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Endothelial dysfunction and serum fatty acid composition in patients with type 2 diabetes mellitus

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Abstract

The aim of this study was to evaluate the possible association between serum fatty acids composition and endothelial dysfunction in patients with type 2 diabetes mellitus. A cross-sectional study was conducted with 125 normo- or microalbuminuric type 2 diabetes mellitus patients with serum creatinine <1.5 mg/dL. Serum fatty acids composition (gas chromatography), serum levels of endothelin-1 (ET-1) (enzyme-linked immunosorbent assay), fibrinogen, serum C-reactive protein, lipids, homeostasis model assessment resistance index (HOMA-R), and 24-hour urinary albumin excretion rate were measured. Serum levels of ET-1 were positively correlated with saturated fatty acids ($r = 0.257$, $P = .025$) and negatively correlated with polyunsaturated fatty acids (PUFAs) ($r = -0.319$, $P = .005$). Serum ET-1 levels were also positively correlated with systolic blood pressure, waist circumference, total cholesterol levels, triglycerides, and HOMA-R. In multiple linear regression models, only saturated fatty acids ($R^2 = 0.317$, $P = .002$) or PUFAs ($R^2 = 0.314$, $P = .001$) remained associated with ET-1 levels. Models were adjusted for systolic blood pressure, HOMA-R, waist circumference, triglycerides, body mass index, and smoking habit. The serum total PUFA levels showed an inverse correlation with urinary albumin excretion rate ($r = -0.248$, $P = .012$). In conclusion, in type 2 diabetes mellitus patients, the serum fatty acids composition was independently related to endothelial function evaluated by serum ET-1. Saturated fatty acids were associated with endothelial dysfunction (high levels of ET-1), whereas PUFAs had a protective role in endothelial function.

1. Introduction

In patients with type 2 diabetes mellitus, endothelial dysfunction, increased urinary albumin excretion, and chronic inflammation are interrelated processes that develop in parallel, and are strongly and independently associated with risk of death [1]. Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by endothelial and vascular smooth muscle cells, and it has been used as a marker of endothelial function [2]. Experimental studies in patients with and without diabetes have consistently shown that ET-1 had a significant correlation with flow-mediated vasodilation of the brachial artery [3]. Endothelial dysfunction increases ET-1 production, leading to vascular hypertrophy, atherogenesis, and glomerulosclerosis in the kidney [2].

We have previously reported that normoalbuminuric, dyslipidemic type 2 diabetes mellitus patients had increased levels of ET-1 [4]; and this was associated with urinary albumin levels and insulin resistance. It has been shown that patients with diabetic nephropathy had higher levels of ET-1 compared with patients without diabetic nephropathy [5].

Serum fatty acids composition has been associated with cardiovascular mortality [6] and sudden death [7]. Patients with serum polyunsaturated fatty acids (PUFAs) in the upper tertile had a lower cardiovascular mortality rate [6]. We have demonstrated that type 2 diabetes mellitus patients with microalbuminuria had a lower proportion of serum PUFAs [8]. Moreover, the replacement of red meat (high content of saturated fatty acids [SFAs]) in the usual diet by chicken meat (high PUFAs content) reduced the urinary albumin excretion rate (UAER) in micro- and macroalbuminuric type 2 diabetes mellitus patients [9,10] and increased the serum levels of PUFAs [10]. Polyunsaturated fatty acids may have a beneficial effect on endothelial function because microalbuminuria appears to represent the glomerular involvement in a state of generalized vascular dysfunction [11,12]. Consequently, PUFAs may have a beneficial effect on endothelial function; but this possible association has not yet been

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analyzed in patients with type 2 diabetes mellitus. Therefore, this study was conducted to evaluate the possible association between serum fatty acids composition and endothelial dysfunction in patients with type 2 diabetes mellitus.

2. Methods

2.1. Patients

One hundred twenty-five patients with type 2 diabetes mellitus (World Health Organization criteria) attending the Endocrine Division's outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil, were selected based on the following criteria: body mass index (BMI) <40 kg/m^2; A_{1c} test <9.0%; triglyceride levels <400 mg/dL; UAER <200 μg/min; serum creatinine ≤1.5 mg/dL; normal liver and thyroid function; absence of urinary tract infection (negative urine culture); and presence of other renal disease, heart failure (class III or IV), or acute cardiovascular event in the preceding 6 months. Treatment with antihypertensive and oral antidiabetic agents was maintained during the study. None of the patients were using hypolipidemic agents. The local Ethics Committee approved the protocol, and patients gave their written informed consent.

Eligible patients entered a run-in period of approximately 1 month, during which they were instructed to perform a 3-day weighed diet record, as previously reported [13]. Briefly, the patient's usual diet was assessed on 2 nonconsecutive week days and 1 weekend day. Patients were issued commercial scales (1-125 g) and measuring cups (25-250 mL), and detailed explanation and demonstration were given to each subject. Compliance with the weight-record technique, besides an interview with the nutritionist, was confirmed by comparison of daily protein intake estimated from the 3-day weighed-diet records (1.20 ± 0.33 g/kg of body weight) and from 24-hour urinary nitrogen output (1.19 ± 0.30 g/kg of body weight, P = .576). During the run-in period, if necessary, changes in medications were prescribed to stabilize glycemic control or blood pressure levels as best as possible. Thereafter, participants were instructed to maintain their medications and usual physical activities and not to make any marked changes in lifestyle throughout the study period.

At the end of the run-in period, patients underwent a clinical and laboratory evaluation. The body weight and height of patients (without shoes or coats) were obtained with an anthropometric scale. Body mass index (weight [in kilograms]/height^2 [in meters]) was then calculated. Waist circumference was measured midway between the lowest rib margin and the iliac crest, near the umbilicus. Flexible, nonstretch fiberglass tape was used for these measurements. Sitting blood pressure was measured twice to the nearest 2 mm Hg, after a 10-minute rest, using a standard mercury sphygmomanometer (phases I and V of Korotkoff). *Hypertension* was defined as blood pressure ≥140/90 mm Hg or use of antihypertensive drugs. The presence of metabolic syndrome was established according to the National Cholesterol Education Program (NCEP) criteria [14].

2.2. Laboratory measurements

Blood samples were collected after a 12-hour overnight fast. For the measurement of plasma ET-1, venous blood (5 mL) was drawn and put into a refrigerated tube containing EDTA. Serum and plasma were separated after centrifugation at 1500g and 4°C for 15 minutes, and stored at −80°C for later measurements. Endothelin-1 was measured by enzyme-linked immunosorbent assay using a commercial kit (R&D Systems, Minneapolis, MN). Plasma glucose was measured by a glucose oxidase method, serum creatinine by the Jaffé reaction, A_{1c} test by ion-exchange high-performance liquid chromatography (Merck-Hitachi L-9100 glycated hemoglobin analyzer; reference range: 4.7%-6.0%; Merck, Darmstadt, Germany), and insulin by a chemoluminescent method (Elecsys 2010, Basel, Switzerland). Insulin resistance was estimated by homeostasis model assessment resistance index (HOMA-R = fasting serum insulin [in microunits per milliliter] × fasting plasma glucose [in millimoles per liter]/22.5) [15]. Fibrinogen was measured by a coagulometric method (STA Compact, Cedex, France) and serum C-reactive protein (CRP) by nephelometry (reference range, 1-4 mg/L). Urinary albumin was measured in 24-hour timed sterile urine samples by immunoturbidimetry (Sera-Pak immunomicroalbuminuria; Bayer, Tarrytown, NY). Microalbuminuria was considered to be present when UAER was 20 to 200 μg/min at least twice in a 6-month period. Serum total cholesterol and triglycerides were measured by enzymatic-colorimetric methods and high-density lipoprotein (HDL) cholesterol by a direct selective inhibition method. Low-density lipoprotein (LDL) cholesterol was calculated by using the Friedewald formula. Fatty acids were determined in total lipids. Lipids were extracted from serum with chloroform-methanol (2:1, by volume) and converted into fatty acid methyl esters by boron trifluoride catalysis as described previously [8]. In brief, 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, Agilent Technologies, Santa Clara, CA). Analysis was performed on a Hewlett-Packard (Santa Clara, CA) 6890 gas chromatograph equipped with a flame ionization detector. Helium was used as carrier gas and nitrogen as makeup 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 minutes and increased to 190°C at a rate of 2°C/min; it was then held at this temperature for 2 minutes and increased again to 220°C at a rate of 1°C/min. The identity of each fatty acid peak was ascertained by comparing the peak retention time with a previously characterized mixture of 24 fatty acids. The relative amount of each fatty acid (proportion of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.

2.3. Statistical analysis

Partial correlation coefficients were used for testing the relationships between the ET-1 levels and serum fatty acids, using LDL, HDL, and triglycerides as covariates. Multiple linear regression models were carried out to test the association of ET-1 (dependent variable) and factors with possible biological relevance or significance at univariate analysis. All independent variables selected were added in block in a single step. Different selected fatty acids were included as independent variables in each model, one at a time.

The serum ET-1 levels and fatty acid composition for patients grouped according to the presence of the metabolic syndrome components were analyzed using the 1-way analysis of variance, followed by a Tukey post hoc test for multiple comparisons. Variables with non-Gaussian distribution were log transformed before analysis. Data were presented as medians and 95% confidence interval or means ± SD unless otherwise stated. P values < .05 were considered statistically significant. The SPSS software version 12.0 for Windows (SPSS, Chicago, IL) was used.

3. Results

3.1. Patient characteristics

The main clinical and laboratory characteristics of the patients are shown in Table 1. The patients presented a reasonable glycemic control, and none were using hypolipemic agents (fibrates or statins). Ninety-three patients (74.4%) met the NCEP criteria for the metabolic syndrome.

Table 1
Clinical and laboratory characteristics of type 2 diabetes mellitus patients

Characteristic	
Age (y)	59. 6 ± 10.7
Sex (male)	62 (49.6%)
Diabetes duration (y)	10.8 ± 7.0
Hypertension	100 (80%)
Microalbuminuria	42 (33.6%)
Diabetes treatment (D/OA/I/I + OA)	6/77/16/26
BMI (kg/m^2)	28.8 ± 4.1
Fasting plasma glucose (mg/dL)	145 ± 52
A_{1C} test (%)	7.1 ± 1.4
Serum creatinine (mg/dL)	0.89 ± 0.20
Total cholesterol (mg/dL)	202 ± 43
HDL cholesterol (mg/dL)	49 ± 10
LDL cholesterol (mg/dL)	124 ± 35
Triglycerides (mg/dL)	134 (40-485)
HOMA-R	3.6 (0.17-74)
Fibrinogen (mg/dL)	388 ± 85
ET-1 (pg/mL)	0.67 ± 0.39
CRP (mg/L)	2.9 (0.18-10)

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

Two women were using hormone replacement therapy, and one was using an oral contraceptive; 9 patients were currently smokers. Most of the patients were not engaged in any physical activities (51%) nor taking light physical exercise (42%). The type of antihypertensive treatment was diuretics (59 patients), direct vasodilators (1 patient), angiotensin-converting enzyme inhibitors (65 patients), angiotensin II receptor antagonist (12 patients), and β-blockers (41 patients). Only one patient did not use antihypertensive drugs.

3.2. Dietary intake and serum fatty acid composition

The mean dietary daily intake of the patients assessed by 3-day weighed diet records was as follows: total energy = 1883 ± 475 kcal, carbohydrates = 46.29% ± 6.57%, proteins = 18.84% ± 3.21%, lipids = 34.88% ± 6.78%, cholesterol = 210 ± 102 mg, SFAs = 9.58% ± 2.52%, monounsaturated fatty acids (MUFAs) = 11.90% ± 2.92%, and PUFAs = 10.37% ± 3.48%. The intake of *trans* fatty acids was 0.97% (0.36%-4.39%) of the total energy intake.

The levels of serum fatty acids (percentage of total fatty acids) were as follows: SFAs = 39.19% ± 5.38%, MUFAs = 23.00% ± 3.93%, PUFAs = 37.79% ± 5.70%, total n-6 PUFAs = 36.83% ± 5.78%, and total n-3 PUFAs = 0.71% (0%-6.21%).

The proportion of serum PUFAs was positively correlated with the intake of the PUFAs (r = 0.320, P = .001). This correlation was particularly evident between the serum n-6 PUFAs and the diet content of n-6 PUFAs (r = 0.305, P = .001). No significant association was observed between serum levels and intake of MUFAs (r = 0.069, P = .467) and SFAs (r = −0.101, P = .288).

3.3. Variables associated with serum ET-1 levels

Serum levels of ET-1 had a positive correlation with systolic blood pressure, waist circumference, serum total cholesterol and triglycerides levels, HOMA-R, and serum CRP concentration. Serum ET-1 levels were also positively correlated with serum SFAs and had a negative correlation with serum PUFAs, especially with the n-6 fatty acids group and with the individual fatty acids linoleic and arachidonic acids (Table 2).

Considering that blood pressure, waist circumference, and serum triglycerides are part of the metabolic syndrome, we also analyzed the association of ET-1 levels with the number of components of the metabolic syndrome (Table 3). A progressive increase in serum ET-1 levels was observed according to the increase in the number of metabolic syndrome components (P < .001). However, significance was only observed between patients with 5 components as compared with the patients without metabolic syndrome (P = .035). Interestingly, a decreased proportion of serum PUFAs (P = .002) and an increase in the proportions of serum MUFAs (P = .001) according to the number of metabolic

Table 2
Partial correlations of serum ET-1 (picograms per milliliter) levels with clinical and laboratory variables in type 2 diabetes mellitus patients

Variables	*R*	*P*
Systolic blood pressure (mm Hg)	0.216	.041
Diastolic blood pressure (mm Hg)	0.129	.225
BMI (kg/m^2)	0.182	.086
Waist circumference (cm)	0.255	.015
Total cholesterol (mg/dL)	0.262	.013
LDL cholesterol (mg/dL)	0.117	.275
HDL cholesterol (mg/dL)	−0.051	.632
Triglycerides (mg/dL)	0.377	<.001
Fasting plasma glucose (mg/dL)	0.127	.237
A_{1c} test (%)	0.156	.145
Creatinine (mg/dL)	−0.118	.269
HOMA-R	0.232	.048
CRP (mg/L)	0.241	.028
Fibrinogen (mg/dL)	0.201	.082
SFAs (%)	0.257	.025
MUFAs (%)	0.075	.521
PUFAs (%)	−0.319	.005
Total n-6 fatty acids (%)	−0.302	.005
Total n-3 fatty acids (%)	0.017	.881
Linoleic acid (18:2n-6)	−0.261	.023
Arachidonic acid (20:4n-6)	−0.227	.049

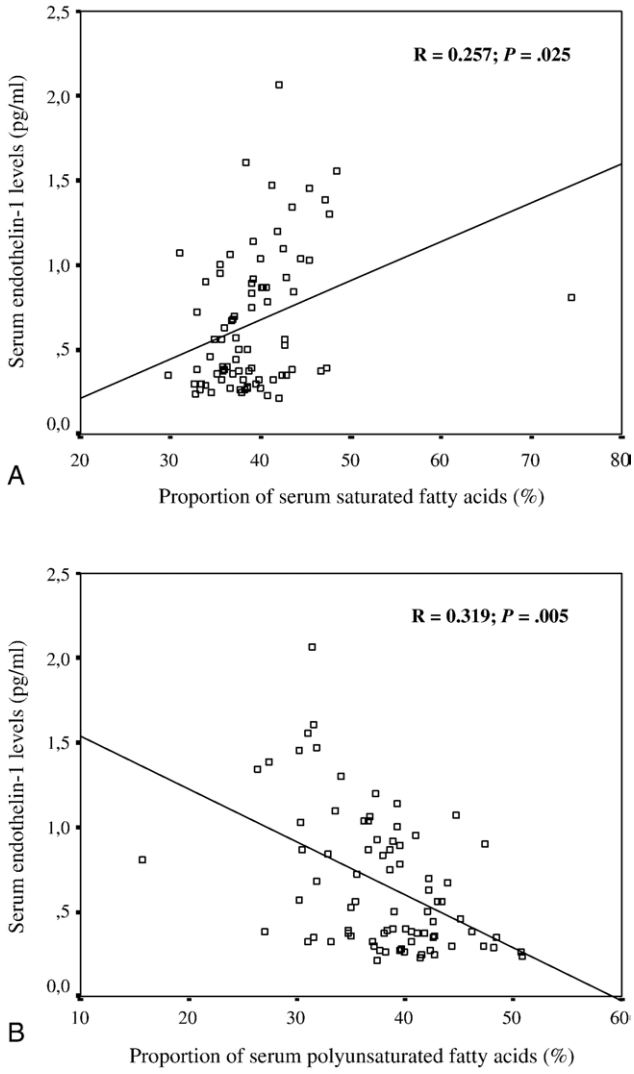

Fig. 1. Partial correlations between serum ET-1 levels and serum fatty acids. A, Saturated fatty acids. B, Polyunsaturated fatty acids.

syndrome components were also observed. Patients with 5 components had a higher proportion of MUFAs and a lower proportion of PUFAs as compared with patients with only 3 components (P = .001 and P = .020, respectively) or with patients without the metabolic syndrome (P = .001 and P = .002). The serum proportion results of total n-6 PUFAs were similar to those of PUFA. However, the proportion of MUFA and PUFA was not significantly different in patients with the 4 components of metabolic syndrome when compared with patients with 3 components or without metabolic syndrome. The n-3 PUFAs and SFAs did not present a significant difference among the patients classified according to the number of components of the metabolic syndrome.

Multiple linear regression analyses were performed with serum ET levels as the dependent variable and serum PUFAs proportion, systolic blood pressure, HOMA-R, waist circumference, triglycerides, BMI, use of angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonist, and smoking as independent variables. Only the proportion of serum PUFAs remained significantly associated with serum ET-1 (R^2 = 0.367, P = .001), especially n-6 PUFAs (R^2 = 0.365, P = .002). The results did not change when systolic blood pressure, HOMA-R, waist circumference,

Table 3
Serum ET-1 levels (picograms per milliliter) and serum fatty acids (percentage) in type 2 diabetes mellitus patients according to the presence of metabolic syndrome and its components

	Without MS (n = 32)	3 Components (n = 42)	4 Components (n = 30)	5 Components (n = 21)	*P*
ET-1	0.52 ± 0.25	0.64 ± 0.35	0.80 ± 0.52	0.86 ± 0.42	.023[a]
SFAs	38.0 ± 4.1	39.3 ± 4.6	40.2 ± 8.2	39.8 ± 4.2	.079
MUFAs	21.9 ± 2.7	22.2 ± 3.8	23.4 ± 4.4	26.1 ± 3.7	.002[b]
PUFAs	40.0 ± 4.7	38.6 ± 5.1	36.4 ± 7.1	34.0 ± 4.6	.001[b]
Total n-6 fatty acids	39.3 ± 4.8	37.4 ± 5.4	35.7 ± 6.9	32.9 ± 4.9	.002[b]
Total n-3 fatty acids	0.79 ± 0.90	1.21 ± 1.29	0.74 ± 0.49	1.06 ± 0.84	.479

Data are expressed as mean ± SD. MS indicates metabolic syndrome.
[a] Five components vs without MS.
[b] Five components vs without MS and 3 components.

triglycerides, and BMI were replaced by the presence or absence of metabolic syndrome (R^2 = 0.270, P < .001). In another model, the proportion of serum SFAs was included instead of PUFAs; and systolic blood pressure, HOMA-R, waist circumference, triglycerides, BMI, and smoking were considered as independent variables. Again, only SFAs remained significantly associated with serum ET-1 levels (R^2 = 0.344, P = .002) (Fig. 1). Once more, when the components of metabolic syndrome, defined according to NCEP [14], were replaced by the presence or absence of the metabolic syndrome, only SFAs were significantly associated with serum ET-1 levels (R^2 = 0.186, P = .004).

3.4. Other associations

The serum proportion of PUFAs and total n-6 PUFAs showed an inverse correlation with UAER (respectively, r = −0.248, P = .012 and r = −0.217, P = .027). The proportion of serum linoleic acid was inversely correlated with HOMA-R (r = −0.254, P = .034). Serum arachidonic acid also correlated with HOMA-R (r = −0.228) but did not reach statistical significance (P = .057).

4. Discussion

In this sample of patients with type 2 diabetes mellitus, it was observed that the serum SFAs had a positive correlation with serum levels of ET-1. The results showed that one third of the variability of ET-1 was independently predicted by levels of serum fatty acids. In addition, an increase in the number of components of the metabolic syndrome was associated with an increase in ET-1 levels and in serum MUFA and SFA levels, and with a decrease in PUFA levels.

Very few studies have analyzed the association of fatty acids and endothelial function in individuals without diabetes; and, as far as we know, there are no data in patients with diabetes. Other authors have observed that the proportion of SFAs had a negative effect on endothelial function assessed by endothelium-dependent vasodilation and venous occlusion plethysmography [16,17]. Interestingly, they also reported that PUFA had a protective effect on the endothelial function. In a study with healthy subjects, palmitoleic acid was inversely associated with the index of endothelial function, whereas stearic and linoleic acids were positively correlated with this index [16]. In young men, elevated serum SFAs and decreased of α-linolenic acid were related to a reduced endothelial vasodilatory function evaluated by venous occlusion plethysmography [17]. Moreover, improved endothelial function mediated by PUFAs could also lead to a beneficial effect on glomerular membrane properties. In fact, in the present study, a negative correlation between serum PUFAs and UAER was observed.

The possible mechanism of the deleterious effect of SFAs on endothelial function is still largely unidentified. It is well known that SFAs may increase serum cholesterol levels [18]; and in fact, we observed a positive correlation between serum cholesterol and ET-1. Moreover, we observed that the aggregation of cardiovascular risk factors was associated with increased serum ET-1 levels and a concomitant increase in serum SFAs. As the association of serum fatty acids and ET-1 remained significant after controlling for the other factors, we may speculate that serum SFAs may have a direct effect on endothelial function. Previous studies have shown that a single meal containing high levels of saturated fat impairs endothelial function for 2 to 6 hours [19]. Unexpectedly, the serum proportion of MUFAs did not correlate with serum ET-1 levels, probably because of the conversion of dietary MUFAs into SFAs in the blood [20].

The serum fatty acids composition depends on the fatty acid content of the diet and on the metabolism of the fatty acid beginning from its synthesis and also taking into consideration the elongation, desaturation, and oxidation steps [21]. In this sample of type 2 diabetes mellitus patients, dietary factors may have made a significant contribution to the serum fatty acid composition because there was a positive correlation between the intake and the serum levels of PUFA. Moreover, the replacement of beef meat by chicken meat reduced the UAER in micro- [9] and macroalbuminuric type 2 diabetes mellitus patients [10] and increased the serum PUFA levels. Chicken meat presents a higher PUFA content than the beef [22]. Although dietary factors may have a significant impact on serum PUFA levels, other factors may also be involved. We observed previously that microalbuminuric type 2 diabetes mellitus patients following a standardized diet had lower levels of PUFA as compared with normoalbuminuric patients [8]. This observation suggests that other factors related to renal involvement associated with diabetes may also influence serum fatty acids. Furthermore, one should take into account that specific genetic polymorphism of the fatty acids binding protein 2 is more frequent in type 2 diabetes mellitus patients with renal disease [23] and may influence the intestinal absorption of dietary fatty acids [24].

The observation that serum SFAs levels increased in parallel with the increase in the number of the components of the metabolic syndrome and that both influence the ET-1 serum levels suggests that the role of SFAs in endothelial function is as deleterious as the presence of metabolic syndrome. The concomitant decrease in serum PUFA under these circumstances may suggest that patients with metabolic syndrome had a low intake of foods rich in PUFA. Alternatively, PUFA, especially linoleic and arachidonic acids, may have a beneficial effect on insulin sensitivity [25].

It should be taken into account that this is a cross-sectional study that allows only for the description of the association between the proportion of serum fatty acids and endothelial function and not for establishing a cause and effect relationship between these parameters.

In conclusion, in type 2 diabetes mellitus patients, the serum fatty acids composition was independently related to

endothelial function evaluated by serum ET-1; but because of the cross-sectional design of this study, it is not possible to conclude that fatty acids regulate endothelial function.

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