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Changes in conjugated linoleic acid and its metabolites in patients with chronic renal failure

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Changes in conjugated linoleic acid and its metabolites in patients with chronic renal failure.

Background. Conjugated linoleic acid (CLA) is a mixture of isomers of linoleic acid with conjugated double bonds that constitutes the most abundant fatty acid with conjugated dienes (CDs) in humans. CLA, erroneously considered in the past as a product of lipoperoxidation, has a dietary origin and has shown to possess anticarcinogenic and anti-atherogenic activity, mainly in animal studies. CLA can be metabolized to conjugated linolenic acid (CD18:3) and to conjugated eicosatrienoic acid (CD20:3) and these metabolites may be implicated in CLA activity. Because of the presence of dyslipidemia and the high incidence of cardiovascular and neoplastic diseases in uremic patients, we evaluated CLA and its metabolites in these patients in order to evaluate their metabolism and site distribution.

Methods. We measured CLA, CD18:3, CD20:3, CD fatty acid hydroperoxides (lipoperoxidation products), and linoleic acid in the plasma, adipose tissue, and red blood cell (RBC) membranes by using high-pressure liquid chromatography in the following groups: (*1*) 23 chronic renal failure (CRF) patients with creatine clearance (C_{Cr}) >10 mL/min (26.2 ± 16.7); (*2*) 21 end-stage CRF patients in conservative treatment with C_{Cr} <10 mL/min (6.8 ± 1.8); (*3*) 30 hemodialysis (HD) patients; and (*4*) 30 healthy controls.

Results. The incorporation of CLA, CD18:3, and CD20:3 in RBC membranes was significantly reduced in group 1 and was even more reduced in groups 2 and 3. CLA significantly increased both in the plasma and adipose tissue of end-stage CRF patients only. CD18:3 and CD20:3 did not change in the plasma and adipose tissue of any group. No significant changes in linoleic acid and CD fatty acid hydroperoxides were found.

Conclusions. The alterations of CD in CRF patients are not due to lipoperoxidation. The increased levels of CLA in plasma and adipose tissue of end-stage CRF patients may be due either to a reduced metabolization of CLA to CD18:3 and CD20:3, or to an altered site distribution with reduced incorporation in cellular membranes and accumulation in the plasma and adipose tissue. The clinical significance of these changes remains to be investigated.

Key words: fatty acids, linolenic acid, eicosatrienoic acid, hemodialysis, end-stage renal disease, adipose tissue.

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The conjugated diene (CD) structure (two double bonds separated by a single bond) is unusual in polyunsaturated fatty acids (PUFAs) [1]. For many years, CDs have been considered as a marker of lipid peroxidation because of the presence of the CD structure in PUFA hydroperoxides, the primary products of lipid peroxidation [2–4]. However, it has been shown that the CD structure can also be formed during partial hydrogenation of fatty acids performed either industrially or by anaerobic bacteria present in the rumen [1]. By this process, it has mainly formed the so-called conjugated linoleic acid (CLA), which refers to a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. CLA constitutes by far the most abundant CD present in foods like ruminant's meat, milk, and dairy products [5–7], and its presence in human tissues is not due to a lipoperoxidative process but to a dietary origin [7].

Conjugated linoleic acid is a cancer-preventive agent. In animal models of chemical carcinogenesis, CLA has been shown to inhibit skin papilloma [8], forestomach neoplasia [9], mammary tumors [10, 11], and colon aberrant crypt foci [12]. A recent epidemiological study in Finland showed that a habitual consumption of whole milk is associated with a reduced risk of breast cancer [13]. In another recent report, CLA protection against breast cancer was assessed by measuring its level in breast adipose tissue as a reflection of the past CLA dietary intake [14]. An inverse association between the CLA level and the risk of breast carcinoma was found, suggesting a protective effect of CLA against breast cancer. The results of these two studies were at least consistent to hypothesize that CLA also may be effective in humans.

Besides the anticarcinogenic activity, it has also been shown that CLA has an anti-atherogenic action [15], to affect fat partitioning in the body [16, 17] and to normalize impaired glucose tolerance in the prediabetic Zucker diabetic fatty (ZDF) rats [18]. Much less is known, however, about the mechanism(s) whereby CLA exerts these effects. One of the current hypotheses of CLA activity stems from its metabolism and influence on tissue lipid metabolism [19]. In fact, we demonstrated in different experimental models that CLA can be metabolized to conjugated linolenic acid (CD18:3) and conjugated eicosatrienoic acid (CD20:3) [20, 21].

Although CLA has been detected in human tissues [22], currently no data are available on CLA metabolites in humans. In a previous article, we detected a higher level of CD, with respect to controls, in the plasma and adipose tissue of end-stage chronic renal failure (CRF) patients [23]. However, the spectrophotometric method used for CD detection in that study did not allow us to separate the different types of CD fatty acids.

The increased incidence of neoplastic [24] and cardiovascular diseases [25], together with the well-known dyslipidemia of uremic patients [26] and the availability of a new method to measure CLA and its metabolites prompted us to evaluate them in lipids extracted from plasma, adipose tissue, and red blood cell (RBC) membranes of CRF patients at different stages of renal function impairment and of hemodialysis (HD) patients, in order to evaluate CLA metabolism and site distribution. In addition, CD fatty acid hydroperoxides were also measured in the same samples in order to evaluate those CDs of lipoperoxidation origin.

METHODS

Patients and controls

The study was carried out on 74 CRF patients selected from those attending the Division of Nephrology and Dialysis of the University Hospital of Modena (Modena, Italy). Thirty healthy control subjects were selected from patients who were admitted to the department of surgery for carpal tunnel syndrome surgery. Exclusion criteria for both patients and controls included subjects with systemic diseases such as diabetes mellitus, liver disorders, neoplasms, collagen-vascular diseases, smokers, plasma total cholesterol or triglycerides higher than 250 mg/dL, blood transfusions within six months prior to the study, or history of recent acute illness. Patients affected by nephrotic syndrome were also excluded. In addition, the selection procedure for controls involved checking for normal renal function. None of the patients were taking lipid-lowering drugs or antioxidant supplements. The drugs they were using included dihydropyridine calcium channel blockers, clonidine, furosemide, vitamin D, and calcium carbonate. The etiology of renal failure was chronic glomerulonephritis (28 cases), chronic pyelonephritis (4 cases), nephrosclerosis (18 cases), autosomal dominant polycystic kidney disease (15 cases), nephrolithiasis (2 cases), and unknown (7 cases).

Forty-four patients were on conservative treatment. In these patients, the predicted creatinine clearance (C_{Cr}) was calculated from serum creatinine according to Cockcroff and Gault formula [27], and the mean value was 17.1 ± 20.4 mL/min (range 3.6 to 51). The use of this formula is known to be less accurate in end-stage renal failure. However, the attempt to use the equations recently proposed by Walser, Drew, and Guldan presented relevant pitfalls [28]; in fact, in some cases (older patients with high serum creatinine levels), the result of the predicted glomerular filtration rate was negative. In a previous study, we showed that total plasma CD increased significantly only in advanced renal failure patients [23]. We found that the statistical discriminant C_{Cr} value was 10 mL/min. Similar results were found in this study concerning the levels of plasma CLA. Therefore, nondialyzed CRF patients were divided into two groups: patients with C_{Cr} >10 mL/min (range 10.1 to 51 mL/min, mean 26.2 ± 16.7, $N = 23$) and patients with C_{Cr} <10 mL/min (range 3.6 to 10 mL/min, mean 6.8 ± 1.8, $N = 21$).

Thirty patients were on standard bicarbonate HD using substituted cellulosic or low-flux synthetic membranes. The mean duration of dialysis treatment was 78.9 ± 65.8 months (range 8 ± 224). They were dialyzed three times per week, and each session lasted four hours. Blood flow was 300 to 450 mL/min, and dialysate flow was 500 mL/min. The ultrafiltration rate was 7 to 16 mL/min, and the dose of unfractionated standard heparin ranged from 2500 to 4500 IU per session.

Patients were chosen because of fistula placement/revision in the group of HD and C_{Cr} <10 mL/min, in order to obtain adipose tissue samples avoiding subcutaneous adipose tissue biopsies. In the group of patients with C_{Cr} >10 mL/min, only 4 patients out of 23 needed fistula placement. The other 19 patients were recruited from outpatients attending our division. In these patients, only blood samples were taken. Control subjects were chosen from those undergoing carpal tunnel syndrome surgery in order to have a sampling site similar to that of the patients.

Dietary protein and phosphate intake were restricted in patients on conservative treatment. However, most of the patients in the group with C_{Cr} >10 mL/min were studied at the time of the discovery of their renal pathology, and for this reason, they were not following any dietary regimen at that time. HD patients were advised to restrict the intake of phosphate and potassium-rich foods and fluid when required. All control subjects were free of prescribed drugs and were without dietary restrictions. Other demographic and clinical characteristics are shown in Table 1.

Table 1. Demographic data, biochemical parameters and dietary assessment of control subjects and patients participating in the study

	Healthy controls	CRF		Hemodialysis
		C_{Cr} >10 mL/min	C_{Cr} <10 mL/min	
Subjects *N*	30	23	21	30
Age *years*	55.8 ± 13.6	56.3 ± 16.1	58.4 ± 15.3	58.8 ± 16.9
Total cholesterol *mg/dL*	209.7 ± 37.7	194.0 ± 46.5	183.4 ± 50.9	186.0 ± 41.7
HDL cholesterol *mg/dL*	51.0 ± 11.6	38.5 ± 12.4[a]	39.0 ± 11.1[a]	41.1 ± 14.1[a]
Triglycerides *mg/dL*	130.6 ± 74.0	130.9 ± 46.6	165.9 ± 71.1	149.0 ± 79.9
Albumin *g/dL*	4.27 ± 0.21	3.86 ± 0.38	3.93 ± 0.48	3.97 ± 0.49
Uric acid *mg/dL*	4.9 ± 1.3	7.7 ± 1.7[a]	7.7 ± 1.8[a]	7.0 ± 1.1[a]
Hemoglobin *g/dL*	14.5 ± 1.4	10.8 ± 2.3[a]	9.9 ± 1.2[a]	10.9 ± 1.8[a]
Ferritin *ng/mL*	80.3 ± 63.7	95.4 ± 79.2	120.2 ± 121.7	152.3 ± 140.3
PTH *pg/mL*	31 ± 27	128 ± 93[a]	189 ± 112[a]	253 ± 273[a]
Energy *kcal/kg/day*	37.3 ± 10.4	33.1 ± 3.8	30.0 ± 4.6[a]	36.0 ± 7.8
Proteins *g/kg/day*	1.18 ± 0.26	1.08 ± 0.24	0.79 ± 0.17[a]	1.17 ± 0.43
Carbohydrates *g/kg/day*	4.76 ± 1.79	4.28 ± 0.63	3.97 ± 1.09	4.55 ± 1.11
Fats *g/kg/day*	1.51 ± 0.31	1.33 ± 0.22	1.19 ± 0.19[a]	1.43 ± 0.38

Abbreviations are: CRF, chronic renal failure; C_{Cr}, creatinine clearance; HDL, high-density lipoprotein; PTH, parathyroid hormone.
[a] $P < 0.05$ vs. controls

Samples and lipid extraction

Blood samples (20 mL) were taken from the antecubital veins of controls and patients after an overnight fast. The blood was heparinized (10 IU/mL) and immediately centrifuged at 2000 × *g* for 10 minutes. Lipids were then extracted from the resulting plasma and stored as previously reported [22, 23].

Adipose tissue (about 200 mg) was taken from the subcutaneous fat of the forearm of CRF patients on conservative treatment at the time of arteriovenous fistula institution and of HD patients during arteriovenous fistula revision. In control subjects, adipose tissue was taken from the forearm subcutaneous fat during surgical procedures for carpal tunnel syndrome. Adipose tissue samples were immediately washed with 0.9% sodium chloride, blotted, and flushed with oxygen-free nitrogen gas. Lipids were then extracted as previously reported [22, 23].

Red blood cell membranes were prepared according to Burton, Ingold, and Thompson and Steck and Kant [29, 30]. Red blood cells were washed three times resuspending the pellet in five volumes of phosphate-buffered saline (centrifugation at 2000 × *g* for 10 min at 4°C). The buffy coat was carefully aspirated from the surface of the pellet. Hemolysis was initiated by rapidly and thoroughly mixing 1 mL of packed RBC with 40 mL of 5 mmol/L phosphate buffer, pH 8, previously refrigerated at 4°C. The membranous ghosts were pelleted by centrifuging at 22,000 × *g* for 10 minutes at 4°C. After removal of the supernatant, the ghosts were resuspended in 40 mL of 2.5 mmol/L phosphate buffer, pH 8, previously refrigerated at 4°C and centrifuged as previously indicated. The ghosts were then washed a second time with an identical procedure using 1.25 mmol/L phosphate buffer, pH 8, refrigerated at 4°C. Lipids were extracted from the obtained ghost as illustrated for adipose tissue previously in this article. All extracted lipids after being dried under a flow of oxygen-free nitrogen gas were stored at −80°C and were assayed for CD within 15 days.

High-pressure liquid chromatography analysis of conjugated diene fatty acids

At the time of the analysis, the dry lipids residues were dissolved in cyclohexane, and 10 mg aliquots of lipids were prepared as previously reported [22]. Free fatty acids were obtained by saponification as described in our previous article [31]. In every step, particular attention was paid to avoid heating or excessive exposition to air and light in order to avoid oxidation of the samples. The solvents used, even in vapor form, can dissolve plastic. Consequently, plastic materials were not used in those steps in which solvents were employed.

Analysis of fatty acids was performed by using a high-pressure liquid chromatography (HPLC) (Hewlett-Packard 1050M HPLC system), equipped with a Hewlett-Packard 1040 photodiode array detector, controlled by a Hewlett-Packard Chemstation software (Hewlett-Packard, Palo Alto, CA, USA). A reverse-phase C-18 column (250 × 4 mm, 5 μm particles size) was used, and the mobile phase was acetonitrile/water/acetic acid (85:15:0.12, vol/vol) with a flow rate of 1.5 mL/min. The first channel of the detector was set at 234 nm for measuring CD fatty acids, whereas the second was set at 200 nm for measuring linoleic acid. Spectra (195 to 315 nm) of the eluate were obtained every 1.28 seconds and were electronically stored. Second derivative spectra were generated by using the chemstation software of the HPLC system. Simple absorption and second derivative spectra of each chromatographic peak allowed us to identify those containing CDs [32]. Once the second derivative of the ab-

sorption spectra is taken, CDs, if present, are revealed as two distinct signals, with a minim value at near 234 and 245 nm [33, 34]. Under these chromatographic conditions, CD fatty acid hydroperoxides eluted at 4 minutes, CD18:3 at 7.6 minutes, CLA at 10.3 minutes, and CD20:3 at 11.6 minutes. Each of these peaks showed the typical characteristics of CDs when subjected to second derivative analysis [32]. Standard solution for CD fatty acid hydroperoxides was [9(S),10E,12Z]-9-hydroperoxyoctadecadien-1-oic acid purchased from Cascade Biochem (Berkshire, UK). The linoleic, linolenic, and eicosatrienoic acid were obtained from Sigma Chemicals (St. Louis, MO, USA). A mixture of standard CLA was obtained from NU Chek Prep, (Elysian, MN, USA). CD18:3 and CD20:3 were prepared by alkalinization from the corresponding fatty acid as previously described [31]. All of the other reagents were of HPLC grade. Concentrations of CD fatty acid were expressed in ng/mg of lipids, and linoleic acid in μg/mg of lipids.

Other measurements

Parathyroid hormone (intact) levels were measured by a radioimmunoassay method, whereas all the other biochemical parameters were assayed by standard laboratory tests.

Dietary intake

Habitual dietary intake was estimated by a trained dietitian by using the dietary history interview. Food photographs were used to represent the estimated portion size. All data regarding the foods were entered into a computer program (Dietapro, Medimatica, S. Benedetto del Tronto, Italy) that is used for calculating the dietary variables (Table 1).

Statistical analysis

Data were expressed as mean values ± SD. One-way analysis of variance was used to test the differences among the means of the groups. When differences were significant, the Student–Newman–Keuls procedure was used for multiple comparison among pairs of groups. Simple linear regression analysis was used to identify, for each of the CD fatty acid, the correlation between the levels in plasma, adipose tissue, and RBC membranes. Multiple linear regression analysis, using the stepwise elimination procedure, was performed in order to assess the influence of the various clinical and biochemical parameters reported in Table 1 on CD fatty acid levels in plasma, adipose tissue, and RBC membranes. Two-tailed tests of significance were used, and a P value of less than 0.05 was considered statistically significant. Statistical analyses were performed by using the Statistical Package for the Social Science (SPSS/PC; SPSS, Inc., Chicago, IL, USA). Nonlinear regression analysis was performed by using the Microsoft Excel 5.0 (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Clinical and laboratory data of control subjects and patients participating in the study are shown in Table 1. In HD patients, the standard deviation of PTH is very high because we included subjects with hyperparathyroidism in the study to evaluate the possible influence of PTH level on the various parameters examined. The results of the dietary assessment are also reported in Table 1. Significant differences were found only in end-stage CRF patients following a low-protein dietary regime. As reported in the **Methods** section, most of the patients with C_{Cr} >10 mL/min at the time of their enrollment in the study were not under dietary restriction, and thus, their dietary regimen was comparable to controls.

Figure 1 shows the levels of CLA and its metabolites together with CD fatty acid hydroperoxides, in lipids extracted from plasma, adipose tissue, and RBC membranes of healthy controls and patients groups. CLA is the most representative fatty acid with CD found in humans; its plasmatic and adipose tissue concentrations were significantly increased only in patients with C_{Cr} <10 mL/min (Fig. 1A). Adipose tissue was obtained only in four patients with C_{Cr} >10 mL/min because it was taken at the time of fistula placement, as described in the **Methods** section. Therefore, data were omitted because of the small sample number. CLA in RBC membranes was significantly reduced in CRF patients with C_{Cr} >10 mL/min, and this reduction became more pronounced in end-stage CRF patients and HD patients. The behavior of CD18:3 (Fig. 1B) and CD20:3 (Fig. 1C) was very similar to that of CLA in RBC membranes, while no significant changes were found in plasma and adipose tissue. The concentrations of CD fatty acid hydroperoxides (Fig. 1D) showed no significant variations in plasma, adipose tissue, and RBC membranes of the various groups studied.

To evaluate whether the variations in CLA content might be due to a similar variation in the content of the non-CLA, we evaluated its concentration in the same samples. No significant differences in linoleic acid content in any of the groups studied were found (data not shown).

Statistically significant correlations between the levels of CD fatty acids in plasma, adipose tissue, and RBC membranes within each group are shown in Table 2. Interestingly, where significant positive correlations were found in the healthy control group, they were not confirmed in any of the patient groups. Peculiarly, in CRF patients with C_{Cr} <10 mL/min and HD patient groups, there was a negative correlation between plasma CLA and RBC CLA, indicating a different incorporation of CLA into lipid classes in these patients with respect to controls.

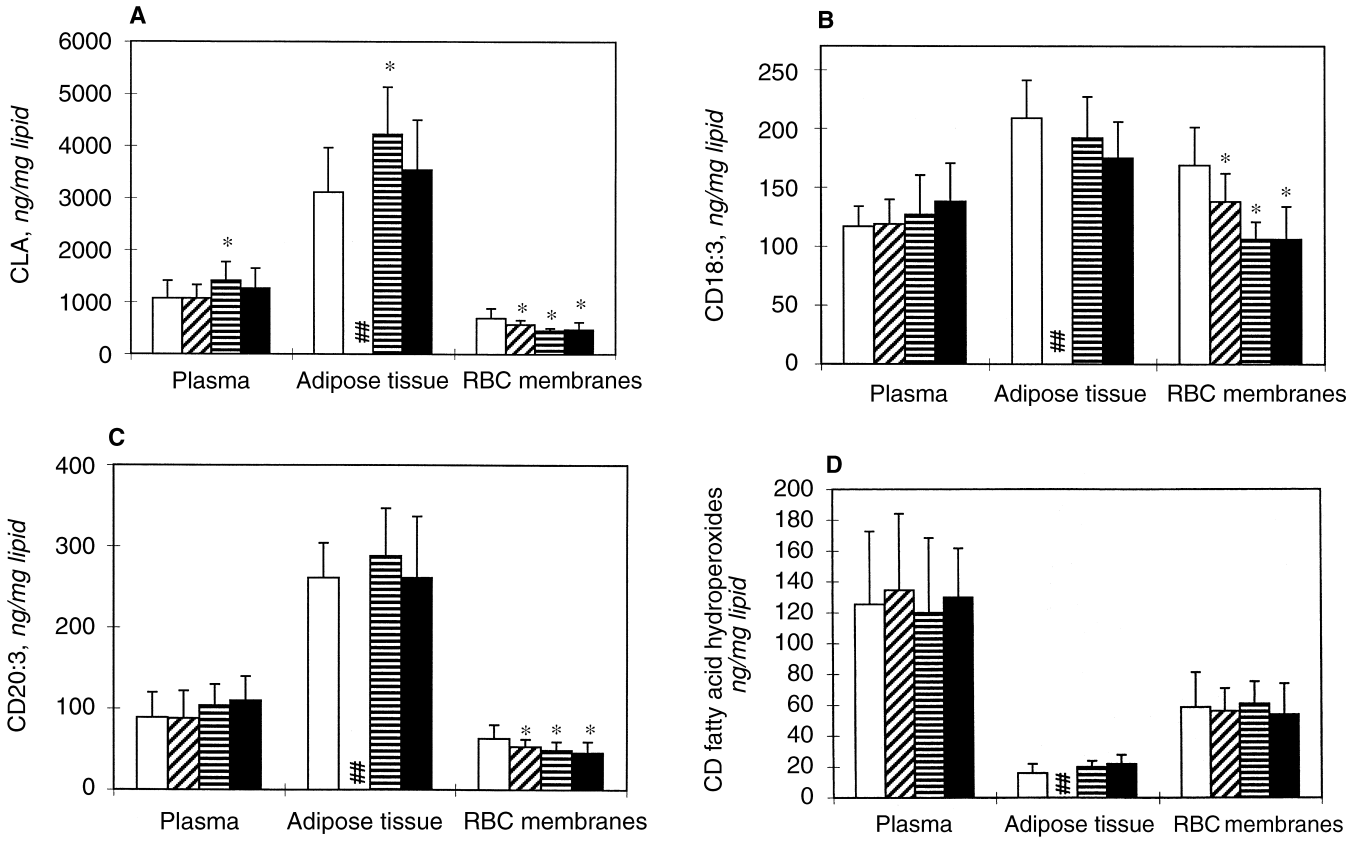

Fig. 1. Concentrations of (*A*) conjugated linoleic acid (CLA), (*B*) conjugated linolenic acid (CD18:3), (*C*) conjugated eicosatrienoic acid (CD20:3), and (*D*) conjugated dienes (CD) fatty acid hydroperoxides in lipids extracted from plasma, adipose tissue, and red blood cell membranes of the various groups studied. Symbols are: (□) controls; (▨) CRF patients with C_{Cr} >10 mL/min; (▤) CRF patients with C_{Cr} <10 mL/min; (■) hemodialyzed patients. *$P < 0.05$ between patients and controls. ##Adipose tissue data from patients with C_{Cr} >10 mL/min were omitted because of the small sample number rendering statistical analysis not reliable.

Table 2. Significant correlations between the levels of the various CD fatty acids in plasma, adipose tissue and red blood cell membranes evaluated by linear regression analysis

		Healthy controls		CRF C_{Cr} >10 mL/min		CRF C_{Cr} <10 mL/min		Hemodialysis	
Variable 1	Variable 2	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Plasma CLA	Adipose tissue CLA	0.61	0.047						
Plasma CLA	Red blood cells CLA	0.56	0.045			−0.47	0.000	−0.46	0.012
Plasma CD18:3	Red blood cells CD18:3	0.61	0.004						

In Table 3, the results of the multiple regression analyses are shown. All variable listed in Table 1, together with linoleic acid, C_{Cr}, and dialytic age were considered as predictor variables, while CLA, CD18:3, CD20:3, and CD fatty acids hydroperoxides in the plasma, adipose tissue, and RBC were considered dependent variables. CRF patients were analyzed as one group and were not divided into two groups with different C_{Cr} in order to have a wide range of C_{Cr} within the group to evaluate the possible influence of C_{Cr} on CD fatty acids better. In the control group and HD patient group, none of the variables considered significantly influenced the level of CD. In CRF patients, C_{Cr} was the only significant predictor variable for plasma CLA, RBC membrane CLA, and RBC membrane CD18:3. When the regression between plasma CLA and C_{Cr} in CRF patients was plotted, it appeared to be nonlinear as shown in Figure 2. The highest R^2 was obtained using the exponential regression. In a previous article [23], we found that in CRF patients with C_{Cr} >20 mL/min, the levels of plasma total CDs were similar to that found in control subjects. This finding was confirmed in the present study by measuring plasma CLA. Therefore, we focused our attention on patients with very advanced renal failure (Fig. 2).

Table 3. Results of multiple linear regression analysis

	Dependent variable	Predictor variables	Adjusted R^2	Constant	B	Beta	*P*
CRF patients	Plasma CLA	C_{Cr}	0.261	1504	−13.6	−0.53	0.000
	Red blood cell CLA	C_{Cr}	0.608	428.7	4.82	0.78	0.000
	Red blood cell CD18:3	C_{Cr}	0.479	100.3	1.19	0.70	0.000

B represents the partial regression coefficient; Beta is the standardized regression coefficient and Constant is sometimes called intercept.

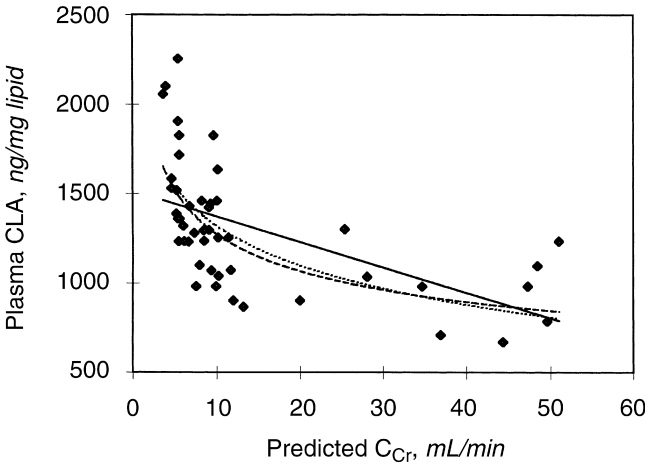

Fig. 2. Relationship between plasma CLA and predicted C_{Cr} in the CRF patients group. The continuous line represents the linear regression ($y = -14.23x + 1514.5$, $R^2 = 0.305$). The dotted line represents the logarithmic regression [$y = -317.9\text{Ln}(x) + 2052.5$, $R^2 = 0.442$], and the dashed line represents the exponential regression ($y = 2292.8x^{-0.2551}$, $R^2 = 0.490$).

DISCUSSION

In the present study, we confirm previous results [22] showing that the major part of CD found in vivo is represented by nonhydroperoxides, and consequently, the measurement of total CD may not be used as a marker of lipid peroxidation in vivo as used in the past [2, 35].

As far as the nonhydroperoxides CD fatty acids are concerned, CLA is the most representative CD found in humans. The National Academy of Sciences has pointed out that CLA is the only fatty acid that has been shown unequivocally to inhibit carcinogenesis in experimental animals [36]. Studies on humans are still in their infancy. While it has been shown that dietary CLA is absorbed and deposited in human tissues, to our knowledge, the present study is the first report showing that CLA metabolites CD18:3 and CD20:3 produced by desaturation and elongation reaction are also present in human plasma and tissues. However, no CD20:4 (the expected main metabolite) was detected. The failure to detect CD20:4 may be explained by either a high dietary intake of linoleic acid, which shares desaturase and elongase enzymes with CLA and thus competes for CD20:4 formation, or too low of an intake of CLA. Our results also showed that CLA and its metabolites' distribution in humans mirrored that previously seen in rats [21], with a preferential incorporation in neutral lipid reach tissues such as adipose tissue.

One of the major results of the present study is that the site distribution of CLA and its metabolites are altered in CRF patients. As far as the CLA is concerned, the earliest alteration found during the progression of CRF is its reduced incorporation in RBC membrane lipids (Fig. 1). This alteration partly could be responsible for the accumulation of CLA in the adipose tissue and for its increase in the plasma of patients with end-stage CRF (Figs. 1 and 2). The accumulation of CLA in the adipose tissue is not likely to be explained by an increased dietary intake of CLA, because of the lower consumption of milk and dairy products by CRF patients. An alteration in the intestinal flora of CRF patients with an increased production of CLA might be possible. Several bacterial species involved in respiratory pathology are also capable of producing CLA in vitro [abstract; Jack et al, *Clin Sci* 81(Suppl 25):S62, 1991].

CD18:3 and CD20:3 were also found to be significantly reduced in RBC membrane lipids of CRF patients (Fig. 1). As these CLA metabolites were not found significantly increased in the adipose tissue of our patients, as it was for CLA, it is possible that a reduced metabolization of CLA, through desaturation and elongation, was present in CRF patients. The possible impaired CLA metabolism, along with a different tissue and/or lipid class distribution of CLA, may explain its increase in plasma and adipose tissue of end-stage CRF patients. The finding of unchanged levels of linoleic acid in the different groups studied indicates that CLA has a different compartmentalization than its parent compound linoleic acid.

Whether changes in the CD fatty acid levels found in renal failure may have clinical significance is difficult to be established at the present stage of our knowledge. However, experimental animal models clearly demonstrated that CLA affects metabolic interconversion of fatty acids in the liver [37]. Particularly, CLA modulates phospholipid arachidonic acid, competing with linoleic acid for Δ6 desaturase, the rate-limiting step in the con-

version of linoleic acid to form arachidonic acid. These data suggest that CLA is likely to influence the synthesis of arachidonic acid-derived eicosanoids, which are well known to modulate tumorigenesis, atherosclerosis, and inflammation [37]. In agreement with this hypothesis, Truitt, McNeill, and Vanderhoek recently demonstrated that CLA may have antiplatelet effects by inhibiting formation of the proaggregatory cyclooxygenase–catalyzed thromboxane H_2 [38].

The present study was conducted in patients without severe dyslipidemia in order to investigate the effect of CRF on CLA metabolism without adding confounding variables. However, these patients represent only part of the CRF patient population.

Future studies will focus on the distribution of CD fatty acids in different lipid classes, that is, triglycerides, phospholipids, and cholesteryl esters in normolipidemic and hyperlipidemic patients, and analyses will be extended to nonconjugated and saturated fatty acids in order to verify whether CRF triggers an imbalance in their incorporation. Actually, changes in PUFA incorporation can influence the availability of PUFA for eicosanoid production, which may eventually favor pathological states such as neoplastic and cardiovascular diseases known to have an increased incidence in CRF patients [24, 25, 39]. In addition, one more point to investigate will be whether the imbalance of CD fatty acids in RBC membrane may be involved in the altered physical properties of RBC in CRF patients [40–42].

The presence of commercially available CLA as an over-the-counter dietary supplement should encourage studies on CLA metabolism in pathological states. In fact, while many studies deal with CLA metabolism in physiological conditions, little is known about the effect of CLA in pathological states.

In conclusion, the results of this study show that the body distribution of CD fatty acids is altered in CRF patients as compared with controls. The incorporation of CLA, CD18:3, and CD20:3 in RBC membranes is reduced in CRF and HD patients. An increase in CLA in both plasma and adipose tissue is present only in end-stage CRF patients. These changes are not accompanied by any variations in linoleic acid and CD fatty acid hydroperoxide content. Therefore, the alterations of CD levels in CRF patients are not due to a lipoperoxidative process, and the increased CD content in plasma and adipose tissue of end-stage CRF patients may be falsely taken for a marker of lipid peroxidation. More studies should be undertaken in order to evaluate whether alteration of CLA incorporation and metabolism in CRF patients may have clinical significance particularly on the incidence of atherosclerotic and neoplastic diseases, which have been demonstrated to have an augmented incidence in these patients.

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