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Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats

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The effect of L-carnitine on lipid peroxidation and antioxidant status has been studied in blood, liver, and kidney of young and aged rats. In the aged rats, level of lipid peroxidation was considerably high, while the antioxidants superoxide dismutase, catalase, glutathione peroxidase, vitamin C, and vitamin E reduced glutathione, and total thiols were low. The L-carnitine was administered by i.p. injection (300 mg/kg body weight/day) for 7, 14, and 21 days. Supplementation of aged rats with L-carnitine demonstrated a time-dependent normalization of abnormally elevated lipid peroxides and of subnormal antioxidant status. Carnitine administration to young rats has brought reduction in lipid peroxidation and elevation in glutathione peroxidase activity and ascorbic acid content. From our observations we conclude that as carnitine is very effective in normalizing age-associated alterations, it can be implemented in the aged to minimize age-associated disorders where free radicals are the major cause. (J. Nutr. Biochem. 9:575–581, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Enzymatic and non-enzymatic antioxidants are engaged in scavenging free radicals produced during cellular metabolism, of which superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), vitamins C and E, reduced glutathione (GSH), and total sulfhydryl groups (TSH) are of important concern.[1] During aging, antioxidant functions decline in almost all mammals.[2] Also, higher levels of free radicals have been reported in aged rats.[3] A rise in free-radical level may be attributed either to its enhanced generation or to the reduction in antioxidant level. The mRNA level for the enzymatic antioxidants such as SOD, CAT, and GPx were quantified in older animals and found to be diminished.[4] Recently, protection rendered by antioxidants in free-radical-mediated pathological conditions has been reviewed.[5]

Recent studies from our laboratory have shown that supplementation of an antioxidant vitamin C to the aged rats has normalized the levels of lipids and lipid peroxidation (LPO), GSH, TSH, vitamin C, vitamin E, and activities of SOD, CAT, and GPx.[6] L-carnitine (vitamin B_T) is one such antioxidant.[7] The major role of L-carnitine is in the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation and subsequent energy production,[8] and it has been reported to decline in aging mice and humans.[9] Of late, the role of carnitine as an antioxidant has been implicated in adriamycin-induced membrane damage,[10] diphtheria toxins,[11] and ischemia-reperfusion injury.[12] Studies on the antioxidant effect of carnitine on aging are sparse and yet to be elucidated.

Because nutritional supplementation with antioxidants has been found to delay the onset of aging and age-associated degenerative diseases, a study of L-carnitine supplementation (a naturally occurring and conditionally essential nutrient) was undertaken.

Methods and materials

L-carnitine (inner salt) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade. Male albino rats of Wistar strain weighing approximately 160 g (young) and 280 g (old) were used. The animals were divided into two major groups, namely, Group I: Normal young rats (3 to 4

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Table 1 Effect of L-carnitine on carnitine status of young and aged rats

Parameter	Young rats				Aged rats			
	Group Ia (Control)	Group Ib (7 days)	Group Ic (14 days)	Group Id (21 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)	Group IId (21 days)
Plasma (nmol/mL)	54.0 ± 4.1	57.4 ± 4.7	58.2 ± 5.1	58.9 ± 5.3	41.0 ± 4.3***	49.3 ± 4.9[1]	52.9 ± 5.5[1]	53.2 ± 5.2[1,2]
Liver (μg/mg protein)	0.54 ± 0.050	0.56 ± 0.052	0.57 ± 0.051	0.56 ± 0.054	0.46 ± 0.049*	0.49 ± 0.048	0.51 ± 0.050	0.53 ± 0.051[1]
Kidney (μg/mg protein)	1.72 ± 0.21	1.79 ± 0.24	1.86 ± 0.23	1.88 ± 0.26[1]	1.29 ± 0.17**	1.51 ± 0.20[1]	1.66 ± 0.22[1]	1.68 ± 0.20[1,2]
Urine (mg/24 hr)	2.5 ± 0.30	2.81 ± 0.21	3.12 ± 0.19[1]	3.61 ± 0.25[1,2]	2.00 ± 0.15**	2.11 ± 0.15	2.22 ± 0.19[1]	2.31 ± 0.21[1,2]

Each value is expressed as mean ±SD for six rats in each group.
[1]Group Ib/IIb versus respective other groups.
[2]Group Ic/IIc versus other groups.
Group Ia versus Group IIa:
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

months old) and Group II: Normal aged rats (above 24 months old). Each group was further subdivided into four groups: one control group (Groups Ia, IIa) and three experimental groups based on the duration of carnitine administration for 7 days (Groups Ib, IIb), 14 days (Groups Ic, IIc), and 21 days (Groups Id, IId). The animals were maintained on commercial rat feed, which contained 5% fat, 21% protein, 55% nitrogen-free extract, 4% fiber (wt/wt), and mineral and vitamin contents were adequate. Each group consisted of six animals and had access to food and water ad libitum. Experimental animals were administered L-carnitine (300 mg/kg body weight/day) i.p. in 0.89% saline at physiological pH. Control animals received saline alone. Body weights of both the young and aged animals were monitored throughout the duration of carnitine therapy, and the changes were found to be insignificant.

On completion of 7, 14, and 21 days of carnitine administration the animals were sacrificed by cervical decapitation. Blood was collected in heparinized tubes. Liver and kidney were excised immediately and immersed in physiological saline. Homogenate (10%) was then prepared in 0.01 M Tris-HCl buffer (pH 7.4). Plasma was separated and hemolysate was prepared.[13] Analyses were carried out in blood for GSH[14] and vitamin C;[15] plasma for carnitine,[16] LPO,[17] vitamin E,[18] and TSH;[19] hemolysate for CAT,[20] SOD,[21] GPx,[22] and liver and kidney homogenates for all the parameters mentioned above (namely LPO, GSH, TSH, vitamins C and E, CAT, SOD, and GPx). All the spectrophotometric readings were recorded using UVIKON 810 KONTRON spectrophotometer.

Statistical analysis

Values are mean ±SD for six rats in each group, and significance of the differences between mean values was determined by one-way analysis of variance (ANOVA) coupled with the Student-Newman-Keul multiple comparison test. Values of $P < 0.05$ were considered to be significant.

Statistical significance of differences between the young control (Group Ia) and aged control (Group IIa) was determined by Student's *t*-test. Levels of significance were evaluated with *P*-values.

Results

Table 1 shows the carnitine status in plasma, liver, kidney, and urine of both normal and L-carnitine-treated young and aged rats. In the aged rats a significant decrease in carnitine level was noticed in the three biological specimens (plasma, liver, and kidney) in comparison with young rats. Upon carnitine supplementation, a time-dependent elevation in carnitine concentration was observed in young and aged rats, with the rise being highly significant in the latter group. Urinary excretion of carnitine was remarkably high in the young treated rats when compared with the aged treated rats.

Table 2 depicts the level of LPO, vitamin E, and TSH in plasma; vitamin C and GSH in blood; and SOD, CAT, and GPx in hemolysate of normal and carnitine-treated young and aged rats. Lipid peroxide level was considerably high, whereas the levels of GSH, TSH, and vitamins C and E were remarkably low in aged rats. Carnitine administration brought down the level of lipid peroxide while elevating the levels of enzymatic and non-enzymatic antioxidants in aged rats. In young rats, carnitine administration exhibited a lowering of lipid peroxides while elevating the glutathione peroxidase activity (Table 2).

Lipid peroxidation and the antioxidant status in the livers of young and aged rats are shown in Table 3. A significant increase in lipid peroxidation and reduction in antioxidant level/activity was noticed in old rats, which was subsequently normalized on prolonged (21 days) carnitine administration. Carnitine administration for 21 days also brought down the level of lipid peroxides and enhanced the level of vitamin C in young rats (Table 3).

Comparisons of lipid peroxidation, and enzymatic and non-enzymatic antioxidants in young and aged rat kidneys before and after carnitine administration are shown in Table 4. Lipid peroxidation was significantly high, whereas the antioxidants were markedly lowered in aged rats as compared to young rats. Lipid peroxide level was significantly reduced while the antioxidants GSH, TSH, vitamins C and E, SOD, CAT, and GPx were significantly increased upon 21 days of carnitine treatment of aged rats. Carnitine administration showed a decrease in lipid peroxidation in young rats (Table 4).

Table 2 Effect of L-carnitine on LPO, GPx, CAT, SOD, GSH, TSH, and vitamins C and E in young and aged rats

Parameter	Young rats				Aged rats			
	Group Ia (Control)	Group Ib (7 days)	Group Ic (14 days)	Group Id (21 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)	Group IId (21 days)
LPO (nmol of MDA released/ mg protein)	2.75 ± 0.631	2.61 ± 0.263	2.43 ± 0.186	2.05 ± 0.216[1]	3.86 ± 0.265**	3.36 ± 0.398[1]	3.00 ± 0.400[1]	2.80 ± 0.260[1–3]
GPx (μmol of GSH oxidized/ min/ mg Hb)	5.33 ± 0.546	5.93 ± 0.575	6.25 ± 0.864	6.76 ± 0.742[1]	2.86 ± 0.366***	3.33 ± 0.398	3.91 ± 0.470[1]	4.68 ± 0.470[1,2]
CAT (μmol of H_2O_2 consumed/ min/mg Hb)	0.66 ± 0.051	0.68 ± 0.075	0.76 ± 0.121	0.81 ± 0.147	0.46 ± 0.081***	0.50 ± 0.126	0.60 ± 0.063[1]	0.65 ± 0.054[1]
SOD (units/min/ mg Hb)	3.21 ± 0.365	3.46 ± 0.550	3.70 ± 0.565	4.01 ± 0.581	2.65 ± 0.207**	2.83 ± 0.233	2.98 ± 0.285	3.13 ± 0.287[1,2]
GSH (mg/dL)	2.13 ± 0.163	2.20 ± 0.236	2.30 ± 0.328	2.43 ± 0.422	1.46 ± 0.206***	1.66 ± 0.186	1.78 ± 0.132[1]	1.93 ± 0.163[1,2]
TSH (μg/mg protein)	1.95 ± 0.187	2.05 ± 0.500	2.23 ± 0.403	2.50 ± 0.424	1.35 ± 0.383**	1.45 ± 0.294	1.78 ± 0.325	1.93 ± 0.175[1]
Vitamin C (mg/dL)	1.18 ± 0.343	1.58 ± 0.381	1.63 ± 0.512	1.86 ± 0.423	0.58 ± 0.047**	0.71 ± 0.075	0.86 ± 0.103[1]	1.10 ± 0.103[1,2]
Vitamin E (mg/dL)	1.42 ± 0.230	1.67 ± 0.270	1.71 ± 0.250	1.76 ± 0.300	0.81 ± 0.080***	0.91 ± 0.120	1.01 ± 0.170	1.09 ± 0.170[1]

LPO, TSH, and vitamin E were analyzed in plasma; GPx, CAT, and SOD in hemolysate; and GSH and vitamin C in whole blood.
Each value is expressed as mean ±SD for six rats in each group.
[1]Group Ib/IIb versus respective other groups.
[2]Group Ic/IIc versus other groups.
[3]Group Id/IId versus Ic/IIc.
Group Ia versus Group IIa:
**$P < 0.01$.
***$P < 0.001$.

Table 3 Effect of L-carnitine on liver LPO, GPx, CAT, SOD, GSH, TSH, and vitamins C and E in young and aged rats

Parameter	Young rats				Aged rats			
	Group Ia (Control)	Group Ib (7 days)	Group Ic (14 days)	Group Id (21 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)	Group IId (21 days)
LPO (nmol of MDA released/ mg protein)	2.55 ± 0.187	2.30 ± 0.477	2.31 ± 0.376	2.05 ± 0.326[1]	3.76 ± 0.531***	3.41 ± 0.376	2.70 ± 0.244[1]	2.61 ± 0.213[1,2]
GPx (μmol of GSH oxidised/min/mg protein)	10.01 ± 1.463	10.70 ± 1.270	11.45 ± 1.080	12.33 ± 1.175	6.18 ± 0.487***	6.71 ± 0.636	7.43 ± 0.625[1]	9.05 ± 0.622[1–3]
CAT (μmol of H_2O_2 consumed/min/ mg protein)	56.00 ± 3.840	57.26 ± 4.243	58.70 ± 5.090	61.81 ± 4.795	44.68 ± 5.88**	49.50 ± 7.858	51.86 ± 4.226	54.30 ± 4.326[1]
SOD (units/min/mg protein)	7.80 ± 0.556	8.31 ± 0.601	8.46 ± 0.653	8.78 ± 0.630	6.11 ± 0.658***	6.40 ± 0.660	6.91 ± 0.577	7.58 ± 0.567[1,2]
GSH (μg/mg protein)	11.96 ± 0.979	12.35 ± 0.845	12.60 ± 0.800	13.38 ± 0.868	8.03 ± 0.769**	9.40 ± 0.635	10.15 ± 0.790[1]	11.48 ± 0.964[1,2]
TSH (μg/mg protein)	23.11 ± 2.853	25.88 ± 2.290	26.20 ± 2.857	28.18 ± 3.014	15.70 ± 2.146***	17.85 ± 2.327	19.45 ± 2.830	21.06 ± 1.680[1,2]
Vitamin C (μg/mg protein)	3.01 ± 0.343	3.16 ± 0.294	3.43 ± 0.344	3.83 ± 0.408[1]	2.20 ± 0.236***	2.55 ± 0.234	2.76 ± 0.206[1,2]	2.96 ± 0.206[1–3]
Vitamin E (μg/mg protein)	2.10 ± 0.300	2.24 ± 0.310	2.43 ± 0.330	2.51 ± 0.350	1.34 ± 0.150***	1.42 ± 0.170	1.56 ± 0.170[1]	1.72 ± 0.210[1,2]

Each value is expressed as mean ±SD for six rats in each group.
[1]Group Ib/IIb versus respective other groups.
[2]Group Ic/IIc versus other groups.
[3]Group Id/IId versus Ic/IIc.
Group Ia versus Group IIa:
**$P < 0.01$.
***$P < 0.001$.

Table 4 Effect of L-carnitine on kidney LPO, GPx, CAT, SOD, GSH, TSH, and vitamins C and E in young and aged rats

Parameter	Young rats				Aged rats			
	Group Ia (Control)	Group Ib (7 days)	Group Ic (14 days)	Group Id (21 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)	Group IId (21 days)
LPO (nmol of MDA released/mg protein)	2.18 ± 0.271	1.85 ± 0.250	1.56 ± 0.250	1.45 ± 0.288[1]	3.81 ± 0.299***	3.30 ± 0.219[2]	2.70 ± 0.316[1]	2.41 ± 0.194[1,2]
GPx (μmol of GSH oxidised/min/mg protein)	9.08 ± 1.135	9.40 ± 0.927	9.60 ± 1.063	9.80 ± 1.063	5.55 ± 0.437***	6.11 ± 0.392[2]	7.01 ± 0.708[1,2]	8.08 ± 0.947[1,2]
CAT (μmol of H_2O_2 consumed/min/mg protein)	43.10 ± 3.897	44.03 ± 4.230	47.00 ± 3.802	48.03 ± 5.096	34.21 ± 2.487***	37.95 ± 3.130	39.13 ± 3.395	41.33 ± 3.433[1]
SOD (units/min/mg protein)	5.55 ± 0.535	6.16 ± 0.585	6.33 ± 0.739	6.63 ± 0.910	4.51 ± 0.299**	4.83 ± 0.557[2]	5.26 ± 0.382[1]	5.41 ± 0.365[1,2]
GSH (μg/mg protein)	8.56 ± 0.847	8.95 ± 1.040	10.13 ± 0.739	10.80 ± 0.828	3.90 ± 0.447***	4.65 ± 0.602	5.61 ± 0.541[1]	6.78 ± 0.679[1,2]
TSH (μg/mg protein)	23.60 ± 2.242	25.83 ± 2.022	26.68 ± 3.139	27.78 ± 4.345	15.31 ± 1.876***	18.01 ± 2.968	19.75 ± 3.629[1]	21.03 ± 3.819[1,2]
Vitamin C (μg/mg protein)	1.90 ± 0.228	2.15 ± 0.187	2.28 ± 0.365	2.48 ± 0.462	0.63 ± 0.163***	0.86 ± 0.216[2]	1.25 ± 0.408[1]	1.63 ± 0.366[1–3]
Vitamin E (μg/mg protein)	1.24 ± 0.160	1.35 ± 0.180	1.41 ± 0.190	1.49 ± 0.230	0.96 ± 0.100**	1.01 ± 0.120	1.14 ± 0.130	1.16 ± 0.140[1]

Each value is expressed as mean ± SD for six rats in each group.
[1]Group Ib/IIb versus respective other groups.
[2]Group Ic/IIc versus other groups.
[3]Group Id/IId versus Ic/IIc.
Group Ia versus Group IIa:
**$P < 0.01$.
***$P < 0.001$.

Discussion

Although the crucial role of carnitine in cellular metabolism is to control the influx of long-chain fatty acids into mitochondria for β-oxidation and subsequent energy production, its role as an antioxidant in the aging process is yet to be elucidated. The present investigation thus focused on the effect of L-carnitine on lipid peroxidation and antioxidant status of aging rats.

Carnitine supplementation increased the carnitine status of both young and aged rats, the increase being highly significant in the aged animals. The possible reason can be that, as the aged rats are carnitine deficient (Table 1), they absorb the administered carnitine readily to meet the requirements of the system. However, compared to absorption, the excretion of carnitine was very high in the young rats, which may be due to the fact that carnitine levels are within the physiological level in the young animals.

Age-associated increase in lipid peroxidation was observed in our study. Lipids act as vital substrates for lipid peroxidation, and the enhancement of lipid profile during aging[23] may be the cause for increased lipid peroxidation. Also, an enhanced level of lipid peroxides in hyperlipidemia was reported,[24] suggesting a causal relationship between lipids and lipid peroxidation. Upon carnitine administration, a decrease in lipid peroxidation was observed. This may have been due to its active role in the transport of fatty acids for energy production, thereby lowering the availability of lipids for peroxidation.

The production of highly reactive oxygen species such as $O_2^{\cdot -}$, H_2O_2, and $OH^{\cdot}$ is also catalysed by free iron through Haber-Weiss reaction.[25] Carnitine and its esters have been shown to partially inhibit iron-induced lipid peroxidation in liposomes[7] by forming complexes with free iron. Thus, the reduction in lipid peroxidation in the present study is due to the iron-chelating property of L-carnitine.

Age-related increase in lipid peroxidation might be a reflection of decrease in enzymatic and non-enzymatic antioxidant protection.[26] The antioxidant defense system is composed mainly of three enzymes—viz. glutathione peroxidase, catalase, and superoxide dismutase. A significant decline in the levels of these three enzymes in aging was observed in our study. GPx catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of reduced glutathione. The lowered GPx activity in aging may be attributed to the decline in glutathione concentration. L-carnitine has been shown to raise the glutathione level in ischemia[27] and thereby enhance the activity of GPx. Also, the age-related decrease in activities of SOD and catalase documented in our study is corroborated by earlier investigators.[4,6] The possible reason could be the decreased synthesis of these enzymes. As enzymes are proteins, the reduced protein synthesis during aging due to decreased ATP production,[28] may be the cause for the reduction in the activities of these enzymes. Carnitine supplementation by virtue of its ability to enhance ATP production[29] might have improved the overall protein (and thus enzyme) synthesis in cells. Moreover L-carnitine, being an antioxidant, can protect these enzymes from further peroxidative damage.

Glutathione and total thiols non-enzymatically reduce peroxides and/or prevent occurrence of peroxidation. In the present study, a significant decrease in these sulfhydryl compounds was noticed in aged rats. The recycling of GSH from GSSG (oxidised glutathione) is catalyzed by the enzyme glutathione reductase using NADPH as a cofactor. NADPH is generated in a pathway involving the enzyme glucose-6-phosphate dehydrogenase (*G6PD*). Age-associated decline in the activity of *G6PD*[30] leading to diminished production of NADPH may be the cause for the observed decrease in GSH level. Carnitine supplementation has improved the glutathione and total thiol status in aged rats. L-carnitine has been shown to exert thiol and methionine sparing activity,[31] and it is suggested that this improved the antioxidant status. Moreover, methionine itself is an antioxidant and also one of the precursors for carnitine biosynthesis.

Decreases in ascorbic acid and α-tocopherol concentrations were significant in aging animals. Ascorbic acid scavenges $O_2^{\cdot -}$ and $OH^{\cdot}$ radicals and converts α-tocopheroxyl radical to α-tocopherol. The resulting dehydroascorbic acid is reduced back to ascorbic acid by glutathione.[32] The observed increase in ascorbic acid status upon carnitine supplementation may be attributed to the enhanced GSH regeneration by carnitine. Also, as ascorbic acid is one of the cofactors in carnitine biosynthesis, supplementation of L-carnitine may spare ascorbic acid, thereby elevating its level. As ascorbic acid has the ability to regenerate α-tocopherol,[33] the concomitant increase in vitamin E concentration of aged rats with carnitine administration in this study could be due to either decrease in oxidative stress or increase in ascorbic acid level.

As the antioxidant status is basically normal in young rats, it is not altered significantly by carnitine supplementation, whereas carnitine administration is found to be very effective in lowering lipid peroxidation and enhancing the antioxidant status in aged rats.

In conclusion, the above observations suggest that the impaired oxidant–antioxidant balance in senescence can be attributed, at least in part, to carnitine deficiency. Hence, carnitine therapy for the aged may consequently reduce age-associated disorders and related diseases where free radicals are the major cause.

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