Withdrawal of red meat from the usual diet reduces albuminuria and improves serum fatty acid profile in type 2 diabetes patients with macroalbuminuria\textsuperscript{1–3}

Vanessa DF de Mello, Themis Zelmanovitz, Magda S Perassolo, Mirela J Azevedo, and Jorge L Gross

ABSTRACT

Background: Replacement of red meat in the diet with chicken has reduced the urinary albumin excretion rate (UAER) and serum cholesterol in microalbuminuric type 2 diabetes patients. The effects of withdrawing red meat are unknown in the more advanced stages of diabetic nephropathy.

Objective: Our objective was to assess the effects of replacing red meat in the usual diet (UD) with chicken (CD) and of consuming a lactovegetarian low-protein diet (LPD) on renal function, fatty acid, and lipid profile in macroalbuminuric type 2 diabetes patients.

Design: A crossover controlled trial was conducted in 17 type 2 diabetes patients with macroalbuminuria (24-h UAER $\geq$ 200 $\mu$g/min). Each patient followed the UD, CD, and LPD in a random order for 4 wk. After each diet, glomerular filtration rate, UAER, serum fatty acid, lipid profile, glycemic control, anthropometric indexes, and blood pressure were measured.

Results: UAER [median CD: 269.4 (range: 111–1128) $\mu$g/min; LPD: 229.3 (76.6–999.3) $\mu$g/min; UD: 312.8 (223.7–1223.7) $\mu$g/min; $P < 0.01$] and mean (±SD) non-HDL cholesterol (CD: 3.92 ± 0.99 mmol/L; LPD: 3.92 ± 0.93 mmol/L; UD: 4.23 ± 1.06 mmol/L; $P = 0.042$) were lower after CD and LPD than after UD. Compared with the UD, an increase in serum total polyunsaturated fatty acids was also observed (CD: 39.8 ± 2.6%; LPD: 39.7 ± 4.4%; UD: 37.3 ± 3.1%; $P = 0.029$).

Conclusion: In macroalbuminuric patients with type 2 diabetes, withdrawing red meat from the diet reduces the UAER. \textit{Am J Clin Nutr} 2006;83:1032–8.

KEY WORDS Type 2 diabetes mellitus, albuminuria, diabetic nephropathy, red meat, chicken-based diet, low-protein diet, lipids, fatty acids

INTRODUCTION

Diabetic nephropathy (DN) develops in $\approx$40% of patients with diabetes and is the leading cause of chronic kidney disease in patients starting renal replacement therapy (1). Furthermore, it is associated with greater cardiovascular mortality (2). The available therapeutic strategies consist of achieving the best glycemic control, treating hypertension, using drugs that block the renin-angiotensin-aldosterone system, and treating dyslipidemia. These strategies are effective in delaying the progression to more advanced stages of nephropathy and also in reducing cardiovascular mortality in diabetes patients (3). Although some patients may regress to early stages of DN, progressive decline of the glomerular filtration rate (GFR) is still observed; therefore, additional strategies are necessary to arrest the progression of macroalbuminuria. A low-protein diet (LPD) slows the decline of renal function in proteinuric patients with type 1 diabetes (4) and reduces the risk of end-stage renal disease or death in these patients (5). However, the long-term nutritional safety of this diet and its benefit on renal function have not been firmly established in type 2 diabetes patients with macroalbuminuria.

Higher intake of fish protein has a protective effect for the development of microalbuminuria in young patients with type 1 diabetes (6). We have previously reported that replacing red meat with chicken in the usual diet (UD) reduced the urinary albumin excretion rate (UAER) by 46% and also improved the serum lipid profile in patients with type 2 diabetes and microalbuminuria (7). The effects of withdrawing red meat from the diet on renal function, serum fatty acid (FA), and lipid profile are unknown in the more advanced stages of DN. Therefore, this study was conducted to assess the effect of replacing the red meat in the diet with chicken and the effect of a lactovegetarian LPD on the renal function, serum FA, and lipid profile in patients with type 2 diabetes and macroalbuminuria.

SUBJECTS AND METHODS

Subjects

This study was conducted in patients with type 2 diabetes defined according to World Health Organization criteria, who attend the Endocrinology Division’s outpatient clinic at the Hospital de Clínicas de Porto Alegre, Brazil. Patients were selected according to the following criteria: age $\leq$ 75 y, body mass index (BMI; in kg/m\textsuperscript{2}) $< 32$, UAER $\geq$ 200 $\mu$g/min confirmed at least twice in a 6-mo period, serum triacylglycerol $< 4.52$ mmol/L, serum glucose $< 7.8$ mmol/L, and systolic blood pressure $< 140$ mmHg. Patients with hypertension or dyslipidemia were randomized to receive angiotensin-converting enzyme (ACE) inhibitors and statins. The study was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre and the Ministry of Health, Brazil. All participants provided written informed consent.

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normal liver and thyroid functions, compliance with diabetes treatment (A1c test < 10%), serum creatinine ≤ 132.6 μmol/L, proteinuria < 3.0 g/24 h, and absence of urinary tract infection (negative urine culture) or other renal diseases, symptomatic autonomic neuropathy, and heart failure. Hypertension was defined as blood pressure ≥ 140/90 mm Hg or use of antihypertensive drugs. None of the patients were using angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or hypolipidemic agents during the study. Eligible patients entered the study period. At the end of each diet, renal function (UAER 3.0 g/24 h, and absence of urinary tract infection) or other renal diseases, symptomatic autonomic neuropathy, and heart failure. Hypertension was defined as blood pressure ≥ 140/90 mm Hg or use of antihypertensive drugs. None of the patients were using angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or hypolipidemic agents during the study. Eligible patients entered a run-in period of ≈2 mo, during which they were oriented to achieve the best possible metabolic control through dietary and oral antidiabetic agents or insulin adjustments and received standardized nutritional guidelines from a nutritionist (VDFdM) according to the American Diabetes Association (8). The usual amount and source of protein were not modified (1.2–1.5 g/kg body wt). At the end of the run-in period, patients underwent clinical and laboratory evaluations. BMI was calculated. Sitting blood pressure was measured twice to the nearest 2 mm Hg after a 10-min rest by using a standard mercury sphygmomanometer (phases I and V of Korotkoff). The antihypertensive and antidiabetic drugs were maintained during the study. The content of the food was prescribed, on the basis of weighed diet records performed during the run-in period and from the diabetes patients’ current menu. Planned meals were prescribed with an exchange list for bread, dairy products, meat (only for the UD), cereal, legumes, fruit, and vegetables, but recipes were not given. The total oil used for cooking and salad dressings and the total amount of margarine used as a spread were prescribed and recorded by the patients during the protocol. No food was supplied to the patients.

After random assignment, compliance with the diets was assessed by means of 2-d weighed diet records and estimation of protein intake from 24-h urinary nitrogen output (9) at the end of the second and fourth weeks. The protein intake estimated by 24-h urinary urea was calculated by using the following formula:

\[
\text{Protein intake (g/d) = nitrogen intake } \times 6.25
\]

The nitrogen intake was estimated by urinary urea nitrogen plus nonurea nitrogen, where

\[
\text{Urinary urea nitrogen = urinary urea/2}
\]

and

\[
\text{Nonurea nitrogen = 0.031 g N } \times \text{ kg body wt }^{-1} \cdot \text{d}^{-1}
\]

assuming that patients were in nitrogen balance (10). We assumed that the report of the source of protein consumed by the patients was accurate if the patient’s report about total protein intake was in accordance with the 24-h urinary nitrogen output (9).

Dietary macronutrients and micronutrients from diet records were analyzed by using NUTRIBASE 98 CLINICAL NUTRITIONAL MANAGER software (version 1.0; Cybersoft, Phoenix, AZ). Nutrient data of frequently consumed foods were updated if necessary (11). The composition of the diets was expressed as a percentage of total daily energy for macronutrients or as an absolute amount.

Laboratory measurements

GFR was measured by using the \(^{51}\text{Cr-EDTA} \) single-injection technique (CV: 12%; GFR reference range: 72–137.5 mL/min \( \cdot \) 1.73 m\(^2\)). Urinary albumin was measured in 24-h timed sterile (negative urine culture) urine samples by using immunoturbidimetry [MicroAlb Sera-Pak immunomicroalbuminuria; Bayer, Tarrytown, NY on Cobas Mira Plus (Roche, Indianapolis, IN); mean intraassay and interassay CVs were 4.5% and 7.6%,

FIGURE 1. Flow of patients. UD, usual diet; CD, chicken diet; LPD, low-protein diet.

Study design

This study followed a crossover, controlled clinical trial design. After the run-in period (Figure 1), consecutive eligible patients were randomly assigned to one of the sequences of the intervention diets as follows: 1) UD, LPD, chicken diet (CD); 2) UD, CD, LPD; 3) LPD, UD, CD; 4) LPD, CD, UD; 5) CD, LPD, UD; and 6) CD, UD, LPD. Each diet was followed for 4 wk with a 4-wk washout period between them. During the washout period, the patients maintained their UD. All participants were instructed to maintain their usual physical activities and not to make any changes in their lifestyles or medications throughout the study period. At the end of each diet, renal function (UAER and GFR), serum FA, lipid profile, glycemic and anthropometric indexes, and blood pressure were measured.

Diet composition and prescription

All prescribed diets (UD, CD, and LPD) were isoenergetic, with the same proportion of lipids [30% (26–35%)], and the UD and the CD were isoprotein [20% (17–25%)]. The UD was the patient’s adjusted run-in diet, and the CD was created by replacing all meat in the UD with dark chicken meat (skinless leg quarter). The protein content of the LPD was 0.5–0.8 g · kg body wt\(^{-1} \cdot \text{d}^{-1}\) (vegetable and dairy protein only) to achieve at least 50% reduction from the customary protein diet intake of each patient.

The content of the food was prescribed, on the basis of weighed diet records performed during the run-in period and from the diabetes patients’ current menu. Planned meals were prescribed with an exchange list for bread, dairy products, meat (only for the UD), cereal, legumes, fruit, and vegetables, but recipes were not given. The total oil used for cooking and salad dressings and the total amount of margarine used as a spread were prescribed and recorded by the patients during the protocol. No food was supplied to the patients.

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respectively. Urinary urea was measured by an enzymatic ultraviolet method (mean intraassay CV: 3.8%).

Blood samples were obtained after a 12-h overnight fast. Plasma glucose was measured by a glucose oxidase method, serum creatinine by the Jaffé reaction, A1c test by ion-exchange HPLC (Merck-Hitachi L-9100 glycated hemoglobin analyzer, reference range: 4.7–6.0%; Merck, Darmstadt, Germany), and fructosamine by a colorimetric method (reference range: 1.87–2.87 mmol/L).

Blood samples for serum lipid analysis were separated after centrifugation at 1500 × g for 15 min and stored at −80 °C for later laboratory measurements. Serum total cholesterol and triacylglycerol were measured by enzymatic-colorimetric methods (Merck Diagnostica, Darmstadt, Germany; Boehringer Mannheim, Buenos Aires, Argentina) and HDL cholesterol by a direct selective inhibition method. LDL cholesterol was calculated by using Friedewald’s formula as follows in the 2 following equations:

\[
\text{LDL (mg/dL)} = \text{total cholesterol} - \text{HDL} - \text{TG}/5 \quad (4)
\]

where TG is triacylglycerols, or

\[
\text{LDL (mmol/L)} = \text{total cholesterol} - \text{HDL} - \text{TG} \times 0.2 \quad (5)
\]

Non-HDL cholesterol was measured according to the difference between total cholesterol and HDL cholesterol. The FA composition on serum total lipids was determined by extraction with chloroform-methanol (2:1; by volume) according to the method of Folch et al (12) and converted into FA methyl esters by boron trifluoride catalysis (13) as described previously (14). Briefly, 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 an HP 6890 gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with a flame ionization detector. The identity of each FA peak was ascertained by comparing the peak retention time with a previously characterized mixture of 20 FAs. The relative amount of each FA (% of total FAs) was quantified by integrating the area under the peak and dividing the result by the total area for all FAs.

Anthropometric indexes

The body weight and height of patients (without shoes or coats) were obtained with an anthropometric scale, with measurements recorded to the nearest 100 g for weight and to the nearest 0.1 cm for height. BMI was then calculated. Triceps skinfold thickness was measured at the midpoint of the back of the upper left arm, between the acromion process and the tip of the olecranon. The measurement of midupper arm circumference was performed at the same midpoint. Midupper arm muscle area was obtained by appropriate calculations according to Frisancho’s indexes (15) by computerized nutrition software (SISTEMA DE APOIO EM NUTRIÇÃO; Centro de Informática em Saúde, Escola Paulista de Medicina; São Paulo, Brazil). Waist circumference was measured midway between the lowest rib margin and the iliac crest, near the umbilicus. Hip circumference was taken at the maximal gluteal protrusion (lateral view). Flexible, nonstretch fiberglass tape was used for these measurements.

Statistical analysis

Sample size was estimated according to the UAER reduction of ≈35% after an LPD in macroalbuminuric patients (16). It was estimated that 15 macroalbuminuric patients had to be included in the study to give 80% power for a P value of 0.05. Differences among diets were tested by using repeated-measures analysis of variance, which was followed by a Bonferroni adjustment test for multiple comparisons. Variables with non-Gaussian distribution were log transformed before analysis. P values < 0.05 were considered significant. Results were expressed as mean ± SD, median (range), or mean (95% CI). SPSS software (version 10.0; SPSS, Chicago, IL) was used for the analyses.

RESULTS

Patients’ characteristics

Sixty-seven eligible patients were invited to enter the study protocol. Twenty-seven patients were not included because they disliked chicken or red meat or refused to participate. Forty patients entered in the run-in period, and 23 of these patients were excluded because of spontaneous regression to microalbuminuria, loss to follow-up, or noncompliance with the protocol. Seventeen patients were then randomly assigned (Figure 1). All randomly assigned patients completed the study protocol and were included in the final analysis.

Patients were aged 59 ± 11 y, had a BMI of 26.2 ± 2.6, a blood pressure of 93.7 ± 8.5 mm Hg, and reasonable metabolic control [fasting glucose: 8.04 ± 3.27 mmol/L; A1c test: 7.6 ± 2.6%; total cholesterol: 5.34 ± 0.93 mmol/L; HDL cholesterol: 1.17 ± 0.18 mmol/L; LDL cholesterol: 3.42 ± 0.91 mmol/L; non-HDL cholesterol: 4.23 ± 1.01 mmol/L; and triacylglycerol: 1.57 (0.88–3.27)]. Fourteen of the 17 patients analyzed were men, 8 were hypertensive, 10 had some evidence of diabetic retinopathy that was proliferative in 4 patients, 4 had a diagnosis of stable coronary artery disease, and only 2 patients were smokers. Two of the 3 women were postmenopausal, but none were using hormone replacement therapy. Most of the patients were treated with oral antidiabetic agents (sulfonylurea, metformin, or both), insulin, or both. Antihypertensive drugs used were calcium channel blockers (n = 3) or a combination of medications: diuretic and calcium channel blockers (n = 1), diuretic and β-blockers (n = 3), and β-blockers and direct vasodilators (n = 1). Neither the antihypertensive drugs the patients were taking nor the doses were changed during the study.

Composition of the diets according to the weighed diet records

Mean daily intakes of nutrients according to the weighed diet records are shown in Table 1. The intake of polyunsaturated FAs (PUFAs) was higher with the CD and with the LPD than with the UD. The ratio of PUFAs to saturated FAs (SFAs) was also higher with the CD than with the UD, and no difference was observed between the LPD and the UD. Intake of SFAs tended to be lower after the CD than after the UD (P = 0.079). Patients’ intake of total energy was lower during the LPD than during the UD and the CD. As expected, protein content was lower during the LPD than during the CD and the UD, but there was no difference between the UD and the CD. During the LPD, the patients’ intakes of carbohydrate and total fiber were higher than during
the UD and the CD. Cholesterol and phosphorus intakes, however, were lower with the LPD than with the other 2 diets. Zinc and iron intakes were higher after the UD than after both the CD and the LPD. Intakes of total fat, monounsaturated FAs (MUFAFs), potassium, and calcium did not differ significantly between the diets.

The mean daily intake of meat recorded during the UD (75% consumed as beef) was 188 ± 63 g/d. This did not differ significantly from the mean daily intake of chicken meat (skinless leg quarter) during the CD, 173 ± 56 g/d (P = 0.341).

Fifty percent of total protein content of the UD was from red meat, 10% from other meats (pork, fish, and mainly chicken), 30% from dairy products, and 10% from vegetables. During the CD, 58% of the total protein content was from chicken meat (skinless leg quarter), 30% from dairy products, and 12% from vegetables.

Protein intake calculated from the 24-h urinary urea excretion as expected was also lower during the LPD (0.80 ± 0.14 g/kg body wt) than during the UD (1.33 ± 0.23 g/kg body wt) and the CD (1.22 ± 0.25 g/kg body wt; P < 0.001), and there was no difference between the UD and the CD. In addition, a positive correlation was observed between total protein intake as assessed by 24-h nitrogen output and by weighed diet records during all diets (r = 0.749; P < 0.001). The sodium intake, as estimated by 24-h urinary sodium excretion, did not differ significantly between diets [UD: 202 mmol/L (135–356 mmol/L); CD: 164 mmol/L (84–373 mmol/L); LPD: 168 mmol/L (85–285 mmol/L); P = 0.205].

Effect of the diets on renal function

Renal markers after the diets are shown in Table 2. The UAER was significantly lower after the CD and the LPD than after the UD. The relative difference of UAER after the CD (20.6%; 95% CI: 4.8%, 36.4%) and the LPD (31.4%; 95% CI: 12.7%, 50%) related to UAER after the UD were both significant (P = 0.014 and P = 0.003, respectively). This reduction in UAER after the CD and the LPD did not differ significantly (P = 0.249). Three patients (17.6%) after the CD and 8 patients (47.1%) after the LPD had a reduction in UAER to the microalbuminuria range (P = 0.143). GFR values did not change after the 3 diets.

| TABLE 2 |
| Renal function and serum lipid profile after test diets in type 2 diabetes patients with macroalbuminuria<sup>4</sup> |

<table>
<thead>
<tr>
<th></th>
<th>Usual diet</th>
<th>Chicken diet</th>
<th>Low-protein diet</th>
<th>P&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAER (µg/min)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>312.8&lt;sup&gt;a&lt;/sup&gt; (223.7–1223.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>269.4&lt;sup&gt;a&lt;/sup&gt; (111–1128&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>229.3&lt;sup&gt;a&lt;/sup&gt; (76.6–999.3&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GFR (mL · min&lt;sup&gt;-1&lt;/sup&gt; · 1.73 m&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>81.8 ± 22.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.3 ± 26.1</td>
<td>81.9 ± 25.3</td>
<td>0.860</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.37 ± 1.18</td>
<td>5.08 ± 0.96</td>
<td>5.06 ± 0.91</td>
<td>0.069</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.14 ± 0.26</td>
<td>1.14 ± 0.23</td>
<td>1.14 ± 0.21</td>
<td>0.989</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.89 ± 0.99</td>
<td>3.64 ± 0.91</td>
<td>3.55 ± 0.84</td>
<td>0.123</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.23 ± 1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.92 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.92 ± 0.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.042</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.46 (0.60–4.73)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.22 (0.50–3.88)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.51 (0.62–7.35)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.012</td>
</tr>
</tbody>
</table>

<sup>1</sup> n = 17. UAER, urinary albumin excretion rate; GFR, glomerular filtration rate. Values in a row with different superscript letters are significantly different, P < 0.05 (Bonferroni adjustment test for multiple comparisons).
<sup>2</sup> Repeated-measures ANOVA.
<sup>3</sup> Tested with log-transformed values.
<sup>4</sup> Median; range in parentheses (all such values) for variables with non-Gaussian distribution.
<sup>5</sup> All values are x ± SD; n = 17. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid. Values in a row with different superscript letters are significantly different.
**TABLE 3**

Serum fatty acid composition (% of total fatty acid content) of total lipids after diets

<table>
<thead>
<tr>
<th></th>
<th>Usual diet</th>
<th>Chicken diet</th>
<th>Low-protein diet</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total SFA</td>
<td>40.6 ± 3.8ᵃ</td>
<td>37.8 ± 2.5</td>
<td>38.8 ± 3.4</td>
<td>0.068</td>
</tr>
<tr>
<td>16:0 Palmitic acid</td>
<td>30.8 ± 9.3ᵇ</td>
<td>28.1 ± 2.2ᵇ</td>
<td>30.5 ± 3.5ᵃ</td>
<td>0.049</td>
</tr>
<tr>
<td>18:0 Stearic acid</td>
<td>9.2 ± 1.4ᵃ</td>
<td>8.8 ± 1.5ᵃ</td>
<td>7.9 ± 1.3ᵇ</td>
<td>0.025</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>22.2 ± 2.6</td>
<td>22.5 ± 2.2</td>
<td>21.6 ± 3.06</td>
<td>0.591</td>
</tr>
<tr>
<td>16:1 Palmitoleic acid</td>
<td>3.5 (0–4.24)ᵇ</td>
<td>2.9 (0–4.84)ᵇ</td>
<td>2.17 (0–3.58)ᵇ</td>
<td>0.094</td>
</tr>
<tr>
<td>18:1 Oleic acid</td>
<td>18.4 ± 1.5</td>
<td>18.6 ± 1.6</td>
<td>19.0 ± 2.6</td>
<td>0.630</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>37.3 ± 3.1ᵃ</td>
<td>39.8 ± 2.6ᵇ</td>
<td>39.7 ± 4.4ᵇ</td>
<td>0.029</td>
</tr>
<tr>
<td>18:2n-6 Linoleic acid</td>
<td>27.6 ± 2.6</td>
<td>28.2 ± 2.4</td>
<td>29.2 ± 4.0</td>
<td>0.248</td>
</tr>
<tr>
<td>18:3n-6 Linolenic acid</td>
<td>0 (0–0.86)ᵇ</td>
<td>0 (0–0.78)ᵇ</td>
<td>0 (0–0.58)ᵇ</td>
<td>0.549</td>
</tr>
<tr>
<td>18:3n-3 Linolenic acid</td>
<td>0.04 (0–0.54)ᵇ</td>
<td>0 (0–1.68)ᵇ</td>
<td>0.44 (0–1.38)ᵇ</td>
<td>0.069</td>
</tr>
<tr>
<td>20:4n-6 Arachidonic acid</td>
<td>5.96 ± 1.4ᵇ</td>
<td>6.43 ± 1.06ᵇ</td>
<td>5.19 ± 1.24ᵇ</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>20:5n-3 Eicosapentaenoic acid</td>
<td>0.40 (0.18–0.82)ᵇ</td>
<td>0.43 (0.16–0.62)ᵇ</td>
<td>0.36 (0.16–0.86)ᵇ</td>
<td>0.538</td>
</tr>
<tr>
<td>22:6n-3 Docosahexaenoic acid</td>
<td>1.64 (0.13–3.8)ᵇ</td>
<td>2.22 (0.85–4.84)ᵇ</td>
<td>1.83 (0.39–7.83)ᵇ</td>
<td>0.415</td>
</tr>
<tr>
<td>Total n-6 fatty acids</td>
<td>34.8 ± 2.7</td>
<td>37.0 ± 1.8</td>
<td>36.3 ± 4.8</td>
<td>0.129</td>
</tr>
<tr>
<td>Total n-3 fatty acids</td>
<td>2.68 (0.19–4.9)ᵇ</td>
<td>2.08 (1.43–5.79)ᵇ</td>
<td>3.1 (1.3–8.24)ᵇ</td>
<td>0.661</td>
</tr>
<tr>
<td>PUFA:SFA</td>
<td>0.96 ± 0.16</td>
<td>1.05 ± 0.11</td>
<td>1.04 ± 0.19</td>
<td>0.184</td>
</tr>
<tr>
<td>MUFA:SFA</td>
<td>0.56 ± 0.10</td>
<td>0.60 ± 0.09</td>
<td>0.56 ± 0.10</td>
<td>0.411</td>
</tr>
</tbody>
</table>

¹ n = 17. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
² Repeated-measures ANOVA. Values in a row with different superscript letters are significantly different, P < 0.05 (Bonferroni adjustment test for multiple comparisons).
³ ± SD (all such values).
⁴ Tested with log-transformed values.
⁵ Median; range in parentheses (all such values) for variables with non-Gaussian distribution.

**Effects of the diets on serum lipids**

In general, the lipid profile improved after the CD and the LPD (Table 2). Non-HDL cholesterol was lower after the CD and the LPD than after the UD. Triacylglycerol was lower only after the UD. Total cholesterol tended to be reduced after the CD than after the LPD and the UD, and the proportion of stearic acid was lower after the LPD than after the UD (P = 0.099). HDL and LDL cholesterol were not different after the diets.

**Fatty acid composition of serum total lipids after diets**

FA composition of serum total lipids is shown in Table 3. The total PUFA proportion was higher after the CD and the LPD than after the UD. The proportion of palmitic acid was lower after the CD than after the UD and the LPD, and the proportion of stearic acid was lower after the LPD than after the UD.

**Effect of diets on glycemic control, blood pressure measurements, and anthropometric indexes**

No significant difference was observed in glycemic control indexes at the end of each diet as evaluated by fasting plasma glucose and fructosamine (Table 4). Mean blood pressure measurements also did not differ significantly among the diets.

BMI did not differ after the UD and the CD, but it was lower after the LPD (Table 4). No other anthropometric indexes used to evaluate nutritional status differed after the diets.

**DISCUSSION**

In this sample of macroalbuminuric type 2 diabetes patients, the CD and the LPD induced a significant reduction in UAER and...
an increase in the serum proportion of PUFAs. Moreover, an improvement was observed in the lipid profile. The UAER reduction observed in this study in macroalbuminuric type 2 diabetes patients after the LPD is in accordance with previous observations in macroalbuminuric patients with type 1 diabetes (4), which reported an average reduction of 30% in UAER after a LPD. Although less remarkable, the decrease in UAER and serum cholesterol after the CD observed in these macroalbuminuric type 2 diabetes patients extends our previous results in patients with microalbuminuria (7).

The reduction of UAER after the LPD and the CD might be related to the increase in the proportion of serum PUFAs because of the withdrawal of red meat from the diet. It is well known that red meat has a high SFA content; therefore, it is expected that the replacement of red meat by chicken or vegetable protein sources would influence serum FA composition. Red meat consumed by our patients had ≈15% fat, and the composition of this fat was 40% SFAs, 45% MUFAs, and 5% PUFAs. The chicken meat consumed (skinless leg quarter) had a similar fat content (12%); however, the composition of this fat was 26% SFAs, 35% MUFAs, and 24% PUFAs (11). It is also possible that the reduction in UAER after the CD and the LPD would have contributed to the increase in the proportion of serum PUFAs. We have previously shown that type 2 diabetes patients with microalbuminuria had a low proportion of PUFAs in serum triacylglycerols after a standardized diet, which suggests that increased albuminuria may reduce the proportion of serum PUFAs independently of dietary FA intake (14).

The observed increase in the proportion of serum PUFAs and UAER reduction after the withdrawal of red meat reinforces the suggestion that a high proportion of PUFAs intake at the expense of a lower proportion of SFA intake may have a favorable effect on the endothelial function and probably on albuminuria. A low proportion of serum PUFAs has been considered a risk factor for coronary artery disease in middle-aged healthy men (17), and it was also negatively associated with inflammatory markers (18) and endothelial dysfunction (19, 20). In type 2 diabetes, increased UAER, endothelial dysfunction, and chronic inflammation are interrelated processes and are strongly associated with the risk of death (21). Higher serum PUFA and linoleic acid are also associated with lower cardiovascular risk, which is also reflected in overall mortality (22). Moreover, evidence suggests that high proportions of serum linoleic acid decreased the risk of type 2 diabetes, probably by improving insulin resistance (23). We previously showed that metabolic syndrome components were associated with DN in patients with type 2 diabetes (24); therefore, a high proportion of PUFAs may reduce the UAER by improving insulin resistance.

The improvement of the lipid profile after the LPD and the CD, as previously shown in microalbuminuric type 2 diabetes patients (7), may also have contributed to the reduction in the UAER. In fact, lipid reduction by hypolipidemic agents may preserve GFR and decrease proteinuria in diabetes patients (25). The decrease in non-HDL cholesterol was probably related to the lower intake of SFAs and the higher intake of PUFAs, which is known to reduce serum cholesterol (26). The reduction in UAER itself might also have contributed to the improvement of the lipid profile, because there is a positive correlation between albuminuria and non-HDL cholesterol in type 2 diabetes patients with DN (27).

Although BMI reduction occurred after the LPD compared with the UD and the CD, this reduction probably did not influence the UAER results. In fact, no correlation was observed between BMI reduction and UAER reduction after the LPD (data not shown). Furthermore, the effect of weight loss on albuminuria reduction was observed only after much higher reductions in BMI (ie, 16) than were seen in the current study (ie, 0.4) (28).

The favorable effect of the withdrawal of red meat on renal function may also be related to the different amino acid composition of the diets. Some amino acids, especially arginine and glycine, may influence renal function (29), and beef meat contains higher amounts of these amino acids than does chicken meat. However, we had previously observed in type 1 diabetes patients that plasma amino acids did not differ after a diet based on chicken and fish meat compared with a diet mainly of beef (30). Furthermore, other researchers found that fish fat, not just fish protein, could also play a role as a protective factor for the development of microalbuminuria (6). To clarify this aspect, further studies analyzing the effect of diets with different FA composition but with the same protein source should be conducted. In fact, we have ongoing studies designed to analyze whether the beneficial effect of withdrawing the red meat from the diet is related to the protein source or FA composition. However, the only dietary recommendation that could be made now to the patients, according to the results of the current study, is to restrict the intake of beef.

Possible limitations of this study were the possibility of a carry-over effect, diet compliance, nonuse of renoprotective medications, measurement of FAs in serum total lipids, and the relatively high proportion of patients not included in the study. The possible carry-over effect probably did not occur because the diets had a 4-wk washout period between them, and this seems to be enough time to wash out from the previous diet. Moreover, plasma lipids and lipoproteins reach stable values after a period of 3–4 wk of dietary modification (31). Compliance with the diets was probably adequate, because its assessment by the weighed diet records method and urea measurements performed during each diet showed a good correlation between these 2 tools. As for the use of renoprotective agents, when this study was designed, the use of angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) was not as firmly established as it currently is, and these medications were not provided to the patients by our local health system. We do not think that this is an important limitation in the study, because no obvious reason was observed that these findings would not be applicable in patients using these medications. Furthermore, this dietary intervention may be useful for type 2 diabetes patients for whom ACE inhibitors or ARBs are not tolerated (3). Another point to consider was the measurement of FAs on total lipids, because their relative degree of saturation varies among cholesterol esters, phospholipids, and triacylglycerols. Therefore, FA measurement on total lipids could be influenced by the variation in each lipid fraction. In the present study, the decrease in triacylglycerol after the CD might have influenced the increase of PUFAs after this diet. However, it is logical to assume that the increased dietary intake of PUFAs would have caused the increased proportion of serum PUFAs, because this was also observed after the LPD that was not associated with triacylglycerol reduction. Finally, the relatively high number of patients excluded because they did not fulfill the selection criteria and the demanding dietary intervention was expected in studies of this.
nature. Moreover, the low drop-out rate observed after random assignment reinforces the meaning of the results.

In conclusion, these results indicate that the withdrawal of red meat from the diet, either by replacing it with chicken or by following a lactovegetarian LPD, promotes a beneficial effect on renovascular and cardiovascular risk factors associated with DN in patients with type 2 diabetes and macroalbuminuria. This benefit may be related to the concomitant reduction in albuminuria concentrations and the rise in serum PUFAs.

VDFdM collected the data, analyzed and interpreted the data, and wrote the manuscript; TZ collected and analyzed the data and gave significant advice about the study design; MSP analyzed and interpreted the data; MJA designed the study, analyzed the data, and wrote and critically reviewed the manuscript. None of the authors had a conflict of interest.

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