carnitine feeding did not affect the total fatty acid profile in the liver or brain (Table 4). The proportion of 22:6n-3 in the

Table 3
Liver and brain α-tocopherol and retinol after an 8-week feeding period

	Control	Carnitine	Significance
Liver			
Total TG (mg/g)	156.9±31.8	96.9±18.3	$P<.01$
α-Tocopherol (nmol/g)	73.3±21.3	126.3±22.1	$P<.01$
Retinol (μmol/g)	2.25±0.63	2.80±0.51	NS
Brain			
α-Tocopherol (nmol/g)	48.7±3.4	51.3±6.1	NS
Retinol (μmol/g)	ND	ND	

Values are expressed as mean±S.D.; control, $n=5$; carnitine, $n=6$.
ND, not measured <1 nmol/g.

Table 4
Comparison of the profiles of major fatty acids in liver and brain [weight (%)]

FAME[a]	Liver		Brain	
	Control	Carnitine	Control	Carnitine
C16:0	31.0±2.0	31.8±2.0	19.2±0.6	19.5±0.6
C16:1n-7	8.7±1.5	9.3±1.4	0.7±0.2	0.7±0.5
C18:0	7.9±2.0	7.4±1.5	23.1±0.5	23.3±0.4
C18:1n-9	28.9±2.4	30.4±2.0	22.0±1.3	21.4±0.5
C18:2n-6	8.7±0.9	8.0±1.1	0.9±0.5	0.7±0.1
C20:4n-6	7.0±2.2	6.4±1.7	11.0±0.7	11.0±0.5
C22:5n-3	0.7±0.2	0.6±0.1	1.7±0.3	1.6±0.2
C22:6n-3	2.0±0.7	1.7±0.6	13.6±0.6	13.8±0.7

Values are expressed as percentage of peak areas, as detected in the gas chromatography (mean±S.D.). Control, $n=5$; carnitine, $n=6$. No statistically significant ($P>.05$) difference between treatments.
[a] Obtained from total lipid extract, derivatization and GC analysis.

brain and liver were unaffected by carnitine feeding. The fatty acid profile for each tissue was relatively constant from animal to animal. Total lipid fatty acid profile suggests that supplemental carnitine did not affect elongation-desaturation pathways of n-3 fatty acids in the brain or liver.

4. Discussion

4.1. Body and tissue weights

The present study is the first to investigate the effect of dietary L-carnitine on fat soluble vitamin status and lipid profiles of various tissues in aging ovariectomized rats. Recent studies showed that dietary carnitine reduces the periepididymal adipose tissue in 2–3-month-old rats [23] and lowers the accumulation of abdominal fat in 46-day-old broiler chickens [24]. In the present study, however, the weight gain and adipose tissue weights were similar in the control and carnitine groups. Our results are consistent with the finding that body weight and abdominal fat did not change in mature ovariectomized rats [25] and in moderately obese premenopausal women [26], in response to supplemental carnitine. Perhaps one factor contributing to the different results is the age of animals. Carnitine supplementation influences nutrient partitioning differently in young vs. old rats [27].

4.2. Tissue and plasma lipids

Our data here show that carnitine significantly lowers plasma TG. Plasma TG generally increases with age, and hypertriglyceridemia is a risk factor for coronary heart disease in both men and women [28]. Our finding and the observations by others [29–31] suggest that supplemental carnitine may lower the risk of coronary heart disease by preventing the age-associated rise in plasma TG. Carnitine may lower plasma TG by increasing the utilization and/or oxidation of fatty acids [31] for energy or possibly by altering very low-density lipoprotein synthesis. The present data showed that the TG levels in the liver exceeded 10% by weight. The diagnosis of steatosis can be made when 5–10% of the liver weight or greater is lipid [32], suggesting the rats in the present study exhibited steatosis. Hepatic steatosis may be fairly common in old rats as total lipid in the liver increases with age and is 2–3-fold greater in old compared to young rats [33]. While liver steatosis was previously thought to be benign, it is now known to be related to several metabolic disorders [32]. L-Carnitine was observed to ameliorate ethanol induced fatty liver in rats [34,35]. Our data show that carnitine markedly lowers TG accumulation in the liver of aging ovariectomized rats, suggesting that supplemental carnitine may decrease the development of hepatic steatosis often associated with aging.

In the current study, L-carnitine supplementation significantly increased α-tocopherol in the liver and tended to increase plasma vitamin E. The improved vitamin E status of our animals may slow the loss of antioxidant function associated with aging. Previous studies have also observed improved body status of vitamin E and antioxidant function with carnitine feeding [36,37]. Carnitine can potentially influence antioxidant status by decreasing oxidative stress and age-related disorders within the cell [38]. In addition to intracellular mechanisms, Zou et al. [12] recently demonstrated that supplemental carnitine significantly increases the lymphatic absorption of α-tocopherol in aging rats. It is unclear how dietary carnitine influences α-tocopherol absorption; it is probably through enhanced fat absorption. Evidence shows that a carnitine-dependent fatty acid transport system in the endoplasmic reticulum is essential for TG synthesis and chylomicron production in the enterocyte [15]. Supplemental carnitine did not affect the α-tocopherol levels in nonhepatic tissues such as the brain, adipose tissue and lungs. Although the reason for difference in tissue response is unknown, it could be attributed to varying tissue functions and requirements for α-tocopherol and carnitine [39].

In contrast to the observations with α-tocopherol, carnitine did not affect the plasma or liver status of retinol. Plasma retinol levels are maintained over a wide range of vitamin A intakes and only change after depletion of vitamin A reserves in the liver, so lack of response in the plasma in the current study was not totally surprising [40].

The amount of retinol in the liver was much greater than vitamin E. Liver retinol levels were elevated but similar to previously observed levels in old rats [33]. When vitamin A levels in the liver are high, as seen in this study, there is an increased conversion of retinol to more polar metabolites for removal from the body in the bile and urine [41,42]. Because of the high levels of retinol in the liver of the rats in this study, the potential for change in liver retinol in response to carnitine treatment was less than the potential for change in α-tocopherol.

4.3. Tissue fatty acids

Our original hypothesis that carnitine supplementation would affect fatty acid elongation and desaturation and,

therefore, DHA levels in tissues was not supported. The percentage of DHA in the total lipid of each tissue type was very constant. As our animals were old with large fat reserves, they may not have been an appropriate model to test this hypothesis. Differences may be seen with young growing animals.

In conclusion, the present study provides evidence that dietary supplementation of L-carnitine improves the utilization of fat, lower the liver and plasma levels of TG and enhances the α-tocopherol status of aging ovariectomized animals. Whether supplemental L-carnitine may be of benefit to postmenopausal women in lowering plasma TG and improving the antioxidant status remains to be studied.

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