Experimental Studies

Changes in the Subcellular Distribution of Free Carnitine and Its Acyl Derivatives in Diabetic Rat Hearts Following Treatment with L-Carnitine

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SUMMARY

Carnitine deficiency has been demonstrated in diabetic hearts, and it is also well known that L-carnitine administration has a beneficial effect on cardiac function. Carnitine treatment would be expected to reduce the accumulation of long-chain acylcarnitine. However, many reports have shown that myocardial long-chain acylcarnitine levels were increased following treatment with L-carnitine in whole-heart studies. Since acylcarnitine exists in both the mitochondrial and cytosolic compartments, it is difficult to investigate changes in subcellular distribution by studying whole-heart preparations. The present study investigated the myocardial subcellular distribution of carnitine and its acyl derivatives in diabetic rats with or without L-carnitine treatment. Approximately 90% of total cellular carnitine was located in the cytosol in the diabetic hearts. Both mitochondrial and cytosolic levels of free carnitine and short-chain acylcarnitine were significantly decreased in the diabetic heart. However, the mitochondrial level of long-chain acylcarnitine was significantly increased. L-carnitine treatment reduced the mitochondrial level of long-chain acylcarnitine, but the cytosolic level of long-chain acylcarnitine was significantly increased. These results show that L-carnitine treatment prevents the accumulation of long-chain acylcarnitine in the mitochondrial space and then reduces the detergent effect of long-chain acylcarnitine on the mitochondrial membrane. We suggest that it is a possible mechanism of the beneficial effect of L-carnitine treatment on the diabetic heart. (Jpn Heart J **34**: 763–772, 1993)

Key Words :
Free carnitine Long-chain acylcarnitine Cellular distribution
Mitochondria Diabetic heart L-carnitine treatment

CHRONIC diabetes mellitus is known to result in myocardial abnormalities. Hearts from diabetic rats show a decreased ability to recover cardiac function after anoxia[1),2)] and ischemia.[3)] The mechanism of these defects in car-

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diac performance is unclear but it is thought that the metabolic cause may be related to a defect in cardiac performance. It is difficult to utilize glucose as an energy substrate in the diabetic heart.[4),5)] Therefore, the diabetic heart must rely almost exclusively on fatty acids for its production of energy.[6)] Fatty acid oxidation requires the presence of both coenzyme A (CoA) and carnitine. CoA is necessary for the cytosolic activation of long-chain free fatty acids to acyl-CoA esters. Since the long-chain acyl-CoA esters cannot penetrate the inner mitochondrial membrane for subsequent β-oxidation,[7)] long-chain acylcarnitine forms from acyl-CoA and free carnitine. The role of carnitine is to transport long-chain fatty acyl moieties across the inner mitochondrial membrane into the mitochondrial matrix.[8)] Thus, adequate carnitine levels are required for normal fatty acid metabolism in heart muscle. On the other hand, lack of sufficient carnitine is the factor responsible for the accumulation of free fatty acid and related intermediates.

Intermediates of fatty acid metabolism, long-chain acyl derivatives of CoA and carnitine, are elevated in diabetic hearts.[9),10)] In addition, total carnitine content is decreased while CoA levels are increased.[11)] Since long-chain acyl CoA and carnitine esters can inhibit a number of enzyme systems,[12)–15)] it is thought that these metabolic abnormalities may be related to the defects in cardiac performance. Therefore, it is suggested that carnitine treatment may ameliorate the alterations in cardiac performance seen during diabetes by decreasing the accumulation of lipid intermediates. In fact, many reports demonstrated improvements in contractile function and in long-chain acyl-CoA and ATP content of diabetic hearts with carnitine treatment.[16)–18)] However, the influence of long-chain acylcarnitine levels is now controversial. Since cytosolic CoA and long-chain free fatty acid levels are increased in the diabetic heart, administration of free carnitine seems to produce the accumulation of long-chain acylcarnitine in the diabetic heart. Paulson et al[19)] demonstrated that administration of L-carnitine increased long-chain acylcarnitine formation in diabetic hearts, despite its protective effect on myocardial function. On the other hand, Lopaschuk et al[20)] reported that the accumulation of long-chain acylcarnitine in the sarcoplasmic reticulum membrane of diabetic rats was prevented by carnitine treatment. Thus, there have been differing results with respect to long-chain acylcarnitine levels in the diabetic heart with carnitine treatment.

Many key reactions related to free fatty acid metabolism are thought to occur in a single cellular compartment. Since, acylcarnitine is located in both the cytosolic and mitochondrial compartments,[21)] it is impossible to investigate changes in its cellular distribution by studying whole-heart preparations. The mitochondria are a site of β-oxidation of free fatty acid and production of ATP. Therefore, it is important to investigate the influence of carnitine treatment on

mitochondrial distribution of long-chain acylcarnitine in the diabetic heart. However, there are no reports of investigation of the changes in mitochondrial levels of long-chain acylcarnitine in the diabetic heart with L-carnitine treatment.

The purpose of the present study is to assess the effect of L-carnitine on the cellular distribution of carnitine and its acyl derivatives in the diabetic rat heart.

Materials and Methods

Animals: Thirty male Wistar rats aged 8 weeks were used. Diabetes was induced by a single injection of streptozocin (STZ 40 mg/kg) dissolved in citrate

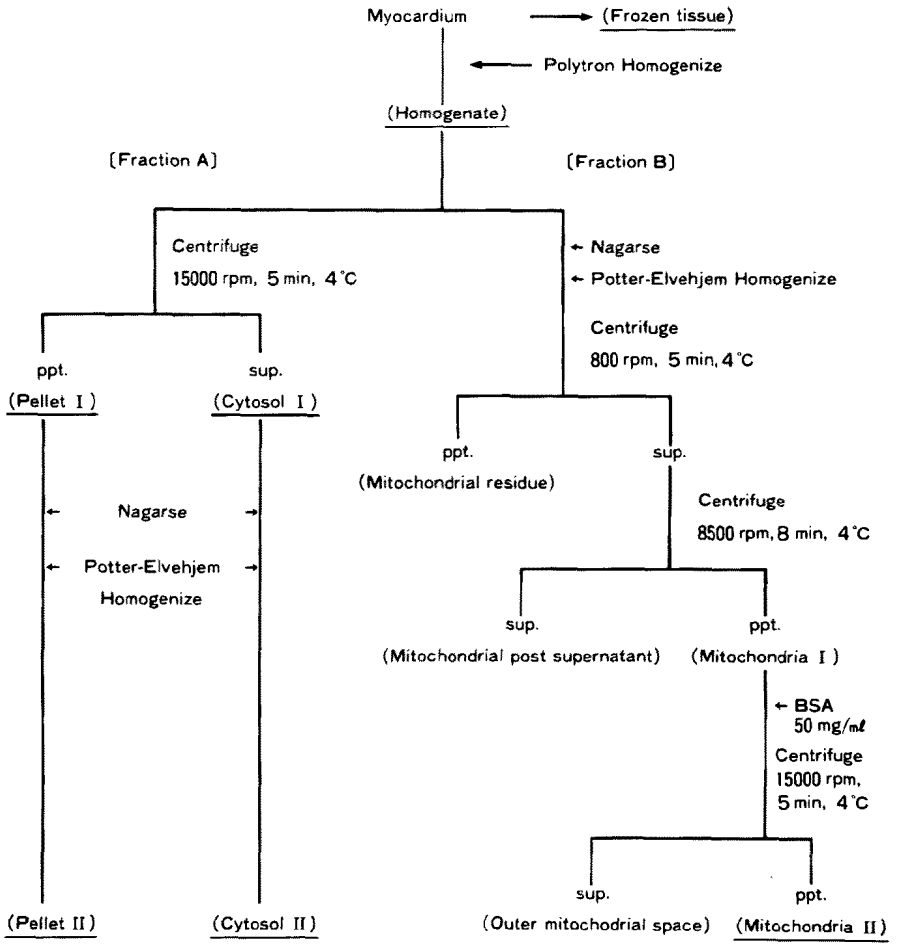

Fig. 1. Preparation of mitochondrial and cytosolic fractions. Mitochondria II and cytosol II were used to measure carnitine and its acyl derivatives.

buffer (pH 4), which was given into the tail vein, while control rats were injected with citrate buffer alone. The rats were maintained at the Institute for Experimental Animals at Hamamatsu University School of Medicine. Food and water were provided ad libitum throughout the study period. The severity of the diabetes was determined by measuring glycosuria with enzymatic test strips. The rats were then divided into three groups: a normal control group (n=10), an untreated diabetic group (n=8), and a L-carnitine-treated diabetic group (n=8). In this last group, L-carnitine salt (3 g/kg/day) dissolved in saline was injected intraperitoneally beginning 3 days after STZ administration. Rats were treated for 6 weeks, and the last injection was given 24 hours before sacrifice. The duration of the study was chosen to coincide with that of the study conducted by Rodrigues et al.[17] The hearts were quickly excised, and a myocardial sample was rapidly freeze-clamped with a Wollenberger clamp cooled in liquid nitrogen for analysis of whole-heart homogenate (Fig. 1; Frozen tissue). The remaining myocardium was immediately chilled in ice-cold isolation buffer containing the following (mM): d-mannitol 225, sucrose 75, HEPES 10, and EGTA 1 (pH 7.4). These myocardial samples were used for isolation of the cytosolic and mitochondrial fractions.

Isolation of the cytosolic and mitochondrial fractions: The methods used for isolation of the cytosolic and mitochondrial fractions have been described previously.[22] In brief, myocardial samples were added to a four-fold volume of ice-cold isolation buffer (pH 7.4) and the tissue suspension was homogenized twice with a Polytron tissue homogenizer (PT-10, Kinematica) for 10 sec at 4°C. The homogenate was then divided into two fractions (Fig. 1: A and B). To obtain fraction A, 2 m*l* of the homogenate were centrifuged at 15,000×g for 5 min at 4°C and the resulting supernatant was immediately stored in liquid nitrogen (cytosolic fraction). To obtain fraction B, the homogenized tissue suspension was first treated with Nagarse. After being allowed to stand at 0°C for 1 min, 13 m*l* of isolation medium were added, and then homogenization was done with a Potter-Elvehjem homogenizer at 500 rpm and 4°C. The homogenate was centrifuged at 800×g for 5 min at 4°C, and the supernatant thus obtained was centrifuged at 8,500×g for 8 min at 4°C. The mitochondrial fraction was then collected from the sediment by centrifugation at 15,000×g for 5 min at 4°C, and immediately frozen in liquid nitrogen.

Assay of carnitine and its acyl derivatives: Each sample was diluted to a 6% perchloric acid solution (v/v) by the addition of 60% perchloric acid, and centrifuged at 12,000×g for 5 min at 0°C. Two hundred microliters of distilled water and 100 μl of 0.5 M HEPES (pH 7.0) were then added to 200 μl of the supernatant thus obtained. After neutralization with KOH, the mixture was centrifuged at 12,000×g for 5 min at 0°C, and the supernatant was used for the free

carnitine assay.

For the measurement of acid-soluble carnitine (containing free carnitine and short-chain acylcarnitine), 200 μl of distilled water and 200 μl of 3 N KOH were added to 200 μl of the supernatant obtained after the addition of 60% perchloric acid to the sample as in the free carnitine assay.

After alkaline hydrolysis for 1 hour at 4°C, 80 μl of 60% perchloric acid and 100 ml of 0.5 M HEPES (pH 7.0) were added to the reaction mixture, and neutralized with KOH. After centrifugation at 12,000×g for 5 min at 0°C, the supernatant thus obtained was used for measurement of the acid-soluble carnitine concentration. The short-chain acylcarnitine concentration was then obtained by subtraction of the free carnitine level from the acid-soluble carnitine level.

After removing the supernatant, the pellet was rinsed twice with 6% perchloric acid solution. After alkaline hydrolysis for 2 hours at 55°C with 0.5 ml of 1.8 N KOH, 130 μl of 60% perchloric acid were added to the reaction mixture, and centrifugation was performed at 12,000×g for 5 min at 0°C. Subsequently, 200 μl of 0.5 M HEPES (pH 7.0) were added to 400 μl of the supernatant and neutralized with KOH. After centrifugation at 12,000×g for 5 min at 0°C, the supernatant was used for the measurement of acid-insoluble carnitine levels (long-chain acylcarnitine). Carnitine was assayed by the radioisotope procedure described by Cederblad et al.[23]

Measurement of the tissue protein concentration was done by the Biuret method[24] using bovine serum albumin as the standard.

Enzyme assays: The activity of citrate synthase (a mitochondrial marker enzyme) was determined by the method of Idell-Wenger et al.[21] Lactate dehydrogenase activity (a cytoplasmic marker enzyme) was determined by the method of Amador et al.[25]

Reagents: STZ was purchased from Sigma (USA) and L-carnitine was purchased from Kongo Chemical Co. (Japan). All other reagents and buffers used were obtained from commercial sources and were of the highest grade available.

Statistical analysis: All results are expressed as the mean ±SE. Significant differences ($p<0.05$) were determined by analysis of variance with Bonferroni's correction for multiple comparisons and by Student's t-test.

Results

Body weight and blood glucose levels: As shown in Table I, both groups of diabetic rats weighed significantly less than the age-matched control rats (both $p<0.01$), but there was no significant difference between the diabetic rats with and without L-carnitine treatment. The blood glucose level was significantly

Table I. Body Weight and Blood Glucose Levels in the Normal and Diabetic Rats

	Normal	Diabetes	
	LC (−) (n=8)	LC (−) (n=8)	LC (+) (n=8)
Body weight (g)	340±10	267±8*	278±6*
Blood glucose (mg/dl)	158±5	496±10*	440±8*

Values are the mean ± SE. Asterisks indicate significant differences ($p<0.01$) vs. the normal group. LC (+) =L-carnitine-treated; LC (−) =untreated.

elevated in the diabetic rats compared with the controls ($p<0.01$), while there was no significant difference between the diabetic rats with and without L-carnitine treatment.

Isolation of the cytosolic and mitochondrial fractions: Mitochondrial protein ranged from 40 to 60 mg of protein/g wet heart muscle in the diabetic rats and the mitochondrial recovery rate was 80%. Lactate dehydrogenase activity (cytoplasmic marker enzyme) was not detected in the mitochondrial fraction and citrate synthase (a mitochondrial marker enzyme) was not extracted from the mitochondria by washing in the isolation buffer. In addition, citrate synthase was not detected in the cytosol fraction.

Changes in carnitine and its acyl derivatives in diabetic rat hearts: Table II shows the concentrations of carnitine and its acyl groups in whole-heart homogenates. The concentrations of free carnitine, short-chain acylcarnitine, and total carnitine were significantly decreased in the diabetic rat hearts compared with the control hearts (all $p<0.01$). On the other hand, the concentration of long-chain acylcarnitine was significantly increased in the diabetic hearts ($p<0.01$). Table III shows the concentrations of carnitine and its acyl derivatives in the cytosolic and mitochondrial fractions of the three groups. In the control rats, approximately 90% of cellular free carnitine, 89% of cellular short-chain acylcarnitine, and 85% of cellular long-chain acylcarnitine were located within

Table II. Changes in the Levels of Carnitine and its Acyl Derivatives in the Whole-heart Homogenates of Normal and Diabetic Rats

	Carnitine (nmol/g wet tissue)			
	Free	Short-chain	Long-chain	Total
Normal rats (n=10)	804±22	385±31	20.1±2.1	1209±37
Untreated diabetic rats (n=8)	330±48*	213±28*	36.3±7.0*	579±62*
L-carnitine-treated diabetic rats (n=8)	1388±111##	765±66##	43.2±3.4#	2196±42##

Values are the mean ±SE. Asterisks indicate significant differences ($p<0.01$) vs. the normal group. ## and # indicate significant differences ($p<0.01$ and $p<0.05$) vs. untreated diabetic rats. free=free carnitine; short-chain=short-chain acylcarnitine; long-chain=long-chain acylcarnitine; total=total carnitine.

the cytosolic compartment. In the untreated diabetic rats, the concentrations of free carnitine and short-chain acylcarnitine in the cytosol and the mitochondria were significantly reduced (both $p<0.01$). On the other hand, the long-chain acylcarnitine concentration was significantly increased in both the cytosol and the mitochondria ($p<0.05$ and $p<0.01$, respectively). The percentage increase in long-chain acylcarnitine was greater in the mitochondrial compartment than in the cytosol (177% vs 80%).

Effects of L-carnitine on the subcellular distribution of carnitine and its acyl derivatives: Table II shows the concentrations of carnitine and its acyl groups in whole-heart homogenates. Administration of L-carnitine significantly increased free carnitine, short-chain acylcarnitine and total carnitine concentrations, respectively (all $p<0.01$). However, the level of long-chain acylcarnitine in the whole-heart homogenate from diabetic rats showed a greater increase following L-carnitine administration ($p<0.05$).

Table III shows the effect of L-carnitine administration on the subcellular distribution of carnitine and its acyl derivatives in diabetic rats. L-carnitine treatment produced differential changes in the carnitine distribution of the cytosolic and mitochondrial compartments. The levels of free carnitine and short-chain acylcarnitine were significantly increased in both the cytosolic and mitochondrial compartments. However, the mitochondrial level of long-chain acylcarnitine was significantly decreased by treatment with L-carnitine, and fell to the same level as

Table III. Subcellular Distribution of Carnitine and its Acyl Groups and Effects of L-Carnitine Treatment on the Subcellular Distribution in Diabetic Rats

	Carnitine (nmol/g wet tissue)	
	Cytosol	Mitochondria
Free carnitine		
Normal rats	721±26	84±9
Untreated diabetic rats	290±46#	41±6#
L-carnitine-treated diabetic rats	1259±103**	129±13**
Short-chain acylcarnitine		
Normal rats	343±30	42±3
Untreated diabetic rats	186±29#	27±7#
L-carnitine-treated diabetic rats	693±62**	72±9**
Long-chain acylcarnitine		
Normal rats	17.0±2.6	3.1±0.8
Untreated diabetic rats	27.7±4.0#	8.6±1.4#
L-carnitine-treated diabetic rats	38.6±3.1**	4.5±0.7*
Total carnitine		
Normal rats	1081±40	129±9
Untreated diabetic rats	503±63#	76±9#
L-carnitine-treated diabetic rats	1991±137**	205±16**

Values are mean ± SE. Asterisks indicate significant differences (** $p<0.01$ and * $p<0.05$) vs. untreated diabetic rats. # indicates significant difference ($p<0.01$) vs. normal rats at each fraction.

that in the control rats. In contrast, the cytosolic level of long-chain acylcarnitine was significantly increased by treatment with L-carnitine.

Discussion

The subcellular distribution of carnitine, CoA, and their acyl derivatives in normal and ischemic rat hearts was first studied by Idell-Wenger et al.[21] Lopaschuk et al[20] examined sarcoplasmic reticulum levels of long-chain acylcarnitine in the diabetic heart. Cardiac sarcoplasmic reticulum levels of long-chain acylcarnitine were significantly elevated, and insulin and D, L-carnitine treatment can lower sarcoplasmic reticulum levels of long-chain acylcarnitine. However, the subcellular distribution (cytosol and mitochondria) of carnitine and CoA has not yet been investigated in the diabetic heart.

Although it may have been preferable to examine the distribution of carnitine, CoA, and their acyl derivatives, there are some problems regarding the loss and redistribution of metabolites during the preparation of mitochondrial and cytosolic fractions. We have already studied the subcellular distribution of carnitine and its acyl derivatives in normal and ischemic canine hearts.[22] Lactate dehydrogenase activity was not detected in the mitochondrial fraction, and the cytosolic fraction contained only about 5% cell membrane contamination. In addition, exogenous [^{14}C]-carnitine and [^{14}C]-palmitoyl carnitine were completely recovered in the post-supernatant mitochondrial fraction and the residual mitochondrial fraction, respectively, whereas neither form of labeled carnitine was detected in the mitochondrial fraction itself. These results indicate that metabolism of carnitine and its acyl derivatives did not occur during preparation of the mitochondrial and cytosolic fractions, and that it is not necessary to correct the values obtained for carnitine.[22]

Approximately 90% of total cellular carnitine was located in the cytosolic compartment in the diabetic rat hearts. Marked changes in the cellular distribution of carnitine and long-chain acylcarnitine occurred in diabetic hearts, and these changes were especially prominent in the mitochondrial compartment. A large percentage of the total intracellular water is in the cytosolic compartment, and only 19% of it is in the mitochondria, the sarcoplasmic reticulum and the other subcellular compartments.[26] Therefore, even a small change in long-chain acylcarnitine level in the mitochondrial compartment could lead to a relatively large effect on long-chain acylcarnitine. Long-chain acylcarnitine has a detergent action and inhibits the activity of enzymes like Na^+, K^+-ATPase.[12-15] Idell-Wenger et al[21] have also suggested that long-chain acylcarnitine may have an adverse detergent effect on the mitochondrial membrane in ischemic hearts. Therefore, it is possible that the detergent effect of long-chain acylcarnitine on

the mitochondrial membrane may be detrimental to the function of mitochondria in diabetic hearts.

L-carnitine treatment would be expected to reduce the accumulation of long-chain acylcarnitine in diabetic hearts. However, myocardial long-chain acylcarnitine levels (whole-heart homogenates) were significantly increased by treatment with L-carnitine. On the other hand, the mitochondrial level of long-chain acylcarnitine was significantly decreased by L-carnitine treatment. These results confirm that L-carnitine treatment reduces the accumulation of long-chain acylcarnitine in the mitochondrial compartment and prevents the detrimental detergent effect on the mitochondrial membrane. In addition, if we had analyzed whole-heart preparations, we could not have detected any reduction of long-chain acylcarnitine in the mitochondrial compartment.

The reduction of long-chain acylcarnitine in the mitochondria may allow better utilization of fatty acids. In support of this hypothesis, Fritz et al have shown that fatty acid oxidation is stimulated by the addition of carnitine to isolated heart mitochondria.[27] However, the addition of carnitine to intact tissue does not always enhance fatty acid uptake and oxidation.[28] The diabetic heart depends on fatty acid for its production of energy. Therefore, better utilization of fatty acids in mitochondria could increase ATP levels and improve myocardial performance. The mechanism by which carnitine treatment can lower mitochondrial levels of long-chain acylcarnitine is as yet unknown. It is possible that carnitine may lower the activity of intracellular long-chain acylcarnitine translocase enzyme in the mitochondrial membrane.

In conclusion, L-carnitine treatment reduced the accumulation of long-chain acylcarnitine in the mitochondrial compartment in diabetic rat hearts. This study indicates that the reduction in mitochondrial levels of long-chain acylcarnitine suppresses the detergent effect of long-chain acylcarnitine on the mitochondrial membrane, and that this mechanism may be related to the beneficial effect of L-carnitine on the diabetic heart.

References

1. Hearse DJ, Steward DA, Chain EB: Diabetes and the survival and recovery of the anoxic myocardium. J Mol Cell Cardiol **7**: 397, 1975
2. Fein FS, Kornstein IB, Strobeck JE, Capasson JM, Sonnenblick EH: Altered myocardial mechanics in diabetic rats. Circ Res **47**: 922, 1980
3. Hearse DJ, Steward DA, Green DG: Myocardial susceptibility to ischemic damage; a comparative study of disease models in the rat. Eur J Cardiol **75**: 437, 1978
4. Miller TB: Cardiac performance of isolated perfused hearts from alloxan diabetic rats. Am J Physiol **236**: H808, 1979
5. Feuvray FS, Idell-Wenger JA, Neely JR: Effects of ischemia on rat myocardial function and metabolism in diabetes. Circ Res **44**: 322, 1979

6. Garland PB, Randle PJ: Regulation of glucose uptake by muscle. 10. Effects of alloxan-diabetes, starvation, hypophysectomy and adrenalectomy and fatty acids, ketone bodies and pyruvate on the glycerol output and concentrations of free fatty acids, long-chain fatty acyl coenzyne A, glycerol phosphate and citrate cycle intermediates in rat heart and diaphragm muscles. Biochem J **93**: 678, 1964
7. Haddock BH, Yates DW, Garland PB: The localization of some enzyme A-dependent enzymes in rat liver mitochondria. Biochem J **119**: 565, 1970
8. Pands SV, Parvin R: Characterization of carnitine acyl carnitine translocase system in heart mitochondria. J Biol Chem **25**: 6683, 1976
9. Fogle PJ, Beiber LL: Effect of streptozotocin on carnitine and carnitine acyl transferases in rat heart, liver, and kidney. Biochem Med **22**: 119, 1976
10. Vary TC, Neely JR: A mechanism for reduced myocardial carnitine levels in diabetic animals. Am J Physiol **243**: H154, 1982
11. Reibel DK, Wyse BW, Berkich DA, Neely JR: Regulation of coenzyme A synthesis in heart muscle; effects of diabetes and fasting. Am J Physiol **240**: H606, 1981
12. Shug AL, Shrago E, Bittar N, Folts JD, Koke JR: Acyl-CoA inhibition of adenine nucleotide translocation in ischemic myocardium. Am J Physiol **228**: 689, 1975
13. Abe M, Yamazaki N, Suzuki Y, Kobayashi A, Ohta H: Effect of palmitoyl carnitine on Na^+, K^+-ATPase and adenylate cyclase activity of canine myocardial sarcolemma. J Mol Cell Cardiol **16**: 239, 1979
14. Wood JM, Bush B, Pitts BJR, Schwartz A: Inhibition of bovine heart Na^+, K^+-ATPase by palmitylcarnitine and palmityl-CoA. Biochem Biophys Res Commun **74**: 677, 1977
15. Katz AM, Messineo FC: Lipid membrane interactions and the pathogenesis of ischemic damage in the myocardium. Circ Res **48**: 1, 1981
16. Pieper GM, Murray WJ: In vivo and in vitro intervention with L-carnitine prevents abnormal energy metabolism in isolated diabetic rat heart; chemical and phosphorous-31 NMR evidence. Biochem Med Metabol Biol **38**: 111, 1987
17. Rodrigues B, Xiang H, McNeill JH: Effect of L-carnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. Diabetes **37**: 1358, 1988
18. Pieper GM, Murray WJ, Salhany JM, Wu ST, Eliot RS: Salient effects of L-carnitine on adeninenucleotide loss and coenzyme A acylation in the diabetic heart perfused with excess palmitic acid. A phosphorous-31 NMR and chemical extract study. Biochim Biophys Acta **803**: 241, 1984
19. Paulson DJ, Schmidt MJ, Traxler JS, Ramacci MT, Shug AL: Improvement of myocardial function in diabetic rats after treatment with L-carnitine. Metabolism **33**: 358, 1984
20. Lopaschuk GD, Tahiliani AG, Vadlamudi RVSV, Katz S, McNeill JH: Cardiac sarcoplasmic reticulum function in insulin- or carnitine-treated diabetic rats. Am J Physiol **245**: H969, 1983
21. Idell-Wenger JA, Grotyohann LW, Neely JR: Coenzyme A and carnitine distribution in normal and ischemic hearts. J Biol Chem **253**: 4310, 1978
22. Fujisawa S, Kobayashi A, Hironaka Y, Yamazaki N: Effect of L-carnitine on the cellular distribution of carnitine and its acyl derivatives in the ischemic heart. Jpn Heart J **33**: 693, 1992
23. Cederblad G, Lindstedt S: A method for the determination of carnitine in the picomole range. Clin Chim Acta **37**: 235, 1972
24. Gornall AG, Bardawill CJ, David MM: Determination of serum proteins by means of the biuret reaction. J Biol Chem **177**: 751, 1949
25. Amador E, Dorfman LE, Wacker WEC: Serum lactic dehydrogenase activity; an analytical assessment of current assays. Clin Chem Acta **9**: 391, 1963
26. Morgan HE, Regen DM, Park CR: Identification of a mobile carrier-mediated sugar transport system in muscle. J Biol Chem **239**: 369, 1964
27. Fritz IB, Yui KTN: Long-chain carnitine acyltransferase and the role of acylcarnitine derivatives in the catalytic increase of fatty acid oxidation induced by carnitine. J Lipid Res **4**: 279, 1963
28. Hulsmann WC, Stam H, Maccari F: The effect of excess (acyl) carnitine on lipid metabolism in rat heart. Biochem Biophy Acta **713**: 39, 1982