Relationship between Plasma Phospholipid Polyunsaturated Fatty Acid Composition and Bone Disease in Renal Transplantation

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To investigate the relationship between polyunsaturated fatty acid (PUFA) and bone metabolism in renal transplant patients, plasma phospholipid (PP) PUFA levels, biochemical markers of bone turnover and bone mineral density (BMD) were determined in 22 recipients of a first renal allograft at baseline and after a mean 24.4 month follow-up. A significant increase in PP n-3 PUFA content, in the [n-3 PUFA/ arachidonic acid] ratio and in BMD values was observed, as well as a close correlation between the increase in PP n-3 PUFA content and femoral neck BMD. Multivariate regression analysis showed that BMD improvement was positively related to PP n-3 PUFA variation and baseline PP eicosapentaenoic acid levels, and negatively to PP arachidonic acid modification. Tacrolimus- versus cyclosporine-treated patients demonstrated a significant increase in femoral neck BMD and PP n-3 PUFA content. This is the first longitudinal study showing a link between PP-PUFA composition and bone disease in renal transplantation.

Keywords: Renal transplantation, Bone disease, Phospholipid polyunsaturated fatty acid.

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Recent epidemiological and clinical data suggest that fatty acids may have a modulatory effect on calcium and bone metabolism (*1*, *2*). Studies on osteoblast-like human cells have demonstrated specific effects of polyunsaturated fatty acids (PUFA) on the gene expression of some cytokines and inducible nitric oxide synthase (iNOS), which are important local regulatory factors of osteoblast and osteoclast development (*3*, *4*). Because renal transplant patients have shown alterations in both bone (*5–7*) and lipid metabolism, including PUFA pathway (*8–12*), the aim of the present longitudinal study was to evaluate if a relationship exists between plasma phospholipid (PP) PUFA composition and bone disease in kidney transplantation.

PATIENTS AND METHODS

Thirty recipients of a first renal allograft (19 males; mean age 44 years, range 22-65 years), selected from those presenting for routine examination at the outpatient department of the transplant unit of Treviso Hospital, were found eligible for our study. The mean age of the transplantation was 16 months (range 8–29). Exclusion criteria included poor renal function (serum creatinine higher than 230 μmol/L), episodes of acute rejection, diabetes, smoking, menopause, drug therapy for hyperlipidemia, treatment with parent vitamin D, calcium supplements and phosphate binders. In accordance with the Helsinki declaration, informed consent was obtained from all of the participants. Six of these (4 males), however, did not grant consent at follow-up and two (1 male) had episodes of acute rejection. All eight were excluded from the final statistical analysis. Immunosuppressive therapy was not modified for at least 2 months before the beginning of the study as well as throughout the follow-up period. The patients were treated with a standard methylprednisolone regimen consisting of oral dosage with 4 mg/day, in association with cyclosporin A (CsA) (14 patients) or with tacrolimus (FK506) (8 patients) with a dose aiming at an approximate level of 150–200 ng/ml and 5–10 ng/ml, respectively; 2 patients also received mycophenolate mofetil at a mean dose of 2g/day. During the 8 week before and throughout the follow-up, all of the patients followed an isocaloric diet providing 55% of the total calories as complex carbohydrates, 20% as proteins, and 20% as fats (7% of which as saturated fatty acids, 10% as monounsaturated, 8% as polyunsaturated) with a cholesterol content of less than 500 mg/day and approximately 800 mg of calcium. At baseline and after a mean follow-up period of 24.4 months (range 20–26) the parameters described in Table 1 were determined. Bone mass was assessed by DEXA at the lumbar spine (L2–L4) and the femoral neck (average of left and right). Results were expressed as BMD (g/cm^2) and as Z-scores. Changes in the parameters at follow up (D) were calculated in percent using the following formula: [(follow-up value−baseline value)/baseline value]×100. Statistical evaluation was carried out with the analysis of variance (ANOVA) for paired variables to compare parameter's values at baseline and at follow-up and with determination of the r coefficient for linear correlation. A forward stepwise multiple regression was used to identify variables associated with BMD changes and ANOVA for unpaired variables was applied to evaluate significant differences in FK506- versus CsA-treated patients.

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TABLE 1. Biochemical parameters of the renal transplant patients at baseline and at follow-up

Variables	Baseline	Follow-up	F value	*P* value
Serum creatinine (μmol/L)	150.28±40.66	137.00±36.24	0.50	0.60
Creatinine clearance (ml/min)	63.30±24.80	65.83±13.47	0.71	0.41
Serum total cholesterol (mmol/L)	6.23±1.17	6.26±1.05	0.02	0.89
Serum triglycerides (mmol/L)	1.51±0.50	1.39±0.65	1.88	0.19
Serum calcium (mmol/L)	2.55±0.17	2.49±0.12	4.23	0.054
Serum phosphorus (mmol/L)	1.09±0.15	1.15±0.19	1.92	0.18
Serum PTH (ng/L)	66.86±51.11	51.59±25.10	3.00	0.098
Serum 1,25 Vit D_3 (pmol/L)	80.30±43.91	76.80±29.24	0.07	0.79
Serum BAP (U/L)	37.27±17.80	27.80±15.60	7.26	0.014
Urine DPD (μg/mmol Cr)	7.60±2.04	3.80±2.06	55.44	<0.0001
Urine calcium (mmol/mmol Cr)	0.17±0.18	0.02±0.02	15.10	<0.001
Urine phosphorus (mmol/mmol Cr)	0.50±0.08	0.40±0.10	0.81	0.30
Plasma phospholipids (mg/dL)	231.27±38.15	235.73±34.57	0.34	0.57
Plasma fatty acid (%)				
C20:4 n-6 (AA)	11.30±1.96	11.63±2.17	0.71	0.41
C20:5 n-3 (EPA)	0.81±0.25	0.94±0.38	2.88	0.10
C22:5 n-3 (DOCA)	0.88±0.28	0.87±0.17	0.01	0.91
C22:6 n-3 (DHA)	3.55±1.08	3.92±1.00	6.21	0.021
n-3 PUFA	5.06±1.11	5.64±1.10	9.73	0.005
n-3 PUFA /AA	0.45±0.09	0.51±0.05	5.84	0.025
Lumbar spine BMD				
g/cm^2	0.94±0.14	1.01±0.22	4.69	0.042
Z score	−1.11±1.02	−0.76±1.12	7.94	0.01
Femoral neck BMD				
g/cm^2	0.89±0.13	0.92±0.11	6.57	0.018
Z score	−0.76±0.89	−0.37±0.80	16.54	<0.001

Data are means ± SD. All urinary determinations were expressed for urine creatinine.

BAP, serum bone-specific alkaline phosphatase; DPD, deoxypiridinoline; BMD, bone mineral density expressed as absolute value g/cm2 and as Z score; AA, arachidonic acid; EPA, eicosapentaenoic acid; DOCA, docosapentaenoic acid; DHA, docosahexaenoic acid; n-3 PUFA, n-3 polyunsaturated fatty acids (EPA+DOCA+DHA).

RESULTS

The clinical and biochemical characteristics of patients at baseline and after follow-up are summarized in Table 1. A significant increase in PP n-3 PUFA content and in the [n-3 PUFA/arachidonic acid –AA-] ratio was observed, as well as in BMD values at the lumbar spine and at femoral neck, while a significant decrease in serum bone alkaline phosphatase (BAP) and in urine excretion of calcium and DPD was found. Direct correlations between the variation of femoral neck BMD, of PP n-3 PUFA content (Fig. 1) and of n-3 PUFA/AA values (Fig. 2) were observed. Moreover, multivariate regression analysis showed that the femoral BMD change, considered as the dependent variable, was positively related (R^2=0.60, F=11.5; P<0.001) to PP n-3 PUFA variation

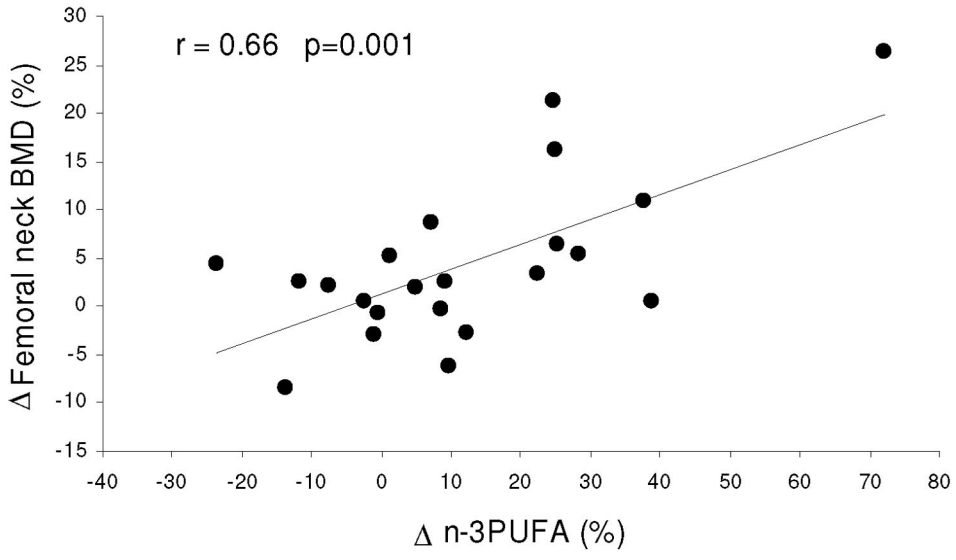

FIGURE 1. Relationship between the longitudinal change in plasma phospholipid n-3 PUFA content and femoral neck BMD values. D, percent change at follow-up [(follow up value− baseline value) / baseline value) 100×]; BMD, bone mineral density, expressed as g/cm^2 ; n-3 PUFA, n-3 polyunsaturated fatty acids (EPA+DOCA+DHA).

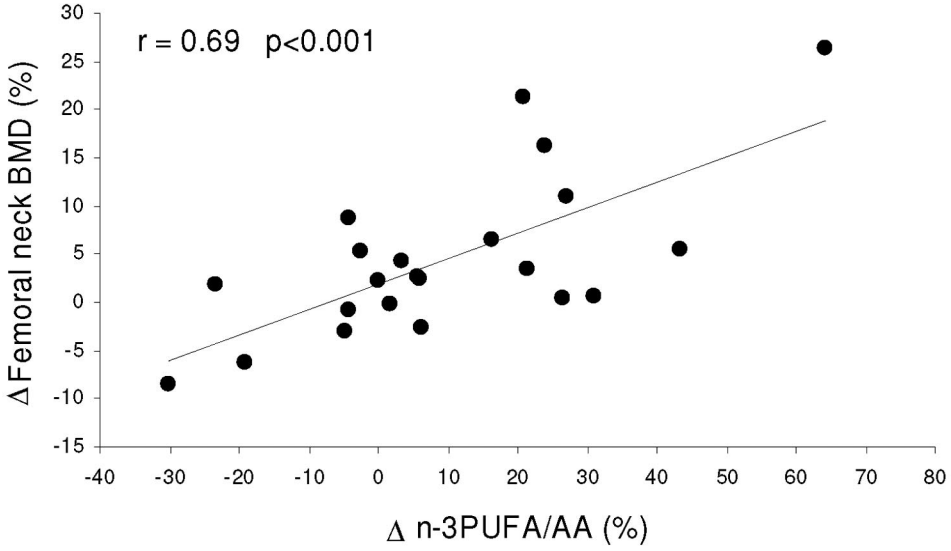

FIGURE 2. Relationship between the longitudinal change in n-3 PUFA/AA and femoral neck BMD values. D, percent change at follow-up [(follow up value−baseline value) / baseline value) 100×]; BMD, bone mineral density, expressed as g/cm^2; n-3 PUFA, n-3 polyunsaturated fatty acids (EPA+DOCA+DHA); AA, arachidonic acid.

(b=0.88, $P<0.001$) and baseline eicosapentaenoic acid (EPA) levels (b=0.37, $P=0.02$), and negatively to PP-AA modification (b=-0.36, $P=0.02$). In addition, FK506- versus CsA-treated patients demonstrated a significant increase in femoral neck BMD (D %: 7.87±9.48 vs. 0.97±4.74; F=5.3, $P=0.03$) and PP n-3 PUFA content (D %: 24.02±21.92 vs. 7.10±14.61; F=4.7, $P=0.04$), in particular of docosahexaenoic (DHA) values (D %: 4.10±19.92 vs. 3.43±11.82; F=9.5, $P<0.01$).

DISCUSSION

Previous studies have indicated that renal transplant patients have altered bone metabolism (*5–7*) and fatty acid pattern (*8*, *9*), including PUFA profile (*10–12*). The association between the two anomalies has never, however, been hypothesized nor investigated. Therefore, the present longitudinal study was designed to evaluate a possible relationship between bone disease and PP-PUFA composition in renal transplant recipients. The results seem to confirm this hypothesis. In effect, in our patients there was a significant improvement in bone metabolism during the follow-up period, as documented by the reduction in serum BAP and in urine excretion of calcium and biochemical markers of bone resorption as well as by an increase in BMD at both the femoral neck and lumbar site. On the other hand, a parallel modification was seen in the PP-PUFA profile, consisting of a significant increase in n-3 PUFA levels, unlike the n-6 PUFA content which remained stable during the longitudinal phase. The two phenomena appear to be closely associated. In fact, the increase in femoral neck BMD values was directly correlated to the increase of n-3 PUFA levels and n-3 PUFA/AA ratio in PP. Moreover, the multiple regression analysis disclosed that the femoral BMD value improvement, considered as a dependent variable, was best explained positively by the PP n-3 PUFA variation and baseline PP-EPA levels and negatively by the PP-AA change.

The significance and the mechanism underlying PUFA's abnormal distribution in renal transplants, as well as the long-term increase in PP n-3 PUFA content, found in the present investigation, are unclear. An anomalous phospholipid PUFA composition in kidney transplantation was previously reported by Cofan et al., whose cross-sectional study reported that renal transplant recipients treated with CsA had higher n-3 PUFA and normal n-6 PUFA levels in the LDL fraction with respect to controls (*11*, *12*).

Looking for the origin of this anomaly, it must be remembered that some long-chain PUFA of tissue and serum lipids are obtained in man by alternating sequences of desaturation and elongation steps of linoleic and α-linolenic acids, their precursors, which cannot be synthesized by the human body and must be supplied by the diet (*13*). Desaturating and elongating activities may be influenced by a number of nutritional, hormonal and metabolic factors, which have been reported to modulate in particular the delta-6-desaturase enzyme activity, the key regulatory step of the biosynthetic pathway of highly unsaturated fatty acids. In this context, the observed anomalous PP-PUFA composition cannot be attributed to diet, because our patients did not vary their dietary habits during the follow-up study, and this was confirmed by the lack of variation in the plasma lipid pattern and urinary urea excretion. PUFA's anomalous composition could be likely related to the posttransplant immunosuppressive therapy, which may selectively modulate different steps in PUFA's biosynthesis. Consistent with this hypothesis, it is known that glucocorticoids and cyclosporine alone or combined may affect the delta 6 and delta 5 desaturase enzyme activity (*14–16*). In addition, CsA, but not FK506, increased AA release in pituitary corticotrope tumor cells (*17*) and different effects of selected immunosuppressive drugs on prostaglandin release have also been reported (*18*).

In the light of these observations, one could speculate that immunosuppressants, such as CsA, FK506 and glucocorticoids, can selectively modulate the desaturating and elongating activities, determining the biosynthesis of a different phospholipid PUFA profile. This could explain the different PP-PUFA composition found in FK506- versus CsA-treated patients.

Whatever the mechanism underlying the anomalous proportion of PUFA in PP of renal transplant recipients, the present study showed a close link between its composition

and bone disease, which remains a serious problem after organ transplantation (*5–7*). Several studies suggest that immunosuppressive therapy plays a key role in posttransplantation bone disease. While glucocorticoids are closely associated with bone loss (*5*, *6*, *19*), the effects of CsA on bone and mineral metabolism are controversial both experimentally and in clinical settings (*7*, *19*). Moreover, it would seem that FK506 per se might have a beneficial effect and better preserve bone mass after renal transplant than CsA (*20*), as confirmed by our results showing a better longitudinal improvement of BMD in FK506- versus CsA-treated patients. It is interesting that different BMD values in the two groups are associated to a parallel difference in the PP n-3 PUFA values, in particular in those of DHA, and this reinforces the hypothesis of a close relation between the two phenomena.

In conclusion, this is the first longitudinal study showing a link between PP-PUFA composition and bone disease in renal transplantation. Further investigations in a larger number of kidney transplant patients evaluated over a longer period of time will be important in confirming these conclusions and in verifying the potential beneficial effects of exogenous supplementation of n-3 PUFA on posttransplant bone remodelling.

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