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Article type: Short communication

Received: January 25, 2020.

Accepted: April 7, 2020.

Published online: April 8, 2020.

ISSN: 0022-9032

e-ISSN: 1897-4279

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Serum phospholipid cis-palmitoleic acid in type 2 diabetic patients with chronic coronary syndrome: an assessment of the relationship with diabetes duration, systemic low-grade inflammation and circulating oxLDL

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Short title:
cis-Palmitoleic acid in type 2 diabetes and chronic coronary syndrome

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Conflict of interest: none declared

The preliminary results of this study were presented at the European Society of Cardiology (ESC) Congress 2018, Munich, Germany (P2532).
INTRODUCTION

There is increasing evidence that *cis*-palmitoleic acid (POA, *cis*-C16:1n-7), a n-7 monounsaturated fatty acid (MUFA) mainly produced by desaturation of palmitic acid via stearoyl-CoA desaturase-1 (SCD1, EC 1.14.99.5) can act as a lipokine and influence systemic metabolism [1-5]. Numerous experimental studies have shown the beneficial effects of a POA on the mechanisms underlying type 2 diabetes (T2D) and atherosclerotic cardiovascular disease (ASCVD) [1,2,5]. However, data from clinical studies are not conclusive [1,2,5,6]. Little is known about the role of POA in the pathogenesis of T2D complications, including diabetic macroangiopathy.

The aim of this study was to assess the association of POA concentration in serum phospholipids with diabetes duration, systemic inflammation and circulating oxidized low-density lipoprotein (oxLDL) in T2D patients with chronic coronary syndrome (CCS) and angiographically proven ASCVD.

METHODS

The study design is described in detail elsewhere [7]. Briefly, 74 patients, including 26 (35.1%) females, aged 65.6 ± 6.8 years, with T2D (median diabetes duration 10 years) and CCS were prospectively enrolled in the study. All subjects had angiographically documented ASCVD, defined as coronary artery disease (CAD) (74 patients) or peripheral arterial disease (26 patients). The study protocol was approved by the University Ethics Committee.

All laboratory tests were performed prior to randomization of patients to the n-3 PUFAs or placebo arm. Standard assay techniques were used in routine laboratory investigations. HbA1C was estimated using turbidimetric inhibition immunoassay. High-sensitivity C-reactive protein (hsCRP) was measured by latex nephelometry (Dade Behring, Marburg, Germany). The serum levels of tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) were evaluated by ELISA (R&D Systems, USA).
Leptin and adiponectin levels were measured by radioimmunoassay kits (DIAsource, Belgium). Insulin levels were assessed using the chemiluminescent immunoassay method (Advia Centaur, Siemens Healthcare, USA). The measurement of circulating peptide C was performed using radioimmunoassay kits (DIAsource, Belgium). The concentration of oxLDL was measured by ELISA (Immundiagnostik AG, Bensheim, Germany).

Serum phospholipid fatty acids were evaluated with gas chromatography (Agilent Technologies 6890N Network GC Systems, Wilmington, De., USA). SCD1 activity was estimated as the ratios of POA to palmitic acid (C16:0) and oleic acid (C18:1n-9) to stearic acid (C18:0), as previously described [1-5]. According to the median value of POA the study participants were grouped into those with POA < 14.9 µmol/L (n = 37) and those with POA ≥ 14.9 µmol/L (n = 37).

**Statistical analysis**

Data were presented as mean (SD) or median (interquartile range [IQR]), as appropriate. Normality was checked using the Shapiro-Wilk test. The Student’s t-test or the Mann-Whitney test were used to assess differences between 2 groups as appropriate. Categorical variables were analyzed using the χ² test or the Fisher exact tests. Correlations were calculated with the Spearman’s Rho correlation coefficient. Stepwise linear regression analysis was performed for determining the independent predictors of serum phospholipid POA. Two-sided P-values < 0.05 were considered statistically significant. Statistical analyses were performed using STATISTICA version 13 (Statsoft Inc, Tulsa, Oklahoma, United States).

**RESULTS AND DISCUSSION**

Individuals with POA < 14.9 µmol/L, compared to patients with POA ≥ 14.9 µmol/L had similar clinical and demographic characteristics, except for longer diabetes duration (11 [8-20] vs 8 [5-10] years; P = 0.01) and lower prevalence of obesity (54.1 vs 78.4%; P = 0.03)
Patients with POA ≥ 14.9 µmol/L had higher levels of insulin, C-peptide, triglycerides and estimated SCD1 activity (Table 1). Moreover, patients with higher POA levels had lower plasma concentration of oxLDL, adiponectin and adiponectin-to-leptin ratio (Table 1). There were no intergroup differences in levels of HbA1c, leptin, total cholesterol, LDL-C, HDL-C, and systemic inflammatory markers (IL-6, TNFα, and hsCRP) (Table 1).

Our study showed that POA was inversely correlated with diabetes duration ($r = -0.29; P = 0.02$) and circulating oxLDL ($r = -0.29; P = 0.02$) and was positively correlated with estimated SCD1 activity ($r = 0.43; P < 0.001$) (Suppl. Table 2). Multivariable linear regression analysis demonstrated that SCD1 activity ($\beta = 0.34; 95\% \text{ CI} 0.13-0.55; P = 0.002$), LDL-C ($\beta = 0.35; 95\% \text{ CI} 0.14-0.56; P = 0.001$) and oxLDL ($\beta = -0.22; 95\% \text{ CI} (-0.43)-(-0.02); P = 0.048$) were independently associated with serum phospholipid POA.

The most important finding of our study is that concentration of POA in serum phospholipids of T2D patients with CCS was related to diabetes duration and circulating oxLDL, although the strength of these associations was of modest magnitude. No significant relationship was found between serum phospholipid POA and biomarkers of systemic inflammation.

Experimental and clinical studies showed that MUFAs have multiple beneficial effects on cardiovascular health and glucose homeostasis [6]. Although the POA content in the average Western diet is low or very low, POA is the second most widespread MUFA, after oleic acid, in fatty tissue and serum phospholipids [1-5]. It has been shown that the content of POA in serum phospholipids depends mainly on the hepatic activity of SCD1 [5]. Furthermore, the $cis$-C16:1n-7/C16:0 index has been shown to better reflect the liver SCD1 activity than the C18:1n-9/C18:0 index [4].

Our study showed an inverse relationship between serum phospholipid POA and circulating oxLDL. This finding is novel and may have important implications for
understanding the beneficial effects of n-7 MUFAs in T2D patients. There is convincing evidence that oxLDL, a strong natural prooxidant derived from native LDL through cellular oxidation, is an early marker of systemic oxidative stress, involved in the pathophysiology of T2D and ASCVD [8,9]. The susceptibility of LDL to oxidation depends mainly on the content of unsaturated fatty acids, especially PUFAs, and antioxidants [10]. Unfortunately, there have been few nutritional trials to date assessing the effect of MUFAs on LDL oxidation in T2D patients, and existing ones have compared such intervention only with a high-carbohydrate diet [10]. The results of these studies, however, are inconclusive [10].

We also found that POA content in serum phospholipids of the study patients is inversely associated with diabetes duration. The Framingham Heart Study showed that duration of T2D was positively associated with the risk of CAD mortality [9]. The relationship between POA concentration in serum phospholipids and diabetes duration seems to be an interesting finding that could be due to alteration in hepatic SCD1 activity in T2D subjects, especially those with longer duration of disease. It has recently been confirmed that in T2D patients, SCD1 mRNA was fivefold and protein expression twofold lower compared to healthy subjects, which may result in altered levels of MUFAs in serum phospholipids [11].

Although several studies have shown the beneficial effects of a MUFA-rich diet on glycometabolic control and cardiovascular risk in T2D patients, data on MUFA effects on systemic inflammation are limited and inconclusive [10]. Importantly, most studies were conducted in experimental models, healthy subjects, or individuals with hypertension, rather than T2D patients [10]. In addition, the main dietary MUFA in most of the cited studies was oleic acid rather than POA [10]. There is growing evidence that individual fatty acids within one class can have different effects on systemic inflammation [12]. In our study, no significant association was found between serum phospholipid POA and inflammatory biomarkers. This finding confirms the complexity of the interaction between endogenously
synthesized POA, hepatic SCD1-mediated Δ9-desaturation and chronic low-grade inflammation in T2D patients with CCS. Further studies are needed on the role of stearoyl-CoA desaturase, its isoforms and Δ9-desaturation products in lipogenic tissues in the pathophysiology of T2D and its complications in humans.

Limitations

Our study had several limitations. First, the cross-sectional nature of the study did not allow us to infer causality. Second, the dietary fat intake including POA was not assessed precisely. Finally, the sample size was relatively small and a larger sample would have provided more robust findings.
References


Table 1. Glycometabolic status, estimated stearoyl-CoA desaturase activity and inflammatory markers in the study patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>n = 74</th>
<th>POA &lt; 14.9 µmol/l</th>
<th>POA ≥ 14.9 µmol/l</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n = 37</td>
<td>n = 37</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.0 (6.6; 7.5)</td>
<td>7.1 (6.7; 7.5)</td>
<td>7.0 (6.6; 7.4)</td>
<td>0.77</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>21.5 (14.6; 33.6)</td>
<td>17.6 (12.1; 25.2)</td>
<td>24.5 (17.1; 35.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>C-peptide (ng/mL)</td>
<td>3.25 (1.40)</td>
<td>2.89 (1.32)</td>
<td>3.60 (1.41)</td>
<td>0.03</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>3.86 (0.91)</td>
<td>3.66 (0.76)</td>
<td>4.06 (1.01)</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>1.91 (1.53; 2.64)</td>
<td>1.77 (1.53; 2.48)</td>
<td>2.16 (1.61; 3.03)</td>
<td>0.11</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.24 (0.38)</td>
<td>1.22 (0.37)</td>
<td>1.26 (0.40)</td>
<td>0.75</td>
</tr>
<tr>
<td>Tg (mmol/L)</td>
<td>1.35 (1.12; 1.92)</td>
<td>1.28 (0.96; 1.54)</td>
<td>1.65 (1.19; 2.47)</td>
<td>0.01</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>83.7 (22.0)</td>
<td>85.2 (27.3)</td>
<td>82.2 (15.6)</td>
<td>0.60</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>78.3 (70.0; 90.0)</td>
<td>78.0 (67.0; 90.0)</td>
<td>86.4 (72.0; 90.0)</td>
<td>0.31</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>1.54 (0.73; 2.71)</td>
<td>1.47 (0.66; 1.99)</td>
<td>1.84 (0.82; 3.10)</td>
<td>0.23</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.99 (1.55; 2.79)</td>
<td>1.82 (1.41; 2.50)</td>
<td>2.11 (1.64; 3.07)</td>
<td>0.11</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>1.48 (1.28; 1.76)</td>
<td>1.48 (1.26; 1.82)</td>
<td>1.48 (1.29; 1.68)</td>
<td>0.79</td>
</tr>
<tr>
<td>ox-LDL (ng/mL)</td>
<td>58.20 (35.40; 128.40)</td>
<td>77.10 (42.10; 175.70)</td>
<td>44.30 (28.10; 79.80)</td>
<td>0.01</td>
</tr>
<tr>
<td>ox-LDL/LDL-C ratio (µg/mmol)</td>
<td>26.90 (18.13; 71.90)</td>
<td>47.82 (22.31; 115.28)</td>
<td>21.59 (11.31; 42.40)</td>
<td>0.01</td>
</tr>
<tr>
<td>ox-LDL/TC ratio (µg/mmol)</td>
<td>14.85 (9.31; 36.20)</td>
<td>25.90 (12.08; 54.11)</td>
<td>11.58 (6.44; 19.46)</td>
<td>0.01</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>3.74 (2.85; 4.82)</td>
<td>4.07 (3.43; 5.84)</td>
<td>3.26 (2.64; 4.00)</td>
<td>0.02</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>3.76 (2.02; 8.48)</td>
<td>3.45 (1.81; 7.05)</td>
<td>4.80 (2.04; 11.04)</td>
<td>0.13</td>
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<tr>
<td>Adiponectin-leptin ratio</td>
<td>0.82 (0.44; 2.49)</td>
<td>1.27 (0.55; 3.12)</td>
<td>0.59 (0.38; 1.51)</td>
<td>0.02</td>
</tr>
<tr>
<td>(µg/ng)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-C16:1n-7/C16:0 index</td>
<td>0.02 (0.01; 0.02)</td>
<td>0.01 (0.01; 0.02)</td>
<td>0.02 (0.02; 0.03)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C18:1n-9/C18:0 index</td>
<td>0.67 (0.59; 0.76)</td>
<td>0.63 (0.58; 0.71)</td>
<td>0.72 (0.65; 0.80)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Data are given as number (percentage) for categorical variables and mean (standard deviation) or median (IQR) for continuous variables.

Abbreviations: eGFR, estimated glomerular filtration rate calculated by the abbreviated MDRD equation; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; ox-LDL, oxidized low-density lipoprotein; POA, cis-palmitoleic acid; TC, total cholesterol; Tg, triglycerides; TNFα, tumor necrosis factor alpha.