SHORT COMMUNICATION

Serum phospholipid *cis*-palmitoleic acid in patients with type 2 diabetes and chronic coronary syndrome

An assessment of the relationship with diabetes duration, systemic low-grade inflammation, and circulating oxidized low-density lipoprotein

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Introduction There is a growing body of 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; KEGG database, EC 1.14.99.5) can act as a lipokine and influences systemic metabolism.[1-5] Numerous experimental studies have shown the beneficial effects of 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 patients with T2D and chronic coronary syndrome (CCS) and angiographically proven ASCVD.

Methods The study design is described in detail elsewhere.[7] Briefly, 74 patients, including 26 women (35.1%), with a mean (SD) age of 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 (74 patients) or peripheral arterial disease (26 patients). The study protocol was approved by the university ethics committee. Participants provided written informed consent.

All laboratory tests were performed prior to randomization of patients to the n-3 polyunsaturated fatty acids (n-3 PUFAs) or placebo arm. Standard assay techniques were used in routine laboratory investigations. Glycated hemoglobin (HbA_{1C}) was estimated using turbidimetric inhibition immunoassay. High-sensitivity C-reactive protein was measured by latex nephelometry (Dade Behring, Marburg, Germany). The serum levels of tumor necrosis factor α and interleukin 6 were evaluated by enzyme-linked immunosorbent assays (ELISA; R&D Systems, Minneapolis, Minnesota, United States).

Leptin and adiponectin levels were measured by radioimmunoassay kits (DIAsource, Louvain-la-Neuve, Belgium). Insulin levels were assessed using the chemiluminescent immunoassay method (Advia Centaur, Siemens Healthcare, Camberley, Surrey, United Kingdom). The measurement of circulating peptide C was performed using radioimmunoassay kits (DIAsource). 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, Delaware, United States). SCD1 activity was estimated as the ratios of POA to palmitic

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acid (C16:0) and oleic acid (C18:1n-9) to stearic acid (C18:0), as previously described.[1-5] Based on the median value of POA, the study participants were grouped into those with POA lower than 14.9 µmol/l (n = 37) and those with POA of 14.9 µmol/l or greater (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 *t* test or the Mann–Whitney test were used to assess differences between 2 groups as appropriate. Categorical variables were analyzed using the χ^2 test or the Fisher exact test. Correlations were calculated with the Spearman rank correlation analysis. Stepwise linear regression analysis was performed for determining the independent predictors of serum phospholipid POA. A 2-tailed *P* value of less than 0.05 was considered significant. Statistical analyses were performed using STATISTICA version 13 (Statsoft Inc., Tulsa, Oklahoma, United States).

Results and discussion Individuals with POA less than 14.9 µmol/l, compared with patients with POA of 14.9 µmol/l or greater had similar clinical and demographic characteristics, except for longer diabetes duration (median [IQR], 11 [8–20] vs 8 [5–10] years; *P* = 0.01) and lower prevalence of obesity (54.1% vs 78.4%; *P* = 0.03) (Supplementary material, *Table S1*). Patients with POA of 14.9 µmol/l or greater had higher levels of insulin, C-peptide, triglycerides, and estimated SCD1 activity (TABLE 1). Moreover, patients with higher POA levels had lower plasma concentrations of oxLDL, adiponectin, and ratio of adiponectin to leptin (TABLE 1). There were no intergroup differences in levels of HbA_{1c}, leptin, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, and systemic inflammatory markers (interleukin 6, tumor necrosis factor α, and high-sensitivity C-reactive protein) (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) (Supplmentary material, *Table S2*). Multivariable linear regression analysis demonstrated that SCD1 activity (β = 0.34; 95% CI, 0.13–0.55; *P* = 0.002), LDL cholesterol (β = 0.35; 95% CI 0.14–0.56; *P* = 0.001), and oxLDL (β = –0.22; 95% CI, –0.43 to –0.02; *P* = 0.048) were independently associated with serum phospholipid POA.

The most important finding of our study is that a concentration of POA in serum phospholipids of patients with T2D and CCS was related to diabetes duration and circulating oxLDL, although the associations were 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 to C16:0 index (POA to palmitic acid ratio) has been shown to better reflect the liver SCD1 activity than the C18:1n-9 to C18:0 index (oleic acid to stearic acid ratio).[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 patients with T2D. 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 patients with T2D, 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 mortality associated with coronary artery disease.[9] The relationship between POA concentration in serum phospholipids and diabetes duration seems to be an interesting finding that could be due to alteration to hepatic SCD1 activity in patients with T2D, especially those with longer history of disease. It has recently been confirmed that in patients with T2D, SCD1 mRNA was 5-fold lower and protein expression 2-fold lower compared with healthy subjects, which may lead to 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 patients with T2D, 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 patients with T2D.[10] In addition, the main dietary MUFA in most of the cited studies was oleic acid rather than

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

Variable	Total (n = 74)	POA <14.9 µmol/l (n = 37)	POA ≥14.9 µmol/l (n = 37)	*P* value
HbA_{1c}, %	7 (6.6–7.5)	7.1 (6.7–7.5)	7 (6.6–7.4)	0.77
Insulin, µIU/ml	21.5 (14.6–33.6)	17.6 (12.1–25.2)	24.5 (17.1–35.3)	0.04
C-peptide, ng/ml	3.25 (1.4)	2.89 (1.32)	3.60 (1.41)	0.03
TC, mmol/l	3.86 (0.91)	3.66 (0.76)	4.06 (1.01)	0.06
LDL-C, mmol/l	1.91 (1.53–2.64)	1.77 (1.53–2.48)	2.16 (1.61–3.03)	0.11
HDL-C, mmol/l	1.24 (0.38)	1.22 (0.37)	1.26 (0.4)	0.75
Tg, mmol/l	1.35 (1.12–1.92)	1.28 (0.96–1.54)	1.65 (1.19–2.47)	0.01
Creatinine, µmol/l	83.7 (22)	85.2 (27.3)	82.2 (15.6)	0.6
eGFR, ml/min/1.73 m^{2a}	78.3 (70–90)	78 (67–90)	86.4 (72–90)	0.31
hsCRP, mg/l	1.54 (0.73–2.71)	1.47 (0.66–1.99)	1.84 (0.82–3.1)	0.23
IL-6, pg/ml	1.99 (1.55–2.79)	1.82 (1.41–2.5)	2.11 (1.64–3.07)	0.11
TNFα, pg/ml	1.48 (1.28–1.76)	1.48 (1.26–1.82)	1.48 (1.29–1.68)	0.79
oxLDL, ng/ml	58.2 (35.4–128.4)	77.1 (42.1–175.7)	44.3 (28.1–79.8)	0.01
oxLDL to LDL-C ratio, µg/mmol	26.9 (18.13–71.9)	47.82 (22.31–115.28)	21.59 (11.31–42.4)	0.01
oxLDL to TC ratio, µg/mmol	14.85 (9.31–36.2)	25.9 (12.08–54.11)	11.58 (6.44–19.46)	0.01
Adiponectin, µg/ml	3.74 (2.85–4.82)	4.07 (3.43–5.84)	3.26 (2.64–4)	0.02
Leptin, ng/ml	3.76 (2.02–8.48)	3.45 (1.81–7.05)	4.8 (2.04–11.04)	0.13
Adiponectin to leptin ratio, µg/ng	0.82 (0.44–2.49)	1.27 (0.55–3.12)	0.59 (0.38–1.51)	0.02
cis-C16:1n-7 to C16:0 index	0.016 (0.013–0.021)	0.014 (0.012–0.015)	0.021 (0.018–0.026)	<0.001
C18:1n-9 to C18:0 index	0.671 (0.588–0.759)	0.626 (0.582–0.712)	0.724 (0.654–0.804)	0.005

Data are presented as median (IQR) or mean (SD).

a Calculated by the abbreviated Modification of Diet in Renal Disease equation

Abbreviations: eGFR, estimated glomerular filtration rate; HbA_{1c}, 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 α

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 patients with T2D and 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 design 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.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/kardiologiapolska.

ARTICLE INFORMATION

NOTE The preliminary results of this study were presented at the European Society of Cardiology (ESC) Congress on August 25 to 29, 2018 in Munich, Germany (P2532) .

CONFLICT OF INTEREST None declared.

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