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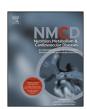
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# Adipose tissue palmitoleic acid is inversely associated with nonfatal acute myocardial infarction in Costa Rican adults

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#### **KEYWORDS**

Costa Rican adults; Nonfatal acute myocardial infarction; Adipose tissue palmitoleic acid; Metabolic syndrome; HDL cholesterol; Case—control study **Abstract** *Background and aims*: Animal models have shown that adipose-derived palmitoleic acid may act as a lipokine by conferring resistance to diet-induced obesity; however, human epidemiologic studies investigating this relationship thus far have not provided data in support of this hypothesis. Because metabolic syndrome and cardiovascular disease are intricately linked with the former being a major risk factor for the latter, we hypothesized that adipose-derived palmitoleic acid may be inversely associated with myocardial infarction. We examined whether adipose tissue palmitoleic acid was associated with nonfatal acute myocardial infarction in a representative population of Costa Rican adults.

Methods and results: Odds ratios of nonfatal acute myocardial infarction by quintiles of adipose tissue palmitoleic acid were calculated using conditional logistic regression in a case—control study of 1828 cases and 1828 controls matched by age, sex, and area of residence. We observed an inverse relationship between nonfatal acute myocardial infarction and adipose tissue palmitoleic acid (OR for highest quintile compared to lowest quintile of palmitoleic acid: 0.55; 95% CI: 0.41, 0.75; *P* for trend: <0.0001). We additionally observed a significant positive association between adipose tissue palmitoleic acid and high-density lipoprotein cholesterol.

Conclusion: These data demonstrate an inverse association between adipose tissue palmitoleic acid and nonfatal acute myocardial infarction; however, further research is required in order to better understand the opposing associations between palmitoleic acid and high-density lipoprotein cholesterol and systolic blood pressure.

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#### Introduction

Cardiovascular disease (CVD) has become one of the leading causes of early mortality among adults over the past few decades. In 2012, CVD accounted for approximately 46.2% of mortality from non-communicable diseases or 17 million deaths, globally [1]. With the global

burden of CVD projected to rise to more than 23.6 million deaths by the year 2030, recent research has focused on identifying mechanisms and understanding risk factors for CVD in an effort to design more effective interventions for its treatment [2]. In particular, investigations into the associations between fatty acids and CVD have become increasingly common for a variety of reasons, one of which

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being a direct translation to intervention [3-6]. In the past decade, research has increasingly focused on palmitoleic acid, a minor monounsaturated fatty acid; in 2008, Cao et al. identified a potential role of palmitoleic acid as a lipokine, a role which they identified via systemic lipid profiling of mice deficient in adipose tissue lipid chaperones aP2 and mal1 [7]. Cao et al. demonstrated the ability of adipose tissue palmitoleic acid to improve muscle insulin signaling and suppress hepatosteatosis, thereby reversing two hallmarks of metabolic syndrome. A protective effect on metabolic syndrome may translate to a similarly protective effect on CVD due to the former being a risk factor for the latter [8]. However, despite these data in mice, evidence in human epidemiologic studies is sparse and controversial. With regards to metabolic syndrome, most data do not support the role of palmitoleic acid as a lipokine [9-11]. For CVD, the data are even less conclusive [12–14]. This research study aims to investigate the relationship between adipose tissue palmitoleic acid and nonfatal acute myocardial infarction (MI) in a large population-based case-control study of Costa Rican adults. This study also aims to evaluate effect modification by sex and assess potential mediators in the relationship between adipose tissue palmitoleic acid and MI.

#### Methods

#### Study population

The subjects in this study were cases of nonfatal acute MI identified in Costa Rica between 1994 and 2004. The catchment area of the study, the Central Valley of Costa Rica, included 34 counties and covered a full range of lifestyles and socioeconomic characteristics. Full details of the study design are provided elsewhere [15]. Briefly, cases were adults who were diagnosed as survivors of a first acute MI by independent examinations of two cardiologists at any of the six recruiting hospitals in the catchment area. All cases met the World Health Organization (WHO) criteria for MI, which includes typical symptoms as well as either elevation in cardiac enzyme concentrations or diagnostic changes in the electrocardiogram [16]. For each eligible case, one population-based control was randomly identified from the underlying source population using data from the National Census and Statistics Bureau of Costa Rica. Controls were matched to cases by age ( $\pm 5$ years), sex, and area of residence (county). All subjects provided written informed consent to participate in the study. This study was approved by the Ethics Committee of the Harvard School of Public Health and the National Institute of Health Research at the University of Costa Rica.

#### Data collection

Information on sociodemographic characteristics and medical histories were collected by trained personnel at the homes of each subject via a questionnaire with closed-ended questions. Anthropometric measurements were collected of subjects in light clothing and without shoes.

All measurements were performed in duplicate, with the average of the two measurements used in data analyses. A bathroom scale (Detecto, Webb City, MO) or a Seca Alpha Model 770 digital scale (Seca, Hanover, MD) accurate to 50 g and a steel anthropometer were used to measure weight and height, respectively. The scales were calibrated biweekly. Self-reported dietary intake was collected via a food-frequency questionnaire developed and validated to reflect fatty acid intake in the Costa Rican population [17]. Biological samples were collected in the subject's home in the morning after an overnight fast. A subcutaneous adipose tissue biopsy was also collected from the upper buttock with a 16-gauge needle and disposable syringe, based on protocols established elsewhere [18]. The tissue specimens were stored in liquid nitrogen tanks at −80 °C for subsequent fatty acid analysis.

#### Fatty acid analysis

Fatty acids from adipose tissue biopsy specimens were quantified by gas—liquid chromatography as previously described [19]. Peak retention times and area percentages of total fatty acids were identified by injecting known standards (NuChek Prep, Elysian, MN) and analyzed with Agilent Technologies ChemStationA.08.03 software (Agilent Technologies, Santa Clara, CA). Twelve duplicate samples, which were indistinguishable from the others, were analyzed throughout the study. The CV for palmitoleic acid was 5.7%.

#### Data analysis

The original sample consisted of 4547 subjects, of whom 2274 were controls and 2273 were cases. After removing subjects with missing information on adipose tissue palmitoleic acid concentrations (n = 732) or confounding variables (n = 34) included in the fully adjusted model (with the exception of income, which was handled via multiple imputation), and subjects who could not be rematched after the aforementioned exclusions (n = 125), 3656 subjects remained, with 1828 cases and 1828 controls matched by age, sex, and area of residence. McNemar's tests were used to assess differences in baseline characteristics between cases and controls for binary variables. Paired t-tests and Wilcoxon signed rank tests were used to assess differences between cases and controls for normally distributed and nonnormally distributed continuous variables, respectively. Multiple imputations were performed on missing data for monthly household income (n = 258; 7.1% of the entire sample), using a multiple Markov chain Monte Carlo method assuming missing at random. Variables used to predict missing income data included palmitoleic acid concentration, current smoking status, histories of diabetes and hypertension, and adipose tissue oleic, linoleic, arachidonic, and alpha-linolenic fatty acid concentrations. Conditional logistic regression models were fit to estimate odds ratios (ORs) and 95% confidence intervals (95% CI) of nonfatal acute MI across quintiles of adipose tissue palmitoleic acid concentrations. Quintiles were used in order to examine non-

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