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Effects of *L*-Carnitine Supplementation on Renal Anemia in Poor Responders to Erythropoietin

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Key Words

L-Carnitine supplementation · Hemodialysis · Renal anemia · Erythropoietin

Abstract

While renal anemia can be successfully treated by use of erythropoietin (EPO) in most hemodialysis (HD) patients, some patients have anemia that is refractory to treatment with a high dose of EPO. We examined whether *L*-carnitine treatment could raise hematocrit (Hct) levels in such patients. Fourteen HD patients who showed a poor response to EPO and no evident factors which inhibit a response to EPO were selected to receive oral *L*-carnitine (500 mg/day) in a 3-month trial. During the study, 36% of the patients showed Hct increases of more than 2%. Statistical analysis revealed significant increases of Hct ($p = 0.003$) and total iron-binding capacity (TIBC) ($p = 0.050$) and a significant decrease of ferritin ($p = 0.005$). In addition, we found that red blood cells (RBCs) in HD patients contained a comparable level of carnitine to normal controls, despite the presence of serum carnitine deficiency, and that RBC carnitine was not removed through HD, in contrast to serum carnitine. These results suggest that RBC carnitine may be essential for RBCs to perform their metabolic function in renal anemia and that oral *L*-carnitine treatment could improve anemia in poor responders to EPO.

Introduction

L-carnitine (γ-trimethyl-ammonium-β-hydroxybutyrate) is a natural substance whose main physiological role is to transport long-chain fatty acids from the cytoplasm to within the mitochondrial matrix for their β-oxidation in various tissues [1]. The presence of adequate carnitine concentrations in the intracellular compartment is essential for normal fatty acid metabolism in human organs that preferentially use fatty acids as primary energy sources, such as skeletal muscle and myocardium. On the other hand, human RBCs, which do not posses mitochondria, have substantial amounts of *L*-carnitine and its esters [2] and *L*-carnitine has been found to affect the stability of RBC membranes under various adverse conditions [3–6].

Several studies have demonstrated that HD patients exhibit a constant loss of plasma carnitine during dialysis and have relative insufficiency of carnitine [7–9]. Against

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this background, some groups have reported beneficial effects of oral supplementation of high-dose *L*-carnitine (2 g/day) on dialysis-associated muscle symptoms [10, 11]. Recently, we have shown that plasma free carnitine deficiency is correlated with months on dialysis, and its low dose supplementation (500 mg/day) had beneficial effects in dialysis-associated muscular symptoms [12].

The most important factor causing renal anemia is the decrease in EPO secretion by the diseased kidney. However, carnitine insufficiency may also contribute to renal anemia. Berad and Jordache [13] reported on 2 hemodialyzed children whose EPO resistance was corrected by *L*-carnitine administration. Labonia [14] showed that *L*-carnitine treatment (1 g intravenously after every dialysis session) promoted a marked reduction in EPO requirements.

The current study was performed to determine whether oral *L*-carnitine supplementation had any effects on renal anemia in HD patients who showed a poor response to EPO. In addition, carnitine levels in serum and RBCs were compared among HD patients with different hematocrit (Hct) levels or EPO requirements.

Materials and Methods

Patients

Thirty-two patients were investigated concerning carnitine status in serum and RBC. They were selected from eight participating centers (21 males and 11 females with a mean age of 59.7 ± 9.8 years) with end-stage renal disease (ESRD) of varying etiologies. All patients had been on HD thrice weekly (4 h each dialysis) for 114 ± 81 months and were taking standard medications including vitamin D_3 and calcium carbonate. Of the 32 patients, 14 patients (group A: 8 males and 6 females; mean age, 63.2 ± 10.5 years; range, 46–78 years; time on dialysis, 120 ± 81 months) showed either no response or a blunted response to EPO: Hct levels of less than 27.5% despite a weekly EPO dose of 9,000 U for 3 months. These patients showed serum ferritin >100 µg/l, and transferrin saturation (serum iron/TIBC) > 0.2. Complication by infection, inflammation, malignancy, blood loss, vitamin B_{12} or folate deficiency, aluminum intoxication, or severe hyperparathyroidism, which could induce a poor response to EPO, were not evident during the 3 months. A further 7 patients (group B: 4 males and 3 females; mean age, 54.9 ± 7.6 years; range, 44–64 years; time on dialysis, 63 ± 46 months) had maintained Hct levels of more than 30% with a weekly EPO dose of 9,000 U for more than 3 months. The remaining 11 patients (group C: 9 males and 2 females; mean age, 58.3 ± 9.1 years; range, 44–75 years; time on dialysis, 138 ± 89 months) had maintained Hct levels of more than 30% without EPO administration for 6 months. HD therapy in all groups was characterized by ultrafiltration control, bicarbonate-base, and cellulose or cellulose acetate membranes. No significant differences between groups were found in weight reduction rates per dialysis session or in Kt/V, which indicates dialysis dose. From more than 3 months before the start to more than 2 months after the end of the carnitine treatment study, drug regimens, including iron and vitamins, and diet remained unchanged and no transfusion was received. All patients gave informed consent for our study. As a control population 15 healthy volunteers (4 males and 11 females; mean age, 58.5 ± 10.3 years; range, 45–83 years) were studied. Five of the 32 patients were randomly selected for investigation of carnitine status in serum and RBCs before and after one dialysis session.

Design of L-Carnitine Treatment Study

There was no placebo-controlled study because the patients were not happy to dose placebo, nonetheless they are carnitine deficient. Before the start of the *L*-carnitine treatment, 14 patients in group A had blood drawn immediately before HD for baseline determination of serum and RBC carnitine concentrations and iron status. These patients were then given a daily dose of 500 mg oral *L*-carnitine each morning on nondialysis days or after dialysis treatment for 3 months. *L*-Carnitine USP (500 mg vanilla-flavored chewable wafers) was purchased from Vitaline Corporation (USA). The routine laboratory assessment and carnitine measurements were repeated monthly. Iron metabolism parameters were measured at the start (T_0) and the end (T_3) of the treatment. Four of the 14 patients were selected randomly for investigation of carnitine levels during and after treatment.

Carnitine Assay in Serum and RBCs

Serum total and free carnitine levels were determined at the Bio-Medical Laboratory (Tokyo, Japan), based on the method described by Deufel [15]. Normal values for serum total and free carnitine were 45–91 and 36–74 µmol/l, respectively. RBC carnitine assay was performed as described previously [2, 16] with minor modification. Briefly, blood was collected into an EDTA tube and fractionated by density gradient centrifugation using Ficoll-Hypaque (ICN Pharmaceuticals, Calif., USA). A pellet of RBCs was washed three times with 0.9% NaCl and resuspended in 0.9% NaCl at a final Hct of 50%. RBCs were deproteinized with an equal volume of 6% perchloric acid. After centrifugation, one-tenth volume of 1 *M* potassium phosphate buffer (pH 7.0) was added to the supernatant. The sample was neutralized to pH 7.0 with 1 *M* potassium hydroxide. After centrifugation, total carnitine concentrations of the supernatants were determined as in the serum assay and corrected for the volume of RBCs.

Laboratory Evaluation

Serum iron, TIBC to estimate transferrin, transferrin saturation, ferritin, and routine parameters, including blood count and biochemical screening, were measured immediately before the dialysis session. Serum iron and TIBC value were obtained by automated methods [17, 18]. The serum ferritin value was determined by enzyme-linked immunosorbent assays established with monoclonal antibodies [19].

Measures of Efficacy

The primary measure of efficacy was an increase in Hct levels. For each patient the average of Hct values at 2 months before (T_{-2}), 1 month before (T_{-1}) treatment, and T_0 was taken as pretreatment Hct, and that at T_3, 1 month after (T_4), and 2 months after (T_5) the end of treatment was taken as posttreatment Hct. The change in Hct level (ΔHct), defined as posttreatment Hct minus pretreatment Hct, was used to assess the efficacy of treatment, as follows: significant efficacy, ΔHct > 2%; marginal efficacy, 1% < ΔHct < 2%; no efficacy, −1% < ΔHct < 1%; negative efficacy, ΔHct < −1%.

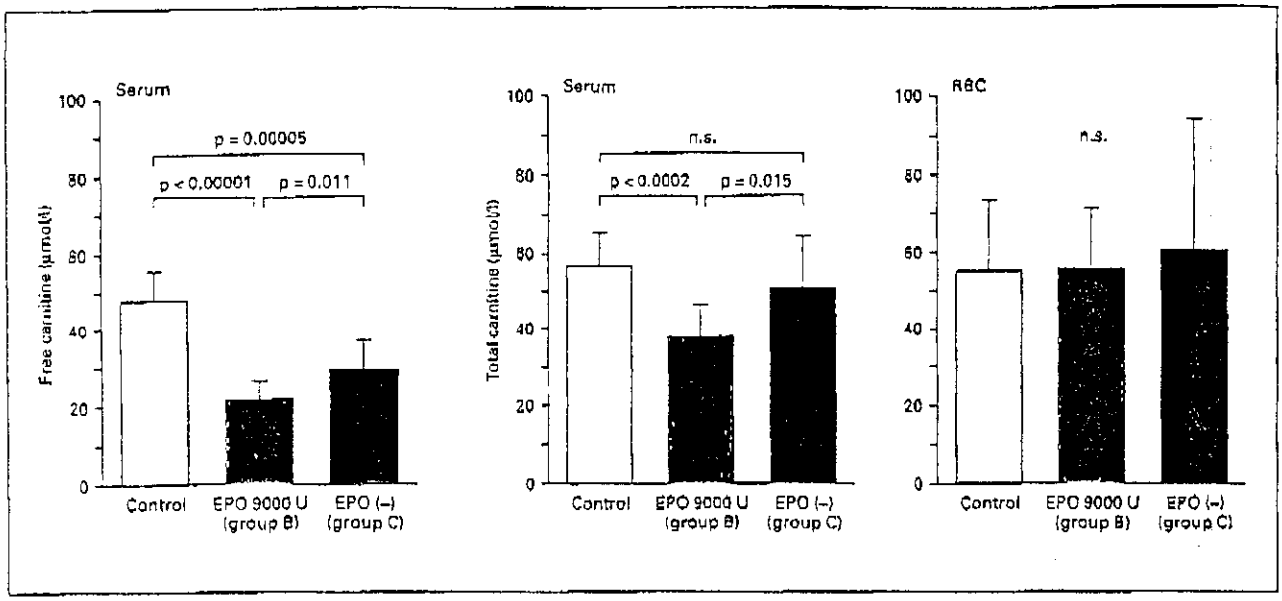

Fig. 1. Serum free (left), serum total (center), and RBC total (right) carnitine concentrations in normal controls and patients with Hct levels >30% (groups B and C). In HD patients, carnitine levels were measured before a dialysis session. Results are expressed as mean values ± SD. Serum carnitine levels in group B were markedly lower than those in group C. RBC carnitine levels showed the same levels in the three groups.

Statistical Analysis

The results were expressed as means ± SD. Paired t tests and unpaired t tests by Fisher's least significant difference method [20] were used to evaluate statistical significance. More than three data points during and after treatment was analyzed using analysis of covariance [20]. Values of $p < 0.05$ were considered statistically significant.

Results

Carnitine Status in Serum and RBCs of Patient Groups with Hct Levels >30%

To elucidate the involvement of carnitine deficiency in EPO requirements, predialysis serum and RBC carnitine levels in two groups who maintained Hct levels of more than 30% (group B with an EPO dose of 9,000 U/week, and group C without EPO) and normal controls were examined. As shown in figure 1, serum free carnitine levels in group B and C were markedly lower than those in controls. When comparing total and free carnitine levels between group B and C, group B showed significantly lower levels than group C. On the other hand, total carnitine concentrations in RBCs showed the same levels in the three groups.

Carnitine Status in Serum and RBCs of Patient Groups Receiving 9,000 U/week of EPO

To investigate the involvement of carnitine deficiency in the poor response to EPO, predialysis serum and RBC carnitine levels were compared in two groups who had been receiving 9,000 U/week of EPO (group A with Hct levels <27.5%; group B with Hct levels >30%) and controls. As shown in figure 2, serum free and total carnitine levels in both groups A and B were significantly decreased compared to controls, while no difference was observed between groups A and B. RBC carnitine concentrations showed no obvious difference among the three groups.

Effects of Oral L-Carnitine Treatment on Hct Levels in HD Patients with a Poor Response to EPO

The clinical and demographic characteristics of poor responders to EPO (group A) are summarized in table 1. The 14 patients were given *L*-carnitine (500 mg/day orally) for 3 months, and 7 of them showed at least some improvement: 5 patients showed an Hct increase of more than 2% and 2 patients showed an increase of 1% to 2% (fig. 3a). There were no patients with negative efficacy. Figure 3b shows the change of Hct levels in each patient of group A. After the *L*-carnitine treatment, 3 of the 14

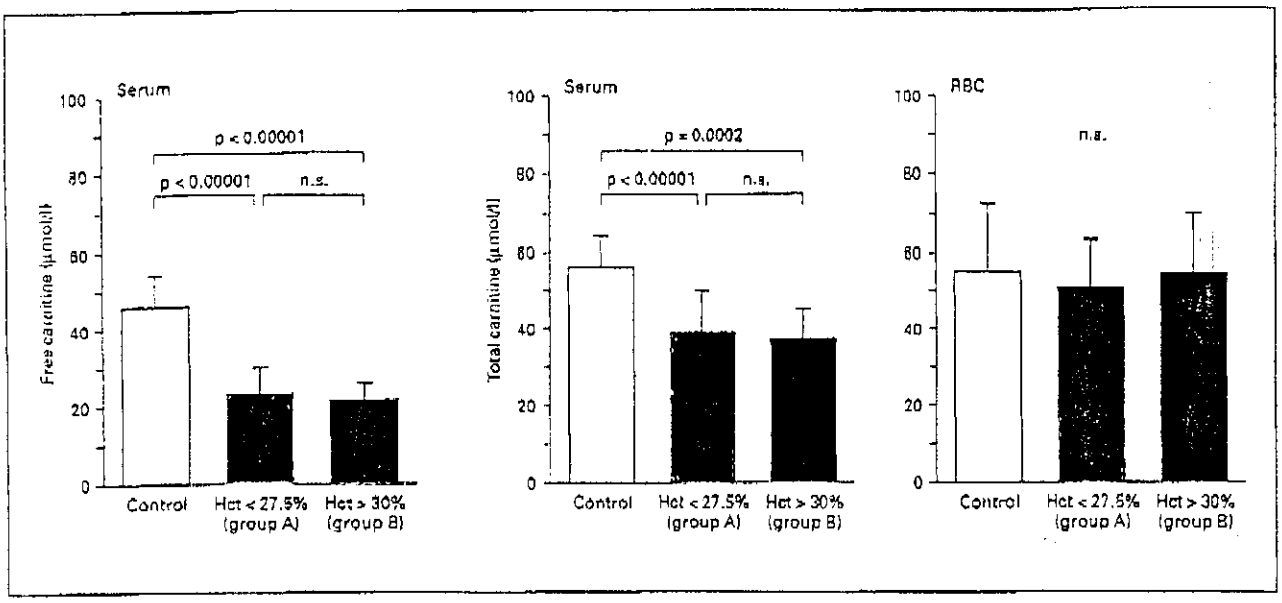

Fig. 2. Serum free (left), serum total (center), and RBC total (right) carnitine concentrations in normal controls and patients receiving 9,000 U/week of EPO (groups A and B). Results are expressed as mean values ± SD. Serum carnitine levels in both group A and group B were significantly decreased compared to controls, while no difference was observed between group A and group B. RBC carnitine levels showed no obvious difference among the three groups.

patients presented Hct levels of more than 27.5%. Figure 3c also shows the kinetics in Hct levels during and after carnitine treatment (from T_0 to T_5). The mean Hct level gradually increased during treatment and, interestingly, it showed a tendency to increase until at least 2 months after the treatment. Analysis of covariance revealed significant increase of Hct in a time-dependent manner ($p = 0.024$). Statistical analysis, as shown in figure 3b, c, indicated significant efficacy of *L*-carnitine supplementation in increasing Hct levels in HD patients with a poor response to EPO.

Effects of Oral L-Carnitine Treatment on Carnitine Levels in Serum and RBCs

Consistent with our previous report [12], serum total carnitine levels were increased during the course of treatment (fig. 4). After 2–3 months of *L*-carnitine treatment, predialysis serum total carnitine increased and reached plateaus at two to three times the baseline level. RBC concentrations also increased with time (fig. 4). After 2–3 months of treatment, total carnitine levels in RBC reached at two to three times the baseline level in all. After the end of the study, carnitine levels in both serum and RBC decreased with time.

Table 1. Clinical characteristics and pretreatment laboratory values in patients with a poor response to EPO (group A, n = 14)[a]

Baseline data	
Male	8
Female	6
Age, years	63.2 ± 10.5
Duration on dilaysis, months	121 ± 81
Primary cause of ESRD	
Glomerulonephritis	7
Diabetes	3
Hypertention	2
SLE	1
Other	1
Serial data	
Albumin, g/dl	3.6 ± 0.4
Total protein, g/dl	6.5 ± 0.3
Iron, μg/dl	67 ± 24
Ferritin, μg/l	500 ± 445
TIBC, μg/dl	211 ± 34
Transferrin saturation, %	34 ± 10
Hct value, %	24.9 ± 2.4
Kt/V	1.33 ± 0.31

[a] Continuous variables are expressed as mean values ± SD.
ESRD = End-stage renal disease; Hct = hematocrit; SLE = systemic lupus erythematosus; TIBC = total iron-binding capacity.

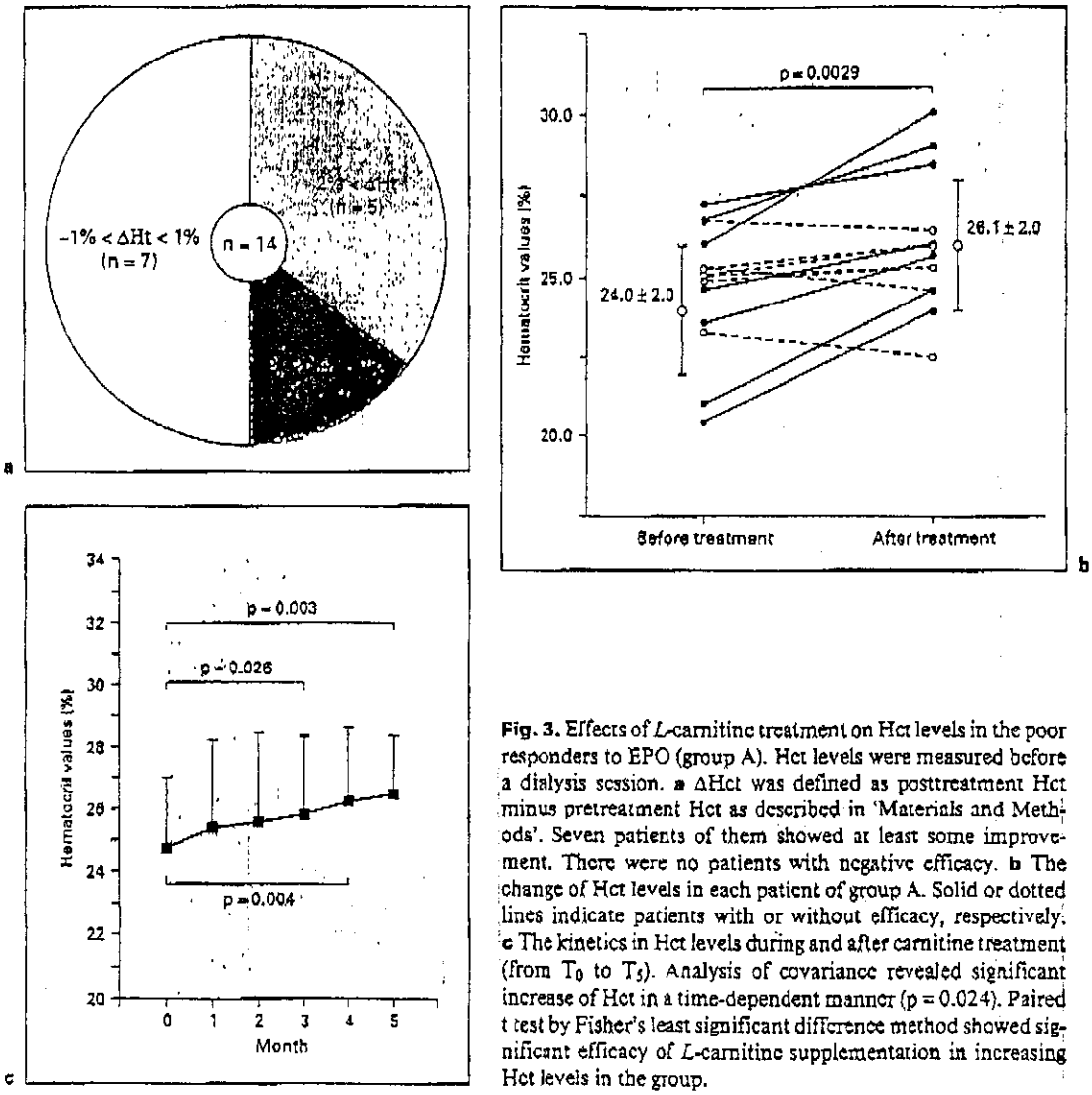

Fig. 3. Effects of *L*-carnitine treatment on Hct levels in the poor responders to EPO (group A). Hct levels were measured before a dialysis session. **a** ΔHct was defined as posttreatment Hct minus pretreatment Hct as described in 'Materials and Methods'. Seven patients of them showed at least some improvement. There were no patients with negative efficacy. **b** The change of Hct levels in each patient of group A. Solid or dotted lines indicate patients with or without efficacy, respectively. **c** The kinetics in Hct levels during and after carnitine treatment (from T_0 to T_5). Analysis of covariance revealed significant increase of Hct in a time-dependent manner ($p = 0.024$). Paired t test by Fisher's least significant difference method showed significant efficacy of *L*-carnitine supplementation in increasing Hct levels in the group.

Effects of Oral L-*Carnitine Treatment on Iron Metabolism in Poor Responders to EPO*

Iron status in the 14 patients treated with *L*-carnitine was investigated by the most widely used methods [21, 22]. Our patients showed serum ferritin levels >100 μg/l and transferrin saturation >0.2. Generally, these reflected enough total body iron stores and better availability of iron to the marrow for erythropoiesis, respectively [23–26]. At the start of *L*-carnitine treatment, patients' TIBC levels were significantly reduced and ferritin levels were extremely high compared with normal ranges (fig. 5). During the *L*-carnitine treatment, serum iron levels (data not shown) and transferrin saturation were not changed, although serum ferritin levels decreased and TIBC values increased significantly (fig. 5). These results may indicate increased release from iron stores to satisfy the requirements of the marrow.

Effects of HD on RBC Carnitine Levels

Postdialysis levels of serum carnitine are known to be reduced significantly compared with predialysis levels [8, 12, 27]. To determine whether RBC carnitine levels are also affected by HD, the difference in RBC carnitine concentrations before and after HD was examined. RBC carnitine levels were not affected by HD, in contrast with serum carnitine levels (fig. 6).

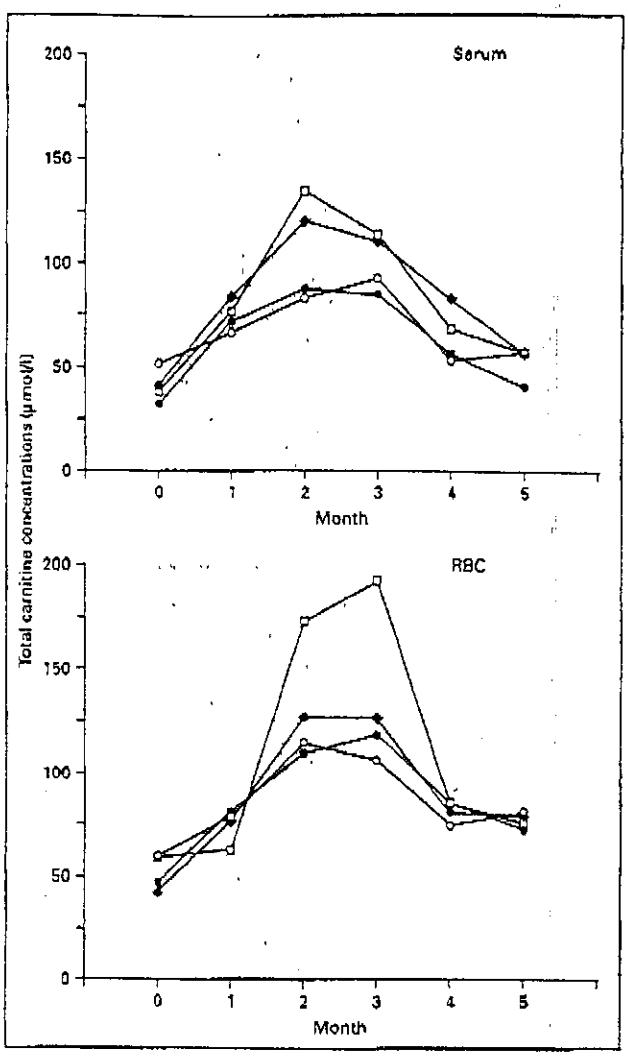

Fig. 4. Effects of *L*-carnitine treatment on total carnitine levels in serum (upper) and RBCs (lower). Four patients from group A were selected randomly. Carnitine levels were measured before a dialysis session. Both carnitine levels increased and reached plateaus during treatment and decreased after treatment.

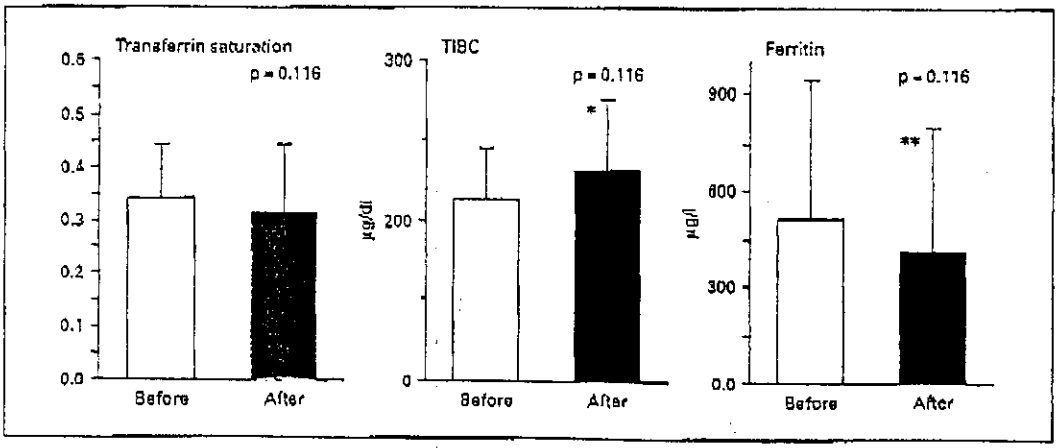

Fig. 5. Effects of *L*-carnitine treatment on transferrin saturation (left), TIBC (center), and ferritin (right) in the poor responders to EPO (group A). TIBC and ferritin values were determined before a dialysis session. Normal values are as follows: TIBC, 250–440 μg/dl; ferritin, 25–270 (male), 4–70 (female) μg/l.

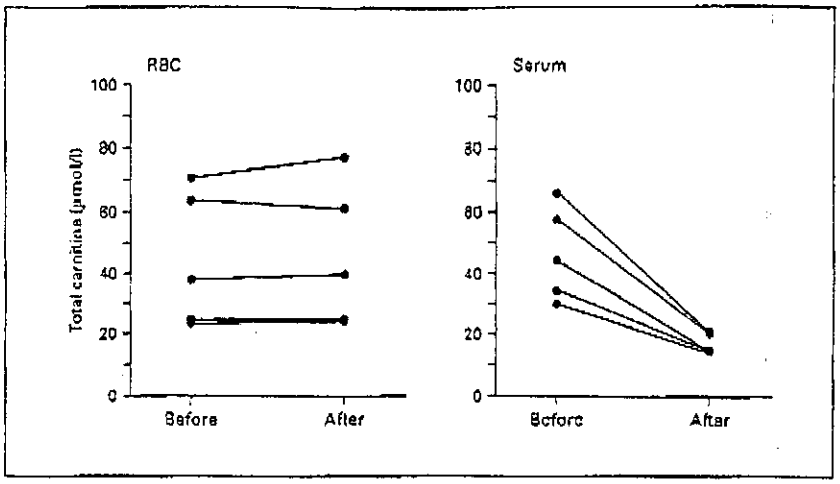

Fig. 6. Effects of HD on serum and RBC carnitine concentrations. Five patients from the 31 HD patients were randomly selected, and their total carnitine levels in serum and RBCs were examined before and after one dialysis session.

Discussion

It is now nearly 10 years since recombinant human EPO became widely used in HD patients with renal anemia in Japan. Successful use of EPO can lead to marked elevation of quality of life in patients. Most patients with renal anemia will respond to EPO, although a small number show either no response or a blunted response. This minority group is important because of the lack of therapeutic efficacy and because they may generally require larger dose of this expensive medicine. A nationwide study of Japan revealed that 10.5% of HD patients were receiving more than 9,000 U/week of EPO in 1996 [28]. The definition of a poor response to EPO is arbitary. Several factors have been shown to inhibit a response to EPO. Major factors are iron deficiency, infection, inflammation, and malignancy. Minor factors include hyperparathyroidism with marrow fibrosis, aluminum toxicity, and vitamin B_{12} or folate deficiency. In the current study, patients without the above problems who exhibited Hct levels of less than 27.5% despite a weekly EPO dose of 9,000 U for 3 months were selected as poor responders to EPO.

One of the main causes of anemia in patients with ESRD is hemolysis due to reduced stability. Na-K pump function and phospholipid (PL) turnover of RBC membranes are crucial in keeping the biconcave disc shape of RBCs [29, 30]. It was reported that the numbers of Na-K pump sites of the youngest RBCs were reduced in the uremic state [31]. Labonia et al. [29] observed that *L*-carnitine supplementation improved RBC Na-K pump activity in HD patients. Also, RBC membrane PLs in uremia are injured by oxidative stress. Acyl carnitine in RBC serves as a reservoir of acyl moieties for the reacylation process of membrane PLs [32]. The reversible transfer of fatty acids from CoA complex to free carnitine and subsequent increase of CoA/Acyl-CoA ratio, which is catalyzed by carnitine palmitoyl transferase (CPT), is critical for the regulation of membrane PL turnover [33, 34]. Recently, it was reported that *L*-carnitine normalized the reduced CPT activity in RBCs from HD patients [35].

In this study, we found a significant difference in serum carnitine levels between patients who maintained Hct levels of more than 30% with an EPO dose of 9,000 U/week and those without EPO requirements. This result indicates that carnitine deficiency may enhance EPO requirements in HD patients with renal anemia, and supports the previous work suggesting that *L*-carnitine treatment promoted a 38.1% reduction in EPO requirements [14]. However, our results showed that serum carnitine levels in poor responders were not reduced compared with the patients with an EPO dose of 9,000 U/week and Hct levels of more than 30%. This may indicate that carnitine deficiency may not be an essential cause of resistance to EPO in HD patients. However, the carnitine treatment study revealed that its supplementation had positive effects on anemia in half of our patients with a poor response to EPO. If carnitine treatment were continued for longer periods, more patients might show increased Hct levels.

With regard to renal anemia, many investigators have observed an increase in Hct levels after *L*-carnitine treatment in HD patients. In theory, *L*-carnitine could modify the lipid composition [33, 34] and intensify Na-K pump

function [29] in RBC membranes, which could eventually improve the RBC half-life. In the current study, we also observed that *L*-carnitine treatment induced the decrease of ferritin and the increase of TIBC in the poor responders to EPO. Labonia [14] has suggested the possibility that *L*-carnitine improves renal anemia through its action on erythroid precursors. Our data, which indicate the probability of enhanced iron utilization and upregulated iron receptors, might reflect the effect of *L*-carnitine on erythropoiesis in marrow or, alternatively, improvement of reduced iron utilization.

The difference in carnitine status and kinetics during HD between RBC and serum is another issue to be addressed. We revealed that RBC in HD patients showed no evident carnitine deficiency despite the presence of serum carnitine deficiency, and that RBC carnitine was not removed in one dialysis session, in contrast with serum carnitine. Shoderbeck et al. [36] showed that the proportion of RBC carnitine to whole blood carnitine content increased with time during pregnancy, although plasma carnitine content decreased gradually. It is possible that RBC has an increased need of carnitine to perform their metabolic function during pregnancy. One could speculate that RBCs in uremia require sufficient carnitine to protect themselves from different types of stress including anemia, circulating uremic toxins, and oxidative substances. The fact that RBC carnitine does not freely exchange with plasma pool carnitine [2, 37], suggests that it is essential to the function of RBCs.

In conclusion, this study has shown that oral *L*-carnitine treatment could improve anemia in poor responders to EPO, which may be attributable to prolonged erythrocyte survival or improved erythropoiesis because of reduced uremic stress. It is our opinion that *L*-carnitine treatment of HD patients might reduce their EPO requirements, thus lowering the cost of dialytic treatment, as has been suggested previously [14, 35, 38]. Further investigations of the difference between responders and nonresponders to *L*-carnitine will contribute to our understanding of EPO resistance.

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