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Serum palmitoleate acts as a lipokine in subjects at high cardiometabolic risk

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Abstract *Background and aim:* Clinical data on the role as a lipokine of *de novo* lipogenesis-derived palmitoleic acid (C16:1n-7*cis*) in serum non-esterified fatty acids (palmitoleate) are scarce. We aimed to assess whether palmitoleate relates to cardiometabolic risk.
Methods and results: In this cross-sectional study we included 358 individuals aged 30–65-years at high cardiovascular risk. We tested the association of palmitoleate (determined by gas chromatography) with metabolic syndrome (MS) and its components (defined by ATPIII criteria), fatty liver index (a surrogate of non-alcoholic fatty liver disease [NAFLD]), and subclinical atherosclerosis (determined as ultrasound-measured carotid intima-media thickness and arterial stiffness). Palmitoleate concentration was higher in women compared with men (median ± range interquartile, 1.36 ± 0.96 vs. 0.97 ± 0.77 μmol/L respectively, $P < 0.001$). In both genders palmitoleate concentration was associated with a higher prevalence of MS: men, odds ratio [OR: 1.12 (95%CI: 1.03; 1.23, $P = 0.010$)]; women [OR: 1.07 (95%CI: 1.03; 1.13, $P = 0.005$)], and all of its components except low HDL-cholesterol and hypertriglyceridemia. Palmitoleate was also associated with increased risk of NAFLD in both men [OR: 1.12 (95%CI: 1.03; 1.29, $P = 0.031$)] and women [OR: 1.11 (95%CI: 1.05; 1.19, $P = 0.001$)]. No associations with subclinical atherosclerosis were detected.
Conclusions: Our observational data supports a relationship between *de novo* lipogenesis-derived circulating palmitoleic acid (palmitoleate) and increased cardiometabolic risk.

Introduction

Increasing evidence supports the observation that compounds released from adipose tissue are involved in systemic carbohydrate and lipid homeostasis. Despite its marginal presence in dietary fats, palmitoleic acid (POA, C16:1n-7*cis*) is the fourth most abundant fatty acid in adipose tissue [1], which suggests that its main source is *de novo* lipogenesis. POA accrues in subcutaneous

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gluteofemoral and upper arm fat depots rather than in the abdominal region [reviewed in 2]. It is believed that such compartmentalisation facilitates a rapid mobilisation of this fatty acid [3], which prompted the notion of POA as a lipokine [4]. POA released from subcutaneous adipose tissue accrues in the plasma non-esterified fatty acid (NEFA) [2].

Clinical data regarding circulating POA in serum NEFA (henceforth palmitoleate) and cardiometabolic health are scarce and controversial, since palmitoleate has suggested to be associated with increased insulin sensitivity in healthy subjects [3,5], but not in obese subjects [6] or patients with type-1 diabetes [7]. A direct association between palmitoleate and nonalcoholic steatohepatitis (a condition linked to insulin resistance) has also been reported [8]. In the present study, we hypothesised that palmitoleate in subjects at increased cardiometabolic risk would relate to the prevalence of metabolic syndrome (MS) and its components, as well as vascular function, and non-alcoholic fatty liver disease (NAFLD).

Methods

Setting

For this cross-sectional study, we recruited 358 individuals at increased cardiometabolic risk attending the Vascular Medicine and Metabolism Unit of our University Hospital. Subjects with chronic lung, renal disease, cancer or any other serious condition were excluded. Data from a complete clinical history and physical examination, including anthropometric measurements, lifestyle variables, and medication use were recorded. Standard biochemical profiles and fatty acid concentrations were measured in fasting plasma after at least a 6 wk washout of lipid-lowering medication (8 wk in the case of fibrates). The study protocol was approved by the ethics committee of the institution and was conducted according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Clinical assessment

Participants were considered diabetic, hyperlipidaemic or hypertensive if they had a previous diagnosis of these conditions and/or they were treated with antidiabetic, hypolipidaemic, or antihypertensive agents, respectively. Smoking status was categorised into never, current or past smoking according to self-reports. Height, weight, and waist circumference were measured with standard methods. Trained personnel measured systolic and diastolic blood pressure with a validated semi-automatic oscillometer (Omron HEM-705CP; Hoofddorp, The Netherlands) in triplicate with a 5-min interval between each measurement, and the mean of these values was recorded. The presence of MS was defined by Adult Treatment Panel III criteria [9].

Laboratory determinations

Fasting blood was immediately centrifuged at 1500 *g* for 15 min at 4° C, and plasma and serum samples were stored at −80 °C until analysis. Total serum lipids were extracted with a mixture of chloroform/methanol (2:1 v/v) and dried under N_2. Palmitoleate (along with other serum NEFA) was isolated by solid-phase extraction as described [10]. Fatty acid methyl esters were prepared by incubation with acidified methanol and were separated by gas chromatography with an Agilent 7890 Gas Chromatograph HP 6890 equipped with a 30 m × 0.25 μm × 0.25 mm SupraWAX-280 capillary column (Teknokroma, Barcelona, Spain), an autosampler, and flame ionisation detection. The intraassay CV for palmitoleate detection (n = 10 replicates) was 1.2. The standard biochemical parameters, lipids, apolipoproteins, and high-sensitive C-reactive protein (CRP) were measured using colorimetric, enzymatic and immunoturbidimetric assays (Spinreact, SA, Spain; Wako Chemicals GmbH, Germany; Polymedco, NY, US) adapted to a Cobas Mira Plus autoanalyzer (Roche Diagnostics, Spain). Plasma glycerol was measured using a commercial kit (Zen-Bio, Inc., NC, US). Fatty acid binding proteins 4 (FABP4), adiponectin, retinol binding protein 4 (RBP4) and insulin levels were determined using commercial ELISA kits (BioVendor Laboratory Medicine Inc., Brno, Czech Republic; R&D Systems, MN, US; RayBiotech, Inc, GA, US; and Mercodia AB, Uppsala, Sweden; AdipoGen Inc., Seoul, Korea). Cholesteryl ester transfer protein (CETP) and lecithin-cholesterol acyltransferase (LCAT) activities were measured using fluorometric assays (BioVision, CA, US and Calbiochem, CA, US, respectively).

Measurement of subclinical atherosclerosis

A MyLab X-50 sonographer (Esaote, Genoa, Italy) equipped with a linear array ultrasound probe (7.5–12 MHz small parts broadband transducer) was used for carotid intima-media thickness (IMT) measurement of the right and left far wall of common carotid arteries, carotid bifurcations and internal carotid arteries [11]. The mean of these six determinations defined the mean IMT (n = 120). Arterial wall functional properties were measured with the QAS software System and a LA533 linear transducer (Quality Arterial Stiffness software). Examination was performed according to a standard protocol [12]. Arterial distensibility, augmentation index and pulse wave velocity were measured directly at the right and left common carotid arteries and were adjusted for arterial pressure, age, gender for pulse wave velocity (PWV) and heart rate and gender for augmentation index (AIx75).

Abdominal fat distribution

A MyLab 50 X-Vision ultrasonographer was used to assess abdominal fat distribution according to the ultrasound image review consensus [13]. Briefly, thicknesses of both abdominal subcutaneous fat and preperitoneal fat were measured by placing the probe perpendicular to the skin in the epigastrium. The thickness of abdominal