

# Effect of L-Carnitine on Brain Lipid Peroxidation and Antioxidant Enzymes in Old Rats

P. Juliet Arockia Rani and C. Panneerselvam

Department of Medical Biochemistry, Dr. Alagappa Mudaliar Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, India.

The effect of L-carnitine on lipid peroxidation and enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, was evaluated in brain regions of young and old rats. In all brain regions except the hypothalamus, lipid peroxidation was higher for old rats than for young control rats. The activity of superoxide dismutase, glutathione peroxidase, and catalase was lower in the striatum, cerebral cortex, and hippocampus, but no difference was observed in the hypothalamus and cerebellum. L-Carnitine administration (intraperitoneally) prevented thiobarbituric acid-reactive substance formation in the cerebral cortex, cerebellum, hypothalamus, hippocampus, and striatum of 24-month-old rats. Administration of L-carnitine reversed the age-associated changes in a duration-dependent manner. Results suggest that the neuroprotective effect on the brains in old rats was achieved by the elevation of antioxidants with L-carnitine.

**A**GING is associated with an array of changes in neuronal function, many of which have been attributed to accumulation of reactive oxygen species (ROS) that arises from either an increased formation of ROS, a compromised ability of the aged brain to cope with stress, or a combination of both factors (1,2). The brain is prone to oxidative damage because of its comparatively high levels of oxygen metabolism and the unique composition of its cellular membrane that contains large amounts of oxidant-sensitive polyunsaturated fatty acids.

L-Carnitine is a quaternary ammonium compound distributed heterogeneously in the brain. All the enzymes necessary for the synthesis of carnitine are present in the brain tissue. L-Carnitine transports long-chain fatty acids through the inner mitochondrial membrane, regulates ketogenesis, and is involved in the metabolism of branched chain amino acids (3). Acetyl L-carnitine (ALCAR) supplementation in old rats reverses markedly the age-associated decline in many indices of mitochondrial function and general metabolic activity (4). ALCAR has the ability to reduce the age-dependent increase of cerebral levels of both sphingomyelin and cholesterol (5). Matsuoka and coworkers (6) reported that, in the mouse, L-carnitine suppressed seizures and impairments of brain metabolism caused by hyperammonemia. These findings suggested that L-carnitine might modulate energy metabolism in the brain. Although carnitine can be synthesized in the body, adults normally obtain carnitine from the diet. The decrease in carnitine concentration in old age is attributed mainly to malnourishment (7). This compound is also used as a therapeutic agent in several conditions such as atherosclerosis, kidney failure, mitochondrial myopathy, and AIDS (8). The current study analyzes the free radical scavenging action of L-carnitine in various brain regions of young and old rats.

## METHODS

### Materials

L-Carnitine (inner salt) was obtained from Sigma (St. Louis, MO). All other chemicals were of reagent grade.

### Animals

Male Wistar albino rats were segregated into two different age groups: young (4 months of age) and old (24 months of age), respectively. Each age group was subdivided into four groups: a control group Ia and three treated groups. Treated groups were based on the days of carnitine administration: 7 days, 14 days, and 21 days. Rats were maintained on commercial rat feed that contained 5% fat, 21% protein, 55% nitrogen-free extract, and 4% fiber (w/w), with adequate mineral and vitamin contents. Each group consisted of six rats. The rats had access to food and water ad libitum. The experimental group was administered L-carnitine (300 mg/kg body weight/day) intraperitoneally in 0.89% physiological saline, whereas the control groups received saline alone. Twenty-four hours after the last injection in the respective groups, all the animals were decapitated; their brains were quickly, but carefully, removed and dissected into the hypothalamus, hippocampus, cortex, cerebellum, and striatum according to the method of Glowinski and Iverson (9) and immersed in saline. Tissue homogenate (10%) was prepared in the ice-cold Tris-EDTA buffer (pH 7.4; 0.1 M Tris-HCl and 1 mM EDTA) using mechanically driven Teflon-fitted Potter Elvehjem-type homogenizer for 1 minute for the total disruption of cells. Tissue homogenates were used to measure superoxide dismutase (SOD) (10), catalase (11), glutathione peroxidase (12), and lipid peroxidation (13). The difference in values of control and experimental groups were analyzed by Student's *t* test.

Table 1. Effect of L-Carnitine on Lipid Peroxidation of Various Brain Regions

Brain Region	Young				Old			
	Control (0 d)	Treated			Control (0 d)	Treated		
		7 d	14 d	21 d		7 d	14 d	21 d
Hippocampus	2.95 ± 0.20	2.90 ± 0.11	2.80 ± 0.08	2.76 ± 0.18	4.81 ± 0.80###	4.30 ± 0.21	3.42 ± 0.60**	3.16 ± 0.36***
Striatum	3.12 ± 0.32	3.10 ± 0.26	3.04 ± 0.56	3.00 ± 0.36	5.50 ± 0.92###	4.90 ± 0.80	4.82 ± 0.64**	3.50 ± 0.55***
Cortex	3.56 ± 0.91	3.40 ± 0.33	3.30 ± 0.74	3.09 ± 0.81	6.10 ± 0.60###	5.40 ± 0.54*	4.10 ± 0.79**	3.91 ± 0.89***
Cerebellum	2.74 ± 0.60	2.61 ± 0.10	2.40 ± 0.54	2.10 ± 0.74	3.71 ± 0.40###	3.58 ± 0.32	3.09 ± 0.38*	3.01 ± 0.31**
Hypothalamus	2.97 ± 0.25	2.80 ± 0.30	2.62 ± 0.48	2.48 ± 0.66	3.35 ± 0.43#	3.21 ± 0.24	3.03 ± 0.33	3.03 ± 0.07*

Notes: Lipid peroxidation was expressed as nmoles of malondialdehyde released/gram of tissue. Values are expressed as the means ± SD for six animals in each group.

Comparison of the 7-d, 14-d, and 21-d groups with the 0-d group (young) and the 7-d, 14-d, and 21-d groups with the 0-d group (old): \* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ . Comparison of aged control with young control: # $p < .05$ ; ## $p < .01$ ; ### $p < .001$ .

## RESULTS

Table 1 shows the status of lipid peroxidation in various brain regions of young and old rats before and after L-carnitine supplementation. For old rats, compared with young control rats, a greater level of lipid peroxidation was observed in the cortex, hippocampus, striatum, and cerebellum, but not the hypothalamus. L-Carnitine supplementation did not induce any alteration in the brains of young rats, whereas in the brains of old rats, lipid peroxidation was reduced in all areas except the hypothalamus. After 21-days ALCAR treatment, highly significant ( $p < .001$ ) changes were observed in the cortex, hippocampus, and striatum in the brains of old rats treated for 21 days, compared with old control rats.

Table 2 presents the effect of L-carnitine on the activity of SOD in the brains of young and old rats. Our observation revealed that the activity of SOD was lower in old control rats when compared with young control rats. Substantial decrements were observed in the cortex and striatum ( $p < .001$ ) and moderate decrements were observed in other brain regions of old rats ( $p < .01$ ). After 21 days of treatment, L-carnitine supplementation restored the enzyme activity to near-normal levels in the brains of old rats.

Table 3 shows the activities of glutathione peroxidase in the brains of young and old rats before and after L-carnitine treatment. As observed for SOD activity, glutathione peroxidase activity was decreased in the cortex, hippocampus, and striatum of old control rats when compared with the same brain regions of young rats ( $p < .001$ ). Apparently,

both L-carnitine supplementations enhanced the activity of glutathione peroxidase in the brains of both young and old rats. The activity of glutathione peroxidase was near normal in the cortex, hippocampus, and striatum of 21-day treated old rats, compared with that of old control rats.

Table 4 shows the efficacy of L-carnitine supplementation on the activity of catalase in the brains of young and old rats. Age-dependent decrements in the activity of catalase were observed in specific brain regions of rats. The activity of catalase decreased in all five brain regions of old rats, with significant changes observed in the cortex, hippocampus, and striatum;  $p < .001$ . Supplementation of carnitine to the old rats partially reversed the age-associated decrements. These effects varied among the brain regions (26%, 16%, and 14% increases in catalase activities, in the cortex, hippocampus, and striatum, respectively).

## DISCUSSION

Aging induces several structural and functional changes in the brain. An imbalance of changes caused by increased generation of free radicals and decreased functional efficiency of the antioxidant system has been suggested to be one of the primary factors that contributes to the aging process (14). Antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase, constitute a natural defense system against the activity of oxidants. The survival of an organism may depend on the ability of the organism to overcome the toxic effects of ROS.

SOD catalyzes the dismutation of the superoxide radical

Table 2. Effect of L-Carnitine on Superoxide Dismutase Activity of Various Brain Regions

Brain Region	Young				Old			
	Ia (0 d)	Ib (7 d)	Ic (14 d)	Id (21 d)	Ila (0 d)	Ilb (7 d)	Ilc (14 d)	Ild (21 d)
Hippocampus	0.470 ± 0.040	0.482 ± 0.020	0.501 ± 0.020	0.522 ± 0.062	0.350 ± 0.070##	0.371 ± 0.020	0.411 ± 0.007*	0.435 ± 0.010**
Striatum	0.540 ± 0.030	0.549 ± 0.015	0.559 ± 0.009	0.567 ± 0.023	0.460 ± 0.050###	0.488 ± 0.040	0.503 ± 0.024*	0.524 ± 0.010**
Cortex	0.340 ± 0.035	0.357 ± 0.020	0.373 ± 0.042	0.389 ± 0.061	0.258 ± 0.020###	0.274 ± 0.030	0.293 ± 0.022**	0.314 ± 0.019***
Cerebellum	0.510 ± 0.050	0.527 ± 0.050	0.539 ± 0.017	0.546 ± 0.150	0.410 ± 0.040##	0.423 ± 0.032	0.441 ± 0.050	0.454 ± 0.041*
Hypothalamus	0.440 ± 0.021	0.451 ± 0.043	0.455 ± 0.043	0.461 ± 0.025	0.345 ± 0.087##	0.357 ± 0.010	0.372 ± 0.041	0.411 ± 0.006*

Notes: Enzyme activity is expressed as units/gram of tissue. Values are expressed as the means ± SD for six animals in each group.

Comparison of the 7-d, 14-d, and 21-d groups with the 0-d group (young) and the 7-d, 14-d, and 21-d groups with the 0-d group (old): \* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ .

Comparison of aged control with young control: ## $p < .01$ ; ### $p < .001$ .

Table 3. Effect of L-Carnitine on Glutathione Peroxidase Activity of Various Brain Regions

Brain Region	Young				Old			
	Ia (0 d)	Ib (7 d)	Ic (14 d)	Id (21 d)	IIa (0 d)	IIb (7 d)	IIc (14 d)	IId (21 d)
Hippocampus	0.870 ± 0.024	0.879 ± 0.012	0.885 ± 0.052	0.894 ± 0.072	0.696 ± 0.045###	0.734 ± 0.014*	0.778 ± 0.048**	0.801 ± 0.036***
Striatum	0.700 ± 0.025	0.723 ± 0.041	0.750 ± 0.076	0.770 ± 0.093	0.590 ± 0.049###	0.621 ± 0.027	0.654 ± 0.014**	0.693 ± 0.061***
Cortex	0.830 ± 0.023	0.840 ± 0.013	0.852 ± 0.034	0.869 ± 0.058	0.598 ± 0.090###	0.650 ± 0.007*	0.742 ± 0.068**	0.810 ± 0.092***
Cerebellum	0.870 ± 0.021	0.890 ± 0.032	0.910 ± 0.061	0.932 ± 0.082	0.800 ± 0.024##	0.815 ± 0.024	0.838 ± 0.008	0.854 ± 0.035*
Hypothalamus	0.670 ± 0.054	0.688 ± 0.027	0.697 ± 0.034	0.714 ± 0.048	0.567 ± 0.068##	0.580 ± 0.041	0.619 ± 0.046*	0.633 ± 0.013**

Notes: Enzyme activity is expressed as  $\mu$ moles of glutathione oxidized/gram of tissue. Values are expressed as the means  $\pm$  SD for six animals in each group.

Comparison of the 7-d, 14-d, and 21-d groups with the 0-d group (young) and the 7-d, 14-d, and 21-d groups with the 0-d group (old): \* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ .

Comparison of aged control with young control: ## $p < .01$ ; ### $p < .001$ .

into hydrogen peroxide ( $H_2O_2$ ). Age-related decline in SOD activity, as documented in this study, was also observed in earlier investigations (15,16). Superoxide radicals affect the lipid phase of the myelin by changing its structure into a more disordered state (17). The superoxide radical also inhibits aconitase activity and thereby affects the tricarboxylic acid cycle (18). Earlier studies (16) showed that carnitine supplementation enhances the dismutation of superoxide radicals by increasing the levels of SOD activities.

Susceptibility to oxidative stress may differ among various regions of the brain. A form of glutathione peroxidase is the major antioxidative enzyme present in the brain. Glutathione peroxidase decomposes  $H_2O_2$  at the expense of the glutathione molecule (19). The activity of this enzyme was low in the brains of old rats. The lowering of glutathione peroxidase activity with aging may be attributed to a decline in glutathione concentration. The enzyme monoamine oxidase oxidizes dopamine with the production of  $H_2O_2$ , leading to an accumulation of  $H_2O_2$  and dopamine metabolites. During aging, the activity of monoamine oxidase is particularly high in the cortex and striatum, which may be responsible for the higher susceptibilities of these regions to ROS stress. L-Carnitine enhanced glutathione peroxidase activity significantly in both of these regions. Apparently L-carnitine promotes energy production particularly in the brains of old rats. The energy-promoting action of L-carnitine was confirmed in the condition of hyperammonemia (20,21). An increase in the activity of catalase after supplementation was observed, confirming that carnitine provides reducing equivalents necessary for converting catalase from the inactive form to the active form.

The greater the degree of unsaturation in fatty acid, the higher the rate of lipid peroxidation. Neuronal membranes, that are rich in unsaturated fatty acids, are highly susceptible to lipid peroxidative damage, an index of ROS stress (22). Increased arachidonic acid turnover may play a vital role in increased ROS stress. Iron is a potent pro-oxidant and a necessary catalyst for in vitro lipid peroxidation (23). The hippocampus contains a large amount of nonheme iron that makes this area of the brain particularly susceptible to free radical-induced lipid peroxidation. Based on the degree of lipid peroxidation, the hippocampus, striatum, and cortex are likely to be highly sensitive to ROS stress. Some age-related increases in lipid peroxidation may be correlated with decreased antioxidant enzyme activities. L-Carnitine supplementation causes a dramatic reduction in malondialdehyde formation in each of the three brain regions. Carnitine supplementation increased overall antioxidant enzyme status as a function of the duration of treatment, thus decreasing the levels of free radicals available for lipid peroxidation. Carnitine can also act as a chelator by decreasing the concentration of cytosolic iron, which plays a very important role in free radical chemistry (24). Koudelova and colleagues (25) showed that the production of lipoperoxides were decreased by carnitine, thereby protecting various tissues from damage. Carnitine and its esters protect cells from ROS damage both by inhibiting free radical propagation and by contributing to the repair of oxidized membrane phospholipid (26). Differential activities of these enzymatic antioxidants in diverse regions may be responsible for different rates of aging in various brain regions.

In summary, the regional variation seen in the action of

Table 4. Effect of L-Carnitine on Catalase Activity of Various Brain Regions

Brain Region	Young				Old			
	Ia (0 d)	Ib (7 d)	Ic (14 d)	Id (21 d)	IIa (0 d)	IIb (7 d)	IIc (14 d)	IId (21 d)
Hippocampus	0.290 ± 0.042	0.312 ± 0.071	0.324 ± 0.064	0.346 ± 0.051	0.240 ± 0.022##	0.251 ± 0.031	0.262 ± 0.017*	0.278 ± 0.032**
Striatum	0.420 ± 0.061	0.438 ± 0.028	0.452 ± 0.014	0.463 ± 0.018	0.360 ± 0.024##	0.382 ± 0.043	0.398 ± 0.019**	0.409 ± 0.017***
Cortex	0.370 ± 0.041	0.381 ± 0.019	0.421 ± 0.073	0.433 ± 0.084	0.280 ± 0.040###	0.308 ± 0.020	0.334 ± 0.048*	0.352 ± 0.051**
Cerebellum	0.360 ± 0.054	0.382 ± 0.044	0.403 ± 0.076	0.424 ± 0.087	0.317 ± 0.004#	0.339 ± 0.058	0.347 ± 0.042	0.354 ± 0.041*
Hypothalamus	0.327 ± 0.074	0.345 ± 0.028	0.363 ± 0.027	0.379 ± 0.011	0.258 ± 0.054#	0.270 ± 0.045	0.284 ± 0.027	0.304 ± 0.031*

Notes: Enzyme activity is expressed as  $\mu$ moles of hydrogen peroxide consumed/gram of tissue. Values are expressed as the means  $\pm$  SD for six animals in each group.

Comparison of the 7-d, 14-d, and 21-d groups with the 0-d group (young) and the 7-d, 14-d, and 21-d groups with the 0-d group (old): \* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ .

Comparison of aged control with young control: # $p < .05$ ; ## $p < .01$ ; ### $p < .001$ .

carnitine on antioxidant status in different brain regions of young and old rats may be caused by regional differences in the uptake and transport of L-carnitine. Our results show that L-carnitine has a capacity to prevent free radical-induced damage, partly because of its antioxidant property.

#### ACKNOWLEDGMENTS

This work was supported in part by a research grant from Lady Tata Memorial Trust (Mumbai, India) and the University Grant Commission (Delhi, India).

Address correspondence to C. Panneerselvam, Department of Medical Biochemistry, Dr. Alagappa Mudaliar Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani-113, India. E-mail: julietibms@yahoo.com

#### REFERENCES

- Reiter RJ. Oxidative process and oxidative defense mechanism in the aging brain. *FASEB J*. 1995;9:526–533.
- Zs-Nagy I. *The Membrane Hypothesis of Aging*. Boca Raton, FL: CRC Press Inc.; 1994.
- Scaglia F, Longo N. Primary and secondary alterations of neonatal carnitine metabolism. *Semin Perinatol*. 1999;23:152–161.
- Hagen TM, Ingersoll RT, Wehr CM, et al. Acetyl-L-carnitine fed old rats partially restores mitochondrial function and ambulatory activity. *Proc Natl Acad Sci USA*. 1998;95:9562–9566.
- Aureli T, Di Cocco ME, Capuani G, et al. Effect of long-term feeding with acetyl-L-carnitine on the age-related changes in rat brain lipid composition: a study by <sup>31</sup>P-NMR spectroscopy. *Neurochem Res*. 2000;25:395–399.
- Matsouka M, Igisu H, Kohriyama K, Inove N. Suppression of neurotoxicity of ammonia by L-carnitine. *Brain Res*. 1991;567:328–331.
- Costell MQ, Connor JE, Grisolia S. Age-associated decrease of carnitine content in muscle of mice and humans. *Biochem Biophys Res Commun*. 1989;161:1135–1143.
- Famularo G, DeSimone C. Carnitine stands on its own in HIV infection treatment. *Arch Intern Med*. 1999;159:1143–1144.
- Glowinski K, Iverson LL. Regional studies of catecholamines in the rat brain. The disposition of [<sup>3</sup>H]dopamine and [<sup>3</sup>H]DOPA in various regions of rat brain. *J Neurochem*. 1996;13:655–699.
- Marklund S, Marklund G. Involvement of the superoxide anion radical in autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*. 1974;47:469–474.
- Sinha AK. Colorimetric assay of catalase. *Anal Biochem*. 1974;47:389–394.
- Rotruck JT, Pope A, Ganther HE. Selenium: biochemical role as a component of glutathione peroxidase, purification and assay. *Science*. 1973;179:588–590.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95:351–358.
- Reiter RJ. Oxidative process and antioxidative defense mechanism in the aging brain. *FASEB J*. 1995;9:351–358.
- Jayachandran M, Jayanthi B, Sundaravadivel B, Panneerselvam C. Status of lipids, lipid peroxidation and antioxidant systems with vitamin C supplementation during aging in rats. *J Nutr Biochem*. 1996;7:272–275.
- Kalaiselvi T, Panneerselvam C. Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. *J Nutr Biochem*. 1998;9:575–581.
- Chia LS, Thompson TE, Mescarello MA. Disorder in human myelin induced by superoxide radical: an *in vitro* investigation. *Biochem Biophys Res Commun*. 1983;117:141–146.
- Gardner PR, Nguyen DD, White CW. Aconitase is a sensitive and critical target of oxygen poisoning in cultured mammalian cells and in rat lungs. *Proc Natl Acad Sci USA*. 1994;91:12248–12252.
- Jain A, Martensson J, Stole E, Auld PAM, Meistar A. Glutathione deficiency leads to mitochondrial damage in brain. *Proc Natl Acad Sci USA*. 1991;88:1913–1917.
- Matsuoka M, Igisu H. Protection of the brain by carnitine. *Brain Res*. 1992;590:334–336.
- Igisu H, Matsuoka M, Iryo Y. Protection of the brain by carnitine. *J Occup Health*. 1995;37:75–82.
- Yu BP, Yang R. Critical evaluation of the free radical theory of aging. *NY Acad Sci*. 1996;786:1–11.
- Backer GT, Martin GR. Biological aging and longevity. Underlying mechanism and potential intervention strategies. *J Aging Activity*. 1984;2:304–328.
- Resnik AZ, Kagan VE, Ramsey R, et al. Antiradical effects of L-propionyl carnitine protection of the heart against ischemia reperfusion injury: the possible role of iron chelation. *Arch Biochem Biophys*. 1992;296:394–401.
- Koudelova J, Mourek J, Drahota Z, Rauchova H. Protective effect of carnitine on lipid peroxide formation in rat brain. *Physiol Res*. 1994;43:387–389.
- Arduini A. Carnitine and its acyl esters as secondary antioxidants? *Am Heart J*. 1992;123:1726–1727.

Received January 3, 2001

Accepted November 19, 2001

Decision Editor: John A. Faulkner, PhD