THE ROLE OF L-CARNITINE IN THE PATHOGENESIS OF CARDIOMEGALY IN PATIENTS WITH CHRONIC HEMODIALYSIS

YASUO KUDOH, M.D., TETSURO SHOJI, M.D., HIROSHI OIMATSU, M.D.
SHIGEO YOSHIDA, M.D., KENJIRO KIKUCHI, M.D., AND OSAMU IIMURA, M.D.

Many reports have suggested that cardiac dysfunction with cardiomegaly is more often observed in patients with chronic hemodialysis. Moreover, cardiac dysfunction has been thought as one of the most important factors affecting the prognosis of these patients. In this study, in order to clarify the role of l-carnitine in the pathogenesis of cardiomegaly, 33 patients with chronic hemodialysis were studied using the multivariate analysis method. Among the factors which may affect cardiac function, the following 10 variables were examined. 1) age, 2) duration of dialysis, 3) plasma carnitine, 4) serum total cholesterol, 5) serum HDL-cholesterol, 6) triglyceride, 7) systolic blood pressure, 8) diastolic blood pressure, 9) left ventricular voltage on a electrocardiogram at rest and 10) hematocrit.

The plasma carnitine levels in these patients were markedly reduced and inversely correlated with the cardiothoracic ratio (CTR) on the chest X-ray films ($r = 0.38$, $p < 0.05$). In principal component analysis, the CTR was closely related to hematocrit and plasma carnitine levels. By multiregression analysis, both hypo-carnitininemia and anemia were independently shown to be important causes of cardiomegaly.

Thus, it is suggested that the cardiomegaly in patients with chronic hemodialysis may be improved by supplemental therapy with l-carnitine, even in cases with severe anemia.

It is well recognized that l-carnitine is an important factor in myocardial fatty acid metabolism, especially in the transport of activated long chain fatty acid to β-oxidation sites in mitochondria and it has also been recently reported that l-carnitine has a direct action on cardiac hemodynamics. In patients with chronic hemodialysis, plasma and muscle carnitine levels were markedly reduced and the relationship between cardiomyopathy and carnitine deficiency has been suggested. However, it has not been clear whether or not the cardiac dysfunction is due to a decreased carnitine level. Because heart failure is a major cause of death in hemodialysis patients, it seems to be important to clarify the significance of l-carnitine on cardiac dysfunction from the points of view of the treatment and the prevention of cardiac failure in these patients.

The purpose of this study is to evaluate the role of l-carnitine on cardiac performance in patients with chronic hemodialysis. Since cardiac function is influenced by many interacting factors, multivariate analysis was performed in this study to clarify the relationships of each variable.

Key Words:
Chronic hemodialysis
Cardiomegaly
L-carnitine
Multivariate analysis

(Received November 24, 1982; accepted June 13, 1983)
The Second Department of Internal Medicine, Sapporo Medical College, Sapporo, Japan
Mailing address: Yasuo Kudoh, M.D., The Second Department of Internal Medicine, Sapporo Medical College, South-1 West-16, Chuo-ku, Sapporo 060, Japan

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MATERIALS AND METHODS

Thirty-three patients (21 females and 12 males), ranging in age from 15 to 70 (mean ± SD, 42.7 ± 13.1), who had undergone regular hemodialysis treatment for a period from 25 to 166 (82.4 ± 37.9) months, were studied. All were outpatients and treated with hemodialysis twice or 3 times every week. The dialysis was carried out for about 4 hours, using a Kolff or a hollowfiber dialyzer. The causes of uremia were chronic glomerulonephritis in 28 patients, and lupus nephritis, polycystic kidney, renal tuberculosis, malignant hypertension and obstructive nephropathy in one each. No patients had evidence of diabetes mellitus. Twenty-one healthy adult subjects (8 females and 13 males), ranging in age from 21 to 71 (39.7 ± 16.5), were examined as a normal or control group.

Blood samples of the patients with chronic hemodialysis were obtained from their dialyzing shunt or fistula immediately before dialysis. In normal controls, blood was drawn from an antecubital vein after an overnight fast.

Among the background factors affecting the cardiac function, the following 10 items were selected:

1) age (year), 2) plasma carnitine (nmol/ml), 3) duration of hemodialysis (month), 4) serum triglyceride (mg/dl), 5) serum total cholesterol (mg/dl), 6) serum HDL-cholesterol (mg/dl), 7) hematocrit (%), 8) systolic blood pressure (SBP, mmHg), 9) diastolic blood pressure (DBP, mmHg) and 10) left ventricular voltage (SV1 + RV5 or RV6) on an electrocardiogram (ECG) at rest (mV), as an index of left ventricular hypertrophy.

Serum triglyceride, total cholesterol and HDL-cholesterol concentrations were determined enzymatically. For determination of plasma carnitine levels, blood sample put into heparinized tubes and centrifuged at 3000 rpm for 10 min at 4°C. Plasma samples were then taken and frozen at -20°C. Plasma carnitine concentration was determined by colorimetric enzymassay12 within 2 weeks. Plasma (1.0 ml) was mixed with 2.0 ml of cold 0.6N perchloric acid, and then centrifuged at 4,500 rpm for 5 min at 4°C after standing for 10 min in ice water. After the addition of 0.05 ml of 0.1M KH2PO4 buffer, 2.0 ml of supernatant was adjusted to pH 6.5 – 7.5 with 4N KOH, and then centrifuged at 4,500 rpm for 5 min at 4°C after standing in ice water for 30 min covered with parafilm. The supernatant (1.0 ml) was used for carnitine (free carnitine) assay. Sample solution (1.0 ml), 0.2 ml of 1M Tris-HCl buffer (PH 7.8 – 8.0), 0.05 ml of 50 mM neutral EDTA, 0.02 ml of 15 mM acetyl-CoA, 0.02 ml of 10 mM DTNB (5, 5'-dithio bis-2-nitrobenzoic acid, 4 mg/ml) dissolved in very dilute KHCO3 and neutralized to pH 7.0 – 8.0, and 0.7 ml of distilled water were mixed. Before and 20 min after an addition of 0.02 ml of carnitine acetyltransferase (1 mg/ml), absorption was read at 412 nm. Carnitine concentration was calculated from the absorbancy changes. In our laboratory, the recovery of free carnitine added to plasma samples was 95.7 ± 6.0 % and the reproducibility (day to day variation) was 5.56 ± 2.64 %. Blood was sampled once a week during the study, and plasma levels were expressed as an average of those determined 4 times within 4 weeks.

As an index of the cardiac function, the cardiothoracic ratio (CTR) on a chest X-ray film was chosen because of its easy repeatability and non-invasive character. In order to ascertain the reproducibility and reliability of the CTR, chest X-ray films were evaluated 2 times before each dialysis at 3-month intervals in 31 cases by 2 different cardiologists without any information about the patients. Multivariate analysis13 including principal component analysis, multiregression analysis, Student's t-test, F test and chi square test were employed in the present study.

![Graph showing plasma carnitine levels in normal subjects and patients with chronic hemodialysis](https://example.com/Graph.png)

\[ p < 0.001 \]

\[ p < 0.01 \]

\[ M = \text{males}, \ F = \text{females} \]

*Japanese Circulation Journal Vol. 47, December 1983*
TABLE I VALUES OF EACH VARIABLE IN 33 PATIENTS WITH CHRONIC HEMODIALYSIS

<table>
<thead>
<tr>
<th>Variables</th>
<th>mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [year]</td>
<td>42.7</td>
<td>13.1</td>
</tr>
<tr>
<td>Duration of dialysis [month]</td>
<td>82.4</td>
<td>37.9</td>
</tr>
<tr>
<td>Plasma carnitine [nmol/ml]</td>
<td>15.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Serum total cholesterol [mg/dl]</td>
<td>181.6</td>
<td>30.4</td>
</tr>
<tr>
<td>Serum HDL cholesterol [mg/dl]</td>
<td>31.5</td>
<td>11.7</td>
</tr>
<tr>
<td>Serum triglyceride [mg/dl]</td>
<td>145.6</td>
<td>50.2</td>
</tr>
<tr>
<td>Hematocrit [%]</td>
<td>23.0</td>
<td>5.4</td>
</tr>
<tr>
<td>SBP [mmHg]</td>
<td>144.5</td>
<td>23.4</td>
</tr>
<tr>
<td>DBP [mmHg]</td>
<td>84.2</td>
<td>12.3</td>
</tr>
<tr>
<td>LVH [mV]</td>
<td>2.9</td>
<td>0.7</td>
</tr>
<tr>
<td>CTR [%]</td>
<td>49.2</td>
<td>3.6</td>
</tr>
</tbody>
</table>

SBP = systolic blood pressure, DBP = diastolic blood pressure, LVH = left ventricular voltage on an electrocardiogram at rest, CTR = cardiothoracic ratio on chest X-ray

RESULTS

The plasma free carnitine levels in normal subjects were 41.8 ± 8.0 (mean ± SD) nmol/ml in males and 30.2 ± 9.4 in females. Thus, in normal subjects, males showed a slight but significantly higher plasma carnitine level than females (p < 0.01). In patients with chronic hemodialysis, the predialysis plasma carnitine level was 14.5 ± 3.4 nmol/ml in males, and 16.2 ± 4.6 in females. However, since chronic hemodialysis resulted in a significant reduction of plasma carnitine levels (p < 0.001), no significant difference was observed in plasma carnitine levels between male and female patients with chronic hemodialysis (Fig. 1). Chi square test showed a normal distribution of plasma carnitine levels in 33 patients (p < 0.05).

Values (mean ± SD) of each variable in 33 patients with chronic hemodialysis are listed in Table I. These patients showed an abnormal finding in lipid metabolism (hypo-carnitinemias.

TABLE II SIMPLE CORRELATION TABLE AMONG THE VARIABLES DETERMINED IN PATIENTS WITH CHRONIC HEMODIALYSIS

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Duration</th>
<th>CAR</th>
<th>HDL</th>
<th>TG</th>
<th>TC</th>
<th>Ht</th>
<th>SBP</th>
<th>DBP</th>
<th>LVH</th>
<th>CTR</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>0.026</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>0.074</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR</td>
<td>-0.074</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HDL</td>
<td>0.045</td>
<td>-0.032</td>
<td>0.185</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>-0.202</td>
<td>0.104</td>
<td>-0.364</td>
<td>-0.218</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TC</td>
<td>-0.074</td>
<td>-0.069</td>
<td>-0.131</td>
<td>0.281</td>
<td>0.422</td>
<td></td>
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<tr>
<td>Ht</td>
<td>0.306</td>
<td>0.088</td>
<td>-0.127</td>
<td>-0.126</td>
<td>-0.186</td>
<td>0.166</td>
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<tr>
<td>SBP</td>
<td>0.406</td>
<td>-0.356</td>
<td>-0.170</td>
<td>-0.157</td>
<td>-0.002</td>
<td>-0.272</td>
<td>-0.321</td>
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<tr>
<td>DBP</td>
<td>0.206</td>
<td>-0.265</td>
<td>-0.016</td>
<td>-0.033</td>
<td>-0.016</td>
<td>-0.202</td>
<td>-0.348</td>
<td>0.690</td>
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<tr>
<td>LVH</td>
<td>-0.264</td>
<td>-0.036</td>
<td>-0.225</td>
<td>0.065</td>
<td>0.056</td>
<td>0.022</td>
<td>-0.354</td>
<td>0.076</td>
<td>0.003</td>
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<tr>
<td>CTR</td>
<td>0.168</td>
<td>0.066</td>
<td>-0.377</td>
<td>0.253</td>
<td>-0.019</td>
<td>0.043</td>
<td>-0.318</td>
<td>0.322</td>
<td>0.271</td>
<td>0.254</td>
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</tbody>
</table>

* p < 0.05  ** p < 0.025  *** p < 0.01
Duration = duration of dialysis, CAR = plasma carnitine, HDL = serum HDL-cholesterol, TG = serum triglyceride, TC = serum total cholesterol, Ht = hematocrit, SBP = systolic blood pressure, DBP = diastolic blood pressure, LVH = left ventricular voltage on an electrocardiogram at rest, CTR = cardiothoracic ratio on chest X-ray

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TABLE III STANDARDIZED PARTIAL REGRESSION COEFFICIENTS

<table>
<thead>
<tr>
<th>Plasma carnitine</th>
<th>−0.546**</th>
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<tbody>
<tr>
<td>Hematocrit</td>
<td>−0.436*</td>
</tr>
<tr>
<td>Serum triglyceride</td>
<td>−0.369</td>
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<tr>
<td>Duration of dialysis</td>
<td>0.286</td>
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<tr>
<td>Serum total cholesterol</td>
<td>0.242</td>
</tr>
<tr>
<td>Serum HDL cholesterol</td>
<td>0.188</td>
</tr>
<tr>
<td>SBP</td>
<td>0.186</td>
</tr>
<tr>
<td>Age</td>
<td>0.096</td>
</tr>
<tr>
<td>DBP</td>
<td>0.088</td>
</tr>
<tr>
<td>LVH</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* 0.05 < p < 0.1  ** p < 0.01
SBP = systolic blood pressure, DBP = diastolic blood pressure, LVH = left ventricular voltage on an electrocardiogram at rest

Fig. 3. Principal component analysis.
CAR = plasma carnitine, DU = duration of dialysis, TC = serum total cholesterol, HDL = serum HDL-cholesterol, TG = serum triglyceride, Ht = hematocrit, SBP = systolic blood pressure, LVH = left ventricular voltage on an electrocardiogram, CTR = cardiothoracic ratio on chest X-ray

Fig. 4. Nomogram relating plasma carnitine levels, hematocrit and the cardiothoracic ratio (CTR)

hypochondro-HDL-cholesterolemia and hypertriglyceridemia) and anemia. Serum electrolyte contents including serum K and Ca were excluded in this study, because these were within normal range in all patients (serum K = 4.8 ± 0.5 mEq/L and serum Ca = 9.6 ± 0.8 mEq/L). Plasma volume was regarded to be well controlled, because changes of body weight between each dialysis (average after one month) were 1.9 ± 0.5 kg and the CTR was 49.2 ± 3.6% (over 55% in only 2 patients). In this study, the CTR was used as an index of cardiac performance, and its reproducibility and reliability were examined. The correlation coefficient between the first and the forth month determination of the CTR was 0.78 and the slope of the regression line was approximately 1.0.

The interrelationships among each variable are shown in Table II as a simple correlation table. As shown in Fig. 2, the CTR inversely correlated with plasma carnitine levels (r = −0.377, p < 0.05), but did not with any other variables. On the other hand, plasma carnitine levels had an inverse correlation only with the serum triglyceride concentration among the various serum lipids (r = −0.364, p < 0.05).

Moreover, in order to clarify the interrelationships among each variable, principal component analysis was applied in this study. In the first component, coefficients of SBP, DBP and CTR were higher than 0.55. On the other hand, the second component was constructed mainly with serum triglyceride concentrations and left ventricular voltage on an ECG at rest (LVH). The cumulative contribution rate to the second component was 39.5%. The symmetrically inverse situation between the CTR and hematocrit or plasma carnitine levels was observed (Fig. 3). By multiregression analysis, it was suggested that the CTR could be predicted using 10 variables. The multiregression coefficient was 0.727 (p < 0.05). The standardized partial regression coefficients

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of plasma carnitine levels and hematocrit (−0.546 and −0.436, respectively) were significantly high (Table III). Next, the best regression formula, which was constructed with the least variable numbers and of which the F value was the highest, was obtained as follows:

CTR (%) = 60.6 − 0.36 x plasma carnitine levels (nmol/ml) − 0.25 x Ht (%) (p < 0.01)

As shown in Fig. 4, although points of individual patients plotted according to plasma carnitine levels and hematocrit were widely distributed, a line calculated by the abovementioned formula divided the 2 groups with a CTR of greater than 50% and a CTR of less than 50%.

DISCUSSION

The recent advance in hemodialysis techniques is shifting our attention from the volume overloading cardiomegaly to the dialysis-resistant-cardiomegaly or uremic cardiomyopathy. Patients with chronic hemodialysis have many risk factors affecting cardiac function, such as anemia, hypertension, abnormal lipid metabolism, electrolyte disorder, hormonal imbalance and so on. However, the interactions or factor loading among them have not as yet been ascertained. On the other hand, it has been reported that the tissue carnitine levels of the myocardium decrease in human heart failure and the administration of L-carnitine is effective for cardiac insufficiency. In animal experiments, it has been also observed that L-carnitine has a positive inotropic action and it decreases coronary vascular resistance. Because plasma and muscle carnitine levels were reduced in patients with chronic hemodialysis, it seems to be important to investigate the interrelationships among the factors, including L-carnitine, which may influence cardiac function, and to clarify the role of L-carnitine in the pathogenesis of cardiomegaly observed in these patients.

In our previous report, the plasma levels of carnitine in patients with chronic renal failure were very high before the commencement of hemodialysis, but gradually decreased during a 2-year period after its commencement, and low plasma levels of carnitine continued thereafter. In addition, left ventricular voltage (SV1 + RV5 or RV6) in an ECG at rest, as an index of left ventricular hypertrophy, was high within 2 years after the commencement of hemodialysis. Thereafter, it decreased, but increased again after 10 years. This "bell shaped" correlation between left ventricular hypertrophy and the duration of hemodialysis has already been reported by Niwa et al using echocardiography. They have suggested that there may be a difference in the causes related to cardiac dysfunction between the early stage (within 2 years of hemodialysis) and the later stage. Because clinical status in patients with hemodialysis may be considered to be unstable for 2 years after the commencement of hemodialysis, in the present study we chose the patients who had undergone hemodialysis treatment for more than 2 years (6.9 ± 3.2 years).

Plasma carnitine levels have been known to decrease markedly after dialysis due to its easy dialyzability (molecular weight 162) but there is still disagreement about the predialysis plasma level of carnitine. In this study, a significant reduction of plasma carnitine level had already been observed before dialysis in the patients with chronic hemodialysis. This result is consistent with that in a previous report from Japan but not with those from other countries in which plasma carnitine levels recovered to almost equal or surpass normal levels until the next dialysis. In Japan, it has been reported that serum triglyceride levels in patients with chronic renal failure are lower, but increase markedly after hemodialysis. Moreover, in the present study a significant inverse correlation was found between plasma carnitine levels and serum triglyceride concentrations. Additionally, considering the fact that meat or protein contains relatively rich L-carnitine, the difference in eating habits may be related in part to the discrepancy in predialysis plasma carnitine levels between Japan and other countries.

L-carnitine is produced not only in the liver but also in the epididymis. Because of the regulation of steroid hormones plasma carnitine levels in normal males may be higher than in females. However, in patients with chronic hemodialysis no significant difference was found between males and females from the strong effects of repeated hemodialysis. It seems likely that patients with chronic hemodialysis should be exposed for a long time to a carnitine depleting state. Recently, Tripp et al have reported that inherited systemic carnitine deficiency may be one of the causes of familial cardiomyopathy. Therefore, it is reasonable to suppose that acquired carnitine deficiency may be one of the causes of dialysis-resistant-cardiomegaly in patients with chronic hemodialysis. In our study,
the CTR was inversely correlated with plasma carnitine levels. This result strongly suggests the above-mentioned hypothesis.

Moreover, we performed multivariate analysis such as principal component analysis and multiple-regression analysis in order to clarify the role of cardiomegaly. In the principal component analysis, the first component was considered to have the specific quality of the hemodynamic state, because it was represented by SBP, DBP, CTR and Ht. Hematocrit and plasma carnitine levels were situated symmetrically to the CTR. In contrast, age and serum total cholesterol concentrations were independent of the CTR. Therefore, it is suggested that anemia and hypo-carnitinemia are closely related with cardiomegaly. The same results were obtained by multivariate analysis, because coefficients of plasma carnitine levels and hematocrit were significantly high. As shown in Fig. 4, each patient was plotted on a nomogram which consisted of plasma carnitine levels, hematocrit and the CTR, and 2 groups of patients having actually measured CTR of more or less than 50% were separated clearly according to a calculated line showing a CTR of 50%. These results suggest that cardiomegaly in the patients with chronic hemodialysis may be improved by supplemental therapy with 1-carnitine, even in with severe anemia.

It has been reported that serum triglyceride levels decreased and serum HDL-cholesterol levels increased by replacement therapy with carnitine in hemodialysis patients. Considering the fact that plasma carnitine level was negatively correlated with serum triglyceride level in our study, 1-carnitine may influence the cardiac function through lipid metabolism.

In conclusion, it was demonstrated in this study that hypo-carnitinemia may be one of the causes of cardiomegaly in regular hemodialysis patients, and it is suggested that cardiomegaly observed in the patients with chronic hemodialysis may be improved by supplemental therapy with 1-carnitine.

Acknowledgements

We thank Dr. Ikuo Watarai for his kind collaboration and encouragement of this work.

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