

Fatty Acid Composition of Serum Lipid Fractions in Type 2 Diabetic Patients With Microalbuminuria

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OBJECTIVE — To determine the fatty acid composition of serum phospholipid, triglyceride, and cholesterol ester fractions and to analyze the lipid profile of microalbuminuric type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — A case-control study was conducted with 72 patients: 37 were normoalbuminuric (urinary albumin excretion rate [UAER] <20 $\mu\text{g}/\text{min}$), and 35 were microalbuminuric (UAER 20–200 $\mu\text{g}/\text{min}$). After 4 weeks of a standardized diet, the fatty acid composition of phospholipid, triglyceride, and cholesterol ester fractions was determined by gas chromatography. Total cholesterol and triglycerides were measured by enzymatic-colorimetric methods; cholesterol HDL by double precipitation with heparin, MnCl_2 , and dextran sulfate; and apolipoprotein B by immunoturbidimetry.

RESULTS — Microalbuminuric patients showed a lower proportion of polyunsaturated fatty acids ($24.8 \pm 11.0\%$), especially of the n-6 family ($21.7 \pm 10.5\%$), in triglyceride fraction than normoalbuminuric patients ($34.1 \pm 11.3\%$, $P = 0.001$ and $31.4 \pm 11.5\%$, $P < 0.001$, respectively). Patients with microalbuminuria also presented higher levels of saturated fatty acids in triglyceride fraction ($43.4 \pm 18.0\%$ vs. $34.7 \pm 13.1\%$, $P = 0.022$). In the logistic regression analysis, only the proportion of polyunsaturated fatty acids in triglyceride fraction remained significantly associated with microalbuminuria (odds ratio [OR] 0.92, 95% CI 0.85–0.98, $P = 0.019$). Total cholesterol, HDL cholesterol, triglyceride, and apolipoprotein B levels were similar in normo- and microalbuminuric patients.

CONCLUSION — Microalbuminuria in type 2 diabetic patients is associated with low polyunsaturated fatty acid contents in serum triglyceride fraction. This association may represent a risk factor for cardiovascular disease and may contribute to the progression of renal disease.

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Microalbuminuria is known to be an independent risk factor for cardiovascular death in type 2 diabetic patients (1,2), but the mechanisms underlying this association have not been clarified. It could be that other cardiovascular risk factors that are frequently asso-

ciated with microalbuminuria, such as hyperglycemia, hypertension (3), and endothelial dysfunction (4), might also contribute to the increased cardiovascular mortality observed in these patients. In addition, dyslipidemia has also been described in type 2 diabetic patients with

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Abbreviations: FAPB2, fatty acid-binding protein type 2; MUFA, monounsaturated fatty acids; OR, odds ratio; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UAER, urinary albumin excretion rate.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

microalbuminuria (2,5,6). Although those studies did not specifically assess the effect of nutrient intake, the effect of dietary habits on the development of dyslipidemia in these microalbuminuric patients cannot be ruled out.

Dietary habits influence serum lipid levels and renal function in patients with diabetes. For example, higher intake of fish protein has been shown to be related to a lower risk for microalbuminuria in type 1 diabetic patients (7), and replacement of red meat with chicken reduces albumin excretion rate and serum cholesterol levels in microalbuminuric type 2 diabetic patients (8). These effects may result from the high content of polyunsaturated fatty acids in fish and chicken meat. An altered fatty acid composition of serum cholesterol esters has been observed to be a risk factor for the development of myocardial infarction in men (9). However, the fatty acid composition of plasma lipid fractions in type 2 diabetic patients with microalbuminuria is virtually unknown.

Therefore, the objective of this study was to determine the fatty acid composition of serum lipid fractions and to describe the lipid profile of type 2 diabetic patients with microalbuminuria following a standardized diet.

RESEARCH DESIGN AND METHODS

Patients

A case-control study was conducted with 72 patients with type 2 diabetes (according to World Health Organization criteria) (10) attending the Endocrine Division's outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil. Patients were selected on the basis of the following criteria: age <75 years; BMI <35 kg/m^2 ; good compliance with diabetes treatment; triglyceride levels <4.52 mmol/l ; urinary albumin excretion rate (UAER) <200 $\mu\text{g}/\text{min}$; normal liver and thyroid function; and absence of urinary tract infection, other renal disease, and cardiac

failure. Treatment with antihypertensive and oral antidiabetic agents was maintained during the study. None of the patients were using hypolipidemic agents. A total of 37 patients were classified as normoalbuminuric (UAER <20 $\mu\text{g}/\text{min}$) and 35 as microalbuminuric (UAER 20–200 $\mu\text{g}/\text{min}$ confirmed at least twice in a 6-month period). The Ethics Committee at Hospital de Clínicas approved the protocol and patients gave their written informed consent before entering the study. Eligible patients entered a run-in period of ~2 months, during which they were oriented to achieve the best possible metabolic control through dietary adjustments and use of oral antidiabetic agents or insulin.

At the end of the run-in period, patients underwent a clinical evaluation. BMI (kg/m^2) was calculated. Sitting blood pressure was measured twice to the nearest 2 mmHg, after a 10-min rest, using a standard mercury sphygmomanometer (phases I and V of Korotkoff). Hypertension was defined as blood pressure $\geq 140/90$ mmHg or use of antihypertensive drugs.

Diet

During the run-in period, each patient received standardized nutritional guidelines developed by a nutritionist following American Diabetes Association recommendations (11) as close as possible. The amount and source of protein from the patient's usual diet was not modified. Patients were also given corn oil [fatty acid composition as indicated by the manufacturer: palmitic acid (10%); stearic acid (2%); oleic acid (31%); linoleic acid (56%); other acids (1%)] to prepare their food during this period. Compliance was assessed by means of 2-day weighed diet records and 24-h urinary nitrogen output at the end of the second and the fourth weeks, as previously reported (12). Patients were assessed after a minimum of 4 weeks following the end of the run-in period.

Laboratory measurements

Blood samples were obtained after a 12-h fast. Serum was separated after centrifugation at 1,500g for 15 min and stored at -80°C for later laboratory measurements. Total HDL cholesterol and its HDL₂ and HDL₃ subfractions were separated by double precipitation with heparin, MnCl_2 , and dextran sulfate. Then, total

cholesterol, total HDL, HDL₂, and HDL₃ cholesterol and triglycerides were measured by enzymatic-colorimetric methods (Merck Diagnostica, Darmstadt, Germany; Boehringer Mannheim, Buenos Aires, Argentina). Non-HDL cholesterol was determined according to the difference between total cholesterol and HDL cholesterol. LDL cholesterol was calculated using Friedewald's formula ($\text{LDL} = \text{total cholesterol} - \text{HDL} - \text{TG}/5$). Apolipoprotein B was determined by immunoturbidimetric assay (Kit Unimate 3; Roche Diagnostic System, Basel, Switzerland; intra-assay coefficient of variation 4.2%).

Fatty acids were determined in phospholipid, triglyceride, and cholesterol ester fractions. Lipids were extracted from serum with chloroform-methanol (2:1 by volume) according to the method of Folch et al. (13). Fatty acid fractions were separated by thin-layer chromatography using a silica gel plate (Silica Gel F240, Merck) and mobile-phase development, using a mixture of hexane, diethyl ether, and acetic acid glacial (80:20:1, respectively, by volume) (14). Fractions were visualized by iodine vapor. Phospholipid, triglyceride, and cholesterol ester bands were scraped into separate tubes, and lipids were extracted from silica with chloroform-methanol and converted into fatty acid methyl esters by boron trifluoride catalysis (15). The methyl esters were then separated and measured by gas chromatography on a 60 m fused silica capillary column with an internal diameter of 0.20 μm (CP-Sil 88). Analysis was performed on a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector. Helium was used as carrier gas and nitrogen as make-up gas. The split-ratio was 5:1. The injection port temperature was 200°C and the detector temperature was 250°C . The column temperature was held at 160°C for 5 min and increased to 190°C at a rate of $2^\circ\text{C}/\text{min}$; it was then held at this temperature for 2 min, and increased again to 220°C at a rate of $1^\circ\text{C}/\text{min}$. The identity of each fatty acid peak was ascertained by comparison of peak retention time with a previously characterized mixture of 20 fatty acids. The relative amount of each fatty acid (% of total fatty acid) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.

Urinary albumin was measured in 24-h timed sterile urine samples by im-

munoturbidimetry (Sera-Pak immuno microalbuminuria; Bayer, Tarrytown, NY; mean intra- and interassay coefficients of variation 4.5 and 11.0%, respectively). Microalbuminuria was considered to be present when UAER measurement was 20–200 $\mu\text{g}/\text{min}$ at least twice in a 6-month period. Plasma glucose level was determined by a glucose oxidase method, serum creatinine level by the Jaffé reaction, GHb by an ion exchange high-performance liquid chromatography procedure (Merck-Hitachi L-9100 GhB analyzer, reference range 2.7–4.3%; Merck, Darmstadt, Germany), and fructosamine by a colorimetric method (normal range 1.87–2.87 mmol/l). Urinary urea was measured by an enzymatic ultraviolet method (mean intra-assay coefficient of variation 3.8%). The protein intake was calculated using 24-h urine by the following formula: protein intake (g/day) = nitrogen intake $\times 6.25$. The nitrogen intake was estimated by urinary urea nitrogen + non-urea nitrogen, where urinary urea nitrogen = urinary urea $\div 2$ and non-urea nitrogen = $0.031 \text{ g N/kg body weight/day}$, assuming patients presented nitrogen balance (16).

Genotype analysis

The genotype analysis of the amino acid variant (A54T) of the fatty acid-binding protein type 2 (FAPB2) gene was performed by PCR amplification using 100 ng of genomic DNA extracted from peripheral leukocytes and specific primers (5'-ACAGGTGTTAATATAGTGAA AAG-3 and 5'-TACCTGAGTTCAGT TCCGTC-3') for exon 2 (22). Each PCR contained 1 $\mu\text{mol}/\text{l}$ of each primer, 2 mmol/l MgCl_2 , 50 mmol/l KCl, 20 mmol/l Tris-HCl, pH 8.4, 0.2 mmol/l of dNTPs and 1 IU *Taq* polymerase in a final volume of 25–50 μl (Gibco). Reactions were performed in a thermal cycler (PTC-100 apparatus, MJ Research, Watertown, MA) at 94°C (1 min), 64°C (1 min), and 72°C (1 min) for 35 cycles; a final extension was carried out for 5 min at 72°C . T-alleles were displayed as an uncut 180-bp fragment and A-alleles were shown as a doublet of 99- and 81-bp bands. Patients were classified in groups of AA or AT and TT according to the presence of the alleles. All amplification reactions were performed twice.

Table 1—Clinical, laboratory characteristics, and lipid profile of type 2 diabetic patients

	Normoalbuminuric	Microalbuminuric	P
n	37	35	
Age (years)	58.9 ± 10.1	56.0 ± 9.3	0.207
Gender (male)	18 (48.6)	20 (57.1)	0.490
Diabetes duration (years)	10.6 ± 7.5	11.5 ± 7.7	0.650
Hypertension	21 (56.8)	22 (62.9)	0.637
Diabetes treatment (D/OA/I/I+OA)	5/28/0/4	2/16/7/10	0.003
BMI (kg/m ²)	26.4 ± 3.5	27.9 ± 2.7	0.052
Fasting plasma glucose (mg/dl)	121 ± 34	124 ± 32	0.698
Fructosamine (mmol/l)	3.07 ± 0.54	3.39 ± 0.54	0.015
GHb (%)	4.97 ± 0.78	5.50 ± 1.28	0.041
Serum creatinine (mg/dl)	0.87 ± 0.19	0.88 ± 0.18	0.587
24 h UAER (μg/min)	6.38 (3.78–7.66)	51.4 (31.9–81.4)	
Apolipoprotein B (mg/dl)	114 ± 31	124 ± 30	0.177
Cholesterol (mg/dl)	196 ± 39	203 ± 35	0.432
Triglycerides (mg/dl)	120 (92–195)	122 (88–175)	0.818
HDL (mg/dl)	52 ± 20	49 ± 16	0.572
HDL ₂ (mg/dl)	14 ± 13	11 ± 12	0.342
HDL ₃ (mg/dl)	37 ± 15	38 ± 13	0.740
LDL (mg/dl)	113 ± 32	124 ± 29	0.157
Non-HDL cholesterol (mg/dl)	144 ± 39	153 ± 35	0.305
Cholesterol/HDL ratio	4.22 ± 1.61	4.36 ± 1.05	0.672

Data are expressed as mean ± SD, median (95% CI), or number of patients (%) with the characteristic. D/OA/I/I+OA, diet only/oral antidiabetic agents/insulin/insulin associated with oral antidiabetic agent.

Statistical analysis

The characteristics of normoalbuminuric and microalbuminuric patients were analyzed using the unpaired Student's *t* test, Mann-Whitney *U* test, χ^2 tests, or Fisher's exact test as appropriate. The Pearson correlation coefficient was used for testing the relationships among the different fatty acid fractions. *P* values <0.05 were considered to be statistically significant. Multiple logistic regression analysis was performed with microalbuminuria as the dependent variable, and possible associated factors were selected according to their significance (*P* < 0.10) in univariate analyses. Results were expressed as medians and 95% CI or means ± SD unless otherwise stated. SPSS 10.0 (SPSS, Chicago, IL) was used for the analyses.

RESULTS

Patient characteristics

The main clinical and laboratory characteristics of normoalbuminuric and microalbuminuric patients are shown in Table 1. All women were postmenopausal and only two (one normoalbuminuric and one microalbuminuric) were using hormone replacement therapy (conjugated estrogens). The microalbuminuric patients were more often treated with

insulin and presented higher levels of fructosamine and GHb than the normoalbuminuric patients. Antihypertensive treatment was calcium channel blockers, diuretics, direct vasodilators, ACE inhibitors, and β -blockers. No difference was observed in the proportion of antihypertensive drugs used by normo- and microalbuminuric patients.

Serum lipid levels

Serum lipid levels are shown in Table 1. No difference was observed between normo- and microalbuminuric patients regarding serum levels of apolipoprotein B, total cholesterol, HDL cholesterol and fractions, LDL cholesterol, non-HDL cholesterol, and triglycerides.

Fatty acid composition

The fatty acid composition of phospholipid, triglyceride, and cholesterol ester fractions is described in Table 2. Polyunsaturated fatty acids (PUFA) tended to be lower and saturated fatty acids (SFA) higher in all fractions of microalbuminuric diabetic patients, but statistical significance was observed only for triglyceride fractions.

In triglyceride fractions, a lower proportion of PUFA was observed in microalbuminuric than in normoalbuminuric patients. This reduction was

mainly due to a decrease in the proportion of n-6 fatty acids in microalbuminuric patients as compared with normoalbuminuric patients. Analyzing individual n-6 family fatty acids in the triglyceride fraction, it was observed that the proportion of linoleic (19.1 ± 10.1% vs. 23.7 ± 10.6%; *P* = 0.066), γ -linolenic [0.23% (0–0.39) vs. 0.54% (0.01–0.93); *P* = 0.054], eicosadienoic (0.69 ± 0.82% vs. 1.30 ± 1.75%; *P* = 0.067), and arachidonic acids [1.09% (0.61–2.21) vs. 1.20% (0.61–2.21); *P* = 0.069] also tended to be lower in microalbuminuric patients, although without statistical significance.

On the other hand, the proportion of SFA in the triglyceride fraction was higher in microalbuminuric than normoalbuminuric patients. The proportion of monounsaturated fatty acids (MUFA) was similar in microalbuminuric patients and normoalbuminuric patients. In a multiple logistic regression analysis, the proportion of PUFA in triglyceride fraction (odds ratio [OR] 0.92, 95% CI 0.85–0.98; *P* = 0.019) was the only factor associated with microalbuminuria. SFA (OR 1.01, 95% CI 0.96–1.06; *P* = 0.647), BMI (OR 1.15; 95% CI 0.97–1.37; *P* = 0.106), and GHb

Table 2—Fatty acid composition (% of the total amount of fatty acids) in lipid fractions in type 2 diabetic patients with normoalbuminuria and microalbuminuria

	Normoalbuminuric	Microalbuminuric	P
<i>n</i>	37	35	
Triglyceride fraction			
SFA	34.70 ± 13.09	43.38 ± 18.04	0.022
MUFA	31.20 ± 13.21	31.83 ± 13.37	0.840
PUFA	34.10 ± 11.31	24.79 ± 11.01	0.001
PUFA n-6	31.36 ± 11.45	21.67 ± 10.55	<0.0001
PUFA n-3	2.09 ± 3.40	2.20 ± 5.50	0.920
Cholesterol ester fraction			
SFA	21.77 ± 9.83	26.15 ± 11.42	0.085
MUFA	17.25 ± 10.20	17.92 ± 12.08	0.799
PUFA	60.98 ± 13.15	55.62 ± 14.95	0.111
PUFA n-6	54.29 ± 14.76	51.72 ± 14.76	0.463
PUFA n-3	5.98 ± 6.56	3.44 ± 3.39	0.044
Phospholipid fraction			
SFA	54.23 ± 14.13	57.29 ± 16.48	0.406
MUFA	11.49 ± 11.01	11.63 ± 11.09	0.958
PUFA	34.28 ± 11.93	30.74 ± 12.85	0.236
PUFA n-6	31.94 ± 10.77	28.44 ± 11.79	0.199
PUFA n-3	1.93 ± 1.89	1.87 ± 1.44	0.876

Data are expressed as mean ± SD.

(OR 1.60; 95% CI 0.86–2.96; $P = 0.137$) were excluded from the model.

In cholesterol ester fraction, the proportion of total PUFAs tended to be lower in microalbuminuric than in normoalbuminuric patients, although without statistical significance ($P = 0.111$). However, the level of n-3 fatty acids was lower in microalbuminuric than in normoalbuminuric patients. No difference was observed between normo- and microalbuminuric patients in terms of other groups of fatty acids (n-6 family, SFA, and MUFA). Analyzing individual fatty acid composition, it was observed that the proportion of γ -linolenic acid (C18:3 n-6) was lower in microalbuminuric patients [0.46% (95% CI 0–0.75)] than in normoalbuminuric patients [0.57% (0.36–1.02); $P = 0.028$]. The proportion of long-chain n-3 fatty acids was similar in both groups of patients, although the median proportion of docosahexaenoic acid (22:6 n-3) tended to be lower ($P = 0.112$) in microalbuminuric [0.76% (0–5.40)] than in normoalbuminuric patients [1.03% (0.40–6.93)].

In phospholipid fraction, fatty acid composition (SFA, MUFA, and PUFA) was similar in normo- and microalbuminuric patients. The analysis of individual fatty acids revealed that the microalbuminuric patients did not present any de-

tectable amount of n-3 α -linolenic acid (C18:3 n-3).

When the proportion of fatty acids was compared in different lipid fractions, we observed a significant positive correlation between triglyceride and phospholipid fractions regarding SFA ($r = 0.356$; $P = 0.003$) and PUFA content ($r = 0.566$; $P < 0.001$). A positive correlation was also observed between triglyceride and cholesterol ester fractions concerning the proportion of SFA ($r = 0.351$; $P = 0.003$) and PUFA ($r = 0.215$). However, the significance of this correlation was marginal for the proportion of PUFA ($P = 0.07$).

Genotype analysis

FAPB2 polymorphism was determined in 69 patients, with the following distribution: AA 29 patients (42.0%); AT 37 patients (51.4%); and TT 3 patients (4.2%). The frequency of allele A was 0.69. The frequency of allele T was 0.31. The observed genotype distribution was in agreement with the Hardy-Weinberg equilibrium. The genotype distribution of FAPB2 gene polymorphism according to the presence of the T allele was similar in microalbuminuric patients (58.8%) and normoalbuminuric patients (57.1%) ($P > 0.05$).

Characteristics of the diet

According to the weighed diet records, normoalbuminuric and microalbuminuric patients had a similar intake of total energy (1,699 ± 436 vs. 1,660 ± 427 kcal); carbohydrate (50.03 ± 6.47 vs. 48.46 ± 5.33%); protein (21.75 ± 3.59 vs. 22.95 ± 3.62%); fat (28.63 ± 5.59 vs. 28.60 ± 5.28%); SFA (8.32 ± 2.30 vs. 8.41 ± 2.05%); MUFA (9.62 ± 2.42 vs. 9.92 ± 2.41%); PUFA (7.89 ± 2.30 vs. 7.49 ± 2.18%); and cholesterol [174 mg (150–224) vs. 186 mg (156–263)].

Total protein intake (g/kg body weight) assessed by nitrogen output and by weighed diet records was similar in normoalbuminuric (1.25 ± 0.3 vs. 1.31 ± 0.3; $P > 0.05$) and microalbuminuric patients (1.31 ± 0.4 vs. 1.25 ± 0.3; $P > 0.05$).

CONCLUSIONS — In this sample of microalbuminuric type 2 diabetic patients, a lower proportion of PUFA, especially from the n-6 family, and a higher proportion of SFA were observed in triglyceride fraction. As far as we know, this is the first study to address fatty acid composition in type 2 diabetic patients with microalbuminuria.

Abnormal fatty acid composition has been described in type 2 diabetic patients in general. The most consistent findings

of previous studies are changes in the n-6 family of fatty acids: lower proportion of PUFA (linolenic acid) in cholesterol ester (17) and in phospholipid fractions (18,19), and higher content of highly unsaturated fatty acids derived from linoleic acid. This has been attributed to an increase in the elongation and desaturation process (18) or to a higher intake of SFA (17). Other studies have reported a decreased level of total PUFA, mainly of the n-6 family, in type 2 diabetic patients with dyslipidemia (20). However, renal status, and especially albuminuria, was not evaluated in those studies (17–20). In addition, alterations in fatty acid composition were described in cholesterol ester and phospholipid fractions, but not in triglyceride fraction. As shown in the present study, the fatty acid alterations in microalbuminuric patients were only significant in triglyceride fraction, although a similar nonsignificant pattern was also observed in cholesterol ester and phospholipid fractions. Moreover, a positive correlation was observed between triglyceride and phospholipid fractions in terms of the proportion of SFA and PUFA and between triglyceride and cholesterol ester fractions in terms of the proportion of SFA, as also demonstrated by others (17). These observations suggest that the fatty acid composition of different lipid fractions behaves similarly.

The composition of fatty acids in triglyceride fraction depends on the diet and on the metabolic process of fatty acid synthesis and elongation, desaturation, and oxidation (21). The lower proportion of PUFA and n-6 family fatty acids in microalbuminuric type 2 diabetic patients was not associated with different fatty acid composition of the diet, since both normo- and microalbuminuric patients followed a similar standardized diet. According to the weighed dietary records, the intake of different fatty acids was similar in normo- and microalbuminuric patients. Therefore, the lower proportion of PUFA in the triglyceride fraction of microalbuminuric patients was probably related to a shift in metabolism, or, alternatively, could have been related to a decrease in the intestinal absorption of these fatty acids.

Absorption of fatty acids across the intestinal mucosa is carried out by FABP2. This intracellular protein is only expressed in the intestine, and it binds to fatty acids in a noncovalent reaction with

saturable kinetics. The G to A polymorphism of codon 54 of the FABP2 gene (A54T) results in the substitution of an alanine (A) for a threonine (T) in FABP2. The presence of the T-54 allele of FABP2 has been associated with increased affinity of FABP2 for long-chain fatty acids (22) and with elevated fasting plasma triglyceride levels in patients with type 2 diabetes (23). In the present study, the proportion of normo- and microalbuminuric patients presenting the codon 54 polymorphism was not different. Therefore, it is very unlikely that our results were influenced by different absorption of fatty acids.

The changes in fatty acid composition observed in this study were mainly related to n-6 fatty acids. Linoleic acid is the precursor of this family of fatty acids (21). The proportion of linoleic acid was similar in normo- and microalbuminuric patients; therefore, the lower content of other n-6 fatty acids was probably due to alterations in the metabolic pathways involved in the synthesis and metabolism of the n-6 family of fatty acids (24).

Microalbuminuric patients had higher levels of fructosamine and GHb, indicating a less well-controlled metabolic state. However, these differences were minimal and probably did not affect the results, since the significance of the association between GHb and microalbuminuria was lost in the multiple regression analysis. Furthermore, other authors have also reported that metabolic control did not influence the fatty acid composition of plasma lipid fractions in diabetic patients (20,25).

The lower proportion of n-6 family PUFA in these microalbuminuric patients might affect renal function through the arachidonic acid cascade. A reduced production of prostacyclin, an arachidonic acid derivative with vasodilatory and platelet inhibition properties, has been described in microalbuminuric patients (26). Furthermore, lower levels of n-6 acids are also associated with altered blood viscosity, decreased membrane fluidity, and endothelial dysfunction (27). In fact, we have previously reported that endothelin 1, a marker of endothelial function, was increased in dyslipidemic type 2 diabetic patients and related to higher levels of albuminuria (28). Prospective studies should be conducted to analyze the possible role of lipid fraction fatty acid composition as a risk factor for the

development of cardiovascular events and/or microalbuminuria in type 2 diabetic patients.

The lipid profile of our microalbuminuric type 2 diabetic patients was not different from that of normoalbuminuric patients. Other studies have reported the presence of dyslipidemia in microalbuminuric type 2 diabetic patients (2,5,6). However, there is no information about the dietary intake in some of those studies (2,6). The increased level of triglycerides and low levels of HDL reported in lean microalbuminuric Japanese patients (5) could be explained (29) in part by a higher carbohydrate intake (60 vs. 50%) as compared with the present study.

In conclusion, type 2 diabetic patients with microalbuminuria presented lower levels of PUFA, especially of the n-6 family, in triglyceride fraction. Lower levels of PUFA in triglyceride fraction may represent a risk factor for cardiovascular disease and may contribute to the progression of renal disease in type 2 diabetic patients with microalbuminuria.

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References

1. Mogensen CE: Microalbuminuria predicts clinical proteinuria and early mortality in maturity onset diabetes. *N Engl J Med* 310:356–360, 1984
2. Mattock MB, Morrish NJ, Viberti GC, Kenn H, Fitzgerald AP, Jackson G: Prospective study of microalbuminuria as predictor of mortality in NIDDM. *Diabetes* 41:736–741, 1992
3. Gall MA, Borch-Johnsen K, Hougaard P, Nielsen FS, Parving HH: Albuminuria and poor glycemic control predict mortality in NIDDM. *Diabetes* 44:1303–1309, 1995
4. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH: Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes* 51:1157–1165, 2002

5. Kasiwaazaki K, Hirano T, Yoshino G, Kurokawa M, Tajima H, Adachi M: Decreased release of lipoprotein lipase is associated with vascular endothelial damage in NIDDM patients with microalbuminuria. *Diabetes Care* 21:2016–2020, 1998
6. Niskanen L, Uusitupa M, Sarlund H, Siitonen O, Voutilainen E, Penttilä I, Pyörälä K: Microalbuminuria predicts the development of serum lipoprotein abnormalities favouring atherogenesis in newly diagnosed type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 33:237–243, 1990
7. Möllsten AV, Dahlquist GG, Stattin EL, Rudberg S: Higher intake of fish protein are related to a lower risk of microalbuminuria in young Swedish type 1 diabetic patients. *Diabetes Care* 24:805–810, 2001
8. Gross JL, Zelmanovitz T, Moulin CC, De Mello V, Perassolo M, Leitão C, Hoefel A, Paggi A, Azevedo ML: Effect of a chicken-based diet on renal function and lipid profile in patients with type 2 diabetes: a randomized crossover trial. *Diabetes Care* 25:645–651, 2002
9. Ohrvall M, Berglund L, Salminen I, Lithel H, Aro A, Vessby B: The serum cholesterol ester fatty acid composition but not the serum concentration of alpha tocopherol predicts the development of myocardial infarction in 50-year-old men: 19 years follow-up. *Atherosclerosis* 127:65–71, 1996
10. American Diabetes Association: *Medical Management of Type 2 Diabetes*. 4th ed. Clinical Educational Series. Alexandria, VA, ADA, 1998
11. American Diabetes Association: Nutrition recommendations and principles for people with diabetes mellitus. *Diabetes Care* 21 (Suppl. 1):S32–S35, 1998
12. Moulin CC, Tiskievicz F, Zelmanovitz, Oliveira J, Azevedo MJ, Gross JL: Use of weighed diet records in the evaluation of diets with different protein contents in patients with type 2 diabetes. *Am J Clin Nutr* 67:853–857, 1998
13. Folch J, Less M, Stanley S: A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509, 1957
14. Skipski VP, Barclay M: Thin layer chromatography of lipids. In *Methods in Enzymology*. Lowenstein JM, Ed. New York, Academic Press, 1969, p. 530–598
15. Morrison MR, Smith LM: Preparation of fatty acids methyl esters and dimethyl acetals from lipids with boron fluoride methanol. *J Lipid Res* 5:600–607, 1968
16. Maroni BJ, Steinman TI, Mitch WE: A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 27:58–65, 1985
17. Salomaa V, Ahola I, Tuomilehto J, Aro A, Pietinen P, Korhonen HJ, Penttilä I: Fatty acid composition of serum cholesterol esters in different degrees of glucose intolerance: a population-based study. *Metabolism* 39:1285–1291, 1990
18. Pelikánová T, Kohout M, Válek J, Base J, Stefka Z: Fatty acid composition of serum lipids and erythrocyte membranes in type 2 (non-insulin-dependent) diabetic men. *Metabolism* 40:175–180, 1991
19. Pelikánová T, Kazdová L, Chvojková S, Base J: Serum phospholipid fatty acid composition and insulin action in type 2 diabetic patients. *Metabolism* 50:1472–1478, 2001
20. Bohov P, Balaz V, Segokova E, Klimes I: The effect of hyperlipidemia on serum fatty acid composition in type 2 diabetics. *Ann N Y Acad Sci* 827:561–567, 1997
21. Jones PJH, Kubow S: Lipids, sterols, and their metabolites. In *Modern Nutrition in Health and Disease*. 9th ed. Schils H, Olson J, Ross AC, Eds. Philadelphia, PA, Williams, Wilkins & Lippincott, 1998, p. 67–94
22. Baier LJ, Sacchettini JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, Mochizuki H, Bennett PH, Bogardus C, Prochazka M: An amino acid substitution in the human intestinal fatty acid binding, increased fat oxidation, and insulin resistance. *J Clin Invest* 95:1281–1287, 1995
23. Georgopoulos A, Aras O, Tsai M: Codon-54 polymorphism of the fatty acid-binding protein 2 gene is associated with elevation of fasting and postprandial triglycerides in type 2 diabetes. *J Clin Endocrinol Metab* 85:3155–3160, 2000
24. Horrobin DF: Fatty acid metabolism in health and disease: the role of Δ -6-desaturase. *Am J Clin Nutr* 57 (Suppl. 5):732S–737S, 1993
25. Faas FH, Dang AQ, Kemp K, Norman J, Carter WJ: Red blood cell and plasma fatty acid composition in diabetes. *Metabolism* 8:711–713, 1988
26. Antonipillai I, Nadler J, Vu EJ, Bughi S, Natarajan R, Horton R: A 12-lipoxygenase product, 12 hydroxyeicosatetraenoic acid, is increased in diabetics with incipient and early renal disease: *J Clin Endocrinol Metab* 81:1940–1945, 1996
27. Bruckner G: Fatty acid and cardiovascular diseases. In *Fatty Acids in Foods and Their Health Implications*. 2nd ed. Chow CK, Ed. New York, Food Science and Technology, 2000, p. 843–863
28. Seligman BG, Biolo A, Polanczyk CA, Gross JL, Clausell N: Increased plasma levels of endothelin 1 and von Willebrand factor in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 23: 1395–1400, 2000
29. Garg A, Bonanome A, Grundy SM, Zhang ZJ, Unger RH: Comparison of a high-carbohydrate diet with a high-monounsaturated-fat diet in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 319:829–834, 1988