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ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1167 – 1172
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

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

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Received 11 April 2007; accepted 7 January 2008

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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doi:10.1016/j.metabol.2008.01.006

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.