Effect of L-Carnitine on Blood Lipid Composition in Atherosclerotic Rats

Anandan DAYANANDAN, Ponnusamy KUMAR, Thangavel KALAISELVI,
and Chinnakkannu PANNEERSELVAM*

*Department of Biochemistry, Dr. ALM Post Graduate Institute of
Basic Medical Sciences, University of Madras, Taramani Campus,
Madras 600 113, India*

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Summary The effect of L-carnitine on serum lipids was studied in rats with experimentally-induced atherosclerosis. L-Carnitine at a dose of 300 mg/kg body weight was given intraperitoneally for 7 days after the induction of atherosclerosis. Carnitine caused a significant reduction in the levels of serum cholesterol, triglycerides, phospholipids, and free fatty acids and elicited a significant increase in HDL cholesterol. The significant reduction observed in the LDL/HDL ratio is prominent from the therapeutic point of view in the treatment against hypercholesterolemia in experimental atherosclerosis.

Key Words: L-carnitine, atherosclerosis, triglycerides, LDL/HDL ratio, hypercholesterolemia

Atherosclerosis is a disease of the arterial wall. Accumulation of lipid-laden foam cells in the subendothelial region of the arterial wall is an early event in the development of atherosclerosis [1, 2]. Carnitine (β -hydroxy- γ -trimethylamino-butyrate) plays a vital role in lipid catabolism and energy production in mammals. In fatty acid catabolism, the inner mitochondrial membrane is impermeable to either long-chain fatty acids or their coenzyme A esters, whereas their acyl carnitine derivatives are able to permeate the membrane. The reactions effecting this transport are catalyzed by carnitine acyl transferases I and II. Failure of this transport mechanism because of carnitine deficiency or abnormalities in the carnitine acyl transferase enzymes will reduce the rate of fatty acid oxidation, resulting in muscle weakness coupled with elevated plasma levels of enzymes of muscle origin [3, 4].

* To whom correspondence should be addressed.

The decreased levels of carnitine in cardiac tissue may also adversely affect myocardial function, and it has been shown that carnitine deficiency can produce cardiomyopathy [5-7]. A lipid-lowering effect of carnitine therapy has been found in patients with hemodialysis [8, 9] and diabetic hyperlipidemia [10]. Abdel-Kader *et al.* [11] reported that carnitine has a lipid-lowering effect on dog sera, whereas Guarnieri *et al.* [8] showed that carnitine has the same effect in chronically uremic patients.

The above studies suggest that carnitine therapy may be beneficial in hyperlipidemia. The present investigation was undertaken to study the effect of carnitine on experimentally-induced atherosclerosis.

MATERIALS AND METHODS

Male albino rats weighing between 150-200 g and having serum cholesterol ranging from 90-110 mg/dl were divided into 3 groups: a control (normal) group of 10 animals and saline- and carnitine-treated atherosclerotic groups, each comprising of 20 rats.

A basal diet was used for them, i.e., a commercial rat feed manufactured by M/s Hindustan Lever Ltd., Bombay, and marketed under the trade name "Gold Mohur Feed." The feed contained 5% fat, 21% protein, 55% nitrogen-free extract, 360 kcal of metabolizable energy/100 g, and 4% fiber. The minerals and vitamin content were also up to the same level of international specifications and universal formulae. Animals were accommodated in well-ventilated spacious cages, and food and water were provided *ad libitum*.

Hypercholesterolemia was induced by giving an atherogenic diet. The diet was prepared by mixing equal amounts of commercial feed powder and atherogenic constituents. The final form of atherogenic diet contained 5% cholesterol, 20% sucrose, 20% hydrogenated vegetable oil, 2% sodium cholate, 2% lactose, 0.4% choline chloride, and 0.15% thiouracil; and these components were mixed with an equal amount (w/w) of commercial feed. The procedure adopted was that of Hahn *et al.* [12] modified by the addition of thiouracil. This mixture was made into a powder form, repelleted, and given to the animals. Serum cholesterol, triglycerides, protein, blood glucose, and blood urea were screened at the end of every month for a period of 4 months in the animals in all the groups and compared.

A pharmacological dose (300 mg/kg body weight) of carnitine dissolved in saline was administered intraperitoneally for 7 days, with the last injection occurring 24 h prior to sacrifice. This procedure was adopted from Brady *et al.* [13] with a slight modification in the dosage of carnitine. After the experimental period, rats were decapitated, and blood was collected for the biochemical investigations.

The investigations done in normal, saline-treated atherosclerotic, and carnitine-treated atherosclerotic rats were serum cholesterol [14], serum triglycerides [15], serum phospholipids [16], serum free fatty acids [17], serum lipo-

proteins [18, 19], serum proteins [20], blood glucose [21], and blood urea [22].

Statistical significance of difference was determined by one-way analysis of variance and least significant difference test or independent Student's *t*-test [23]. $p < 0.05$ was regarded as significant.

RESULTS

There was a highly significant increase in serum levels of cholesterol and triglycerides when rats were fed the atherogenic diet (Table 1). During the first 30–60 days of the diet, both cholesterol and triglycerides were significantly increased but no substantial increase was seen in the later stages (between 90–120 days). The increase was slowly retarded due to the saturation of these lipids, possibly by a self-regulating mechanism. The atherogenic diet did not have any significant or

Table 1. Levels of whole blood and serum parameters during atherogenic feeding.

Parameter	Atherogenic diet	Initial	30 days	60 days	90 days	120 days
Cholesterol (mg/dl)	—	78 ± 6	80 ± 10	94 ± 7	92 ± 8	97 ± 12
	+	—	160 ± 10**	210 ± 16***	228 ± 19***	258 ± 21***
Triglycerides (mg/dl)	—	110 ± 11	113 ± 13	106 ± 9	138 ± 8	125 ± 12
	+	—	180 ± 14***	224 ± 17***	235 ± 8***	246 ± 17***
Protein (g/dl)	—	8.4 ± 1	7.6 ± 1.2	8.3 ± 1	8.2 ± 1.2	9.0 ± 1.4
	+	—	8.1 ± 1.3	8.6 ± 1.3	8.3 ± 1.4	9.1 ± 1.0
Glucose (mg/dl)	—	66 ± 6	68 ± 7	64 ± 6	67.0 ± 7	70 ± 5.5
	+	—	66 ± 7	69 ± 6	68.5 ± 7	69 ± 7.0
Urea (mg/dl)	—	36.5 ± 7	37 ± 6	37 ± 5	37.0 ± 4	38 ± 8.0
	+	—	40 ± 5.4	38 ± 4	36.0 ± 5	37 ± 6.0

Serum was used for the analysis of parameters except glucose and urea, which were estimated in whole blood. Values are expressed as mean ± SD. For statistical evaluation of significant variations, normals were compared with the group fed the atherogenic diet. ** $p < 0.01$, *** $p < 0.001$: statistically significant alteration from the initial value.

Table 2. Levels of serum cholesterol, triglycerides, phospholipids and free fatty acids in normal and experimental animals.

Group	Serum			
	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	Fatty acids (mg/dl)
I. Control	112 ± 5.2	110 ± 7.8	120 ± 6.8	21 ± 3.5
II. Saline-treated atherosclerotic	246 ± 9***	228 ± 6***	241 ± 12.2***	105 ± 12.1***
III. Carnitine-treated atherosclerotic	172 ± 13.1***	164 ± 9.7***	230 ± 15*	76 ± 4.3***

Values are expressed as mean ± SD. For statistical evaluation of significant variations, Group I was compared with Group II; and Group II was compared with Group III. Alterations were statistically significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 3. Levels of serum LDL, VLDL, HDL, and LDL/HDL ratio in normal and experimental animals.

Group	Serum			
	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	LDL/HDL ratio
I. Control	41 ± 5.1	16 ± 8.2	40 ± 4.3	1.025 ± 1.1
II. Saline-treated atherosclerotic	80 ± 8.2***	62 ± 9.7***	78 ± 9.3***	1.025 ± 0.8
III. Carnitine-treated atherosclerotic	41 ± 4.8***	34 ± 11.8***	96 ± 14.2*	0.427 ± 0.33***

Values are expressed as mean ± SD. For statistical evaluation of significant variations, Group I was compared with Group II; and Group II was compared with Group III. Statistical significant alterations were statistically significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

adverse effect on serum proteins, blood glucose, and urea.

There was a highly significant decrease in serum levels of cholesterol, triglycerides, and free fatty acids and a significant reduction in phospholipid levels after the administration of carnitine in the atherosclerotic rats, as shown in Table 2.

After carnitine treatment of the atherosclerotic rats, the low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels decreased significantly (Table 3), whereas a significant increase ($p < 0.05$) occurred in the high-density lipoprotein (HDL). Thus the LDL/HDL ratio (coronary risk index) decreased dramatically.

DISCUSSION

Carnitine is required to maintain normal cardiac function. This finding was first observed by Wittels and Bressler [24] in diphtheria-infected guinea pigs. The diphtheria toxin produced a depletion of myocardial carnitine, which resulted in cardiomyopathy. Carnitine treatment partially prevented the reduction in myocardial performance in these animals [25]. A number of subsequent studies have now shown that carnitine deficiency is closely correlated with cardiomyopathy. Wittels and Bressler [24] suggested that an 80% myocardial carnitine depletion adversely affects the contractile function. However, recent studies have shown that even a 60% depletion can adversely affect the heart [5].

Carnitine has also been shown to have a lipid-lowering effect on prolonged administration in dogs [11]. Further, a decrease in serum total lipids in diabetics after carnitine therapy was reported by Bekaert and Deltour [10]. A decrease in serum triglyceride concentrations had been reported in type-IV hyperlipoproteinemia patients treated with carnitine [26]. Hosein and Bextan [27] reported the ability of carnitine to decrease liver and serum triglycerides as well as the serum level of glutamic oxaloacetic transaminase in ethanol-fed rats. The effects of carnitine on lipid metabolism in hyperlipidemia encouraged us to study the effect of carnitine on blood lipid patterns in experimental atherosclerosis.

The highly significant decrease in serum cholesterol in carnitine-treated atherosclerotic rats, which is a prominent finding in this work, is in agreement with the results of Bekaert and Deltour [10]. Also, the highly significant decrease found in the level of triglycerides agrees with the results of Abdel-Kader *et al.* [11] in dogs and Macbashi *et al.* [26] in patients with type-IV hyperlipoproteinemia. This reduction in the triglyceride level by carnitine may be attributed to enhance fatty acid oxidation [28]. It was also reported that carnitine inhibited fatty acid incorporation into triglycerides in tissue homogenates or slices [29]. Thus the lowering effect of carnitine on serum triglycerides in experimental atherosclerosis indicates that carnitine should be of great therapeutic value.

Cholesterol and phospholipids of plasma and erythrocytes are in equilibrium due to the active exchange between the two pools of these compounds [25]. A significant decrease in serum phospholipids is another prominent finding in this work on experimental atherosclerosis after carnitine administration. The highly significant decrease in free fatty acids observed after the administration of carnitine may be attributed to the effect of carnitine-stimulated fatty acid oxidation in the mitochondria, and these data agree with the results of Abdel-Aziz *et al.* [30] and Fritz [28].

The significant change in the levels of lipoproteins in our study was due to the opposite effect of carnitine on serum cholesterol components of HDL. The highly significant decrease in the LDL evident in our study was due to the lowering effect of carnitine on the serum cholesterol level, which was discussed before, as the main component of LDL is cholesterol. So carnitine should be of great therapeutic value in hyper- β -lipoproteinemia type II, the main risk of which is premature atherosclerotic vascular lesions [31].

The highly significant reduction in the level of VLDL is due to the lowering effect of carnitine on serum triglycerides, which was evidenced by this work. Thus the effect of carnitine on both LDL and VLDL in atherosclerosis suggest the potentially important therapeutic value of carnitine in type-IV hyperlipoproteinemia.

Increased LDL and decreased HDL have been found to be associated with arteriovascular diseases and an increased incidence of cardiovascular diseases [32, 33]. So carnitine through its reversing effect on these changes should be of great value in treating these diseases. The significant decrease in the LDL/HDL ratio (coronary risk index) in this work was due to the lowering effect of carnitine on serum LDL and significant increasing effect on HDL. This effect is of great importance as the incidence of coronary heart disease is known to parallel an increase in the LDL/HDL ratio [33].

In our study we found that carnitine has a lipid-lowering effect on all blood lipid components except HDL in atherosclerotic rats. This effect is very important from the therapeutic point of view as hyperlipidemia leads to atherosclerosis, the main risk factor for coronary heart disease and other arteriovascular diseases.

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