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Comparison of predictive performance of various fatty acids for the risk of cardiovascular disease events and all-cause deaths in a community-based cohort

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ABSTRACT

Background: The issue of whether saturated fats and trans fats are superior predictors of all-cause death and cardiovascular disease than n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), remains a matter of contention. Furthermore, few studies have examined the relationship between fatty acids and the outcomes of cardiovascular disease (CVD) in Asian populations. The aim of this study was to compare the effectiveness of various plasma fatty acids as predictors for all-cause death and CVD events in an ethnic Chinese population.

Methods: This study assembled a community-based prospective cohort, comprising 1833 participants (60.6 ± 10.5 yrs, 44.5% women) who underwent a comprehensive evaluation of fatty acids in blood using gas chromatography. None of the subjects had a history of CVD at the time of recruitment.

Results: A total of 568 individuals died and 275 individuals developed CVD during the follow-up period (median of 9.6 years; interquartile range of 8.9–10.5 years). Following adjustment for established cardiovascular risk factors, the relative risk of all-cause death in the highest quartile, compared with the lowest quartile, was 1.33 for saturated fats (95% confidence interval [CI], 1.01–1.75, test for trend, $P = 0.015$), 1.71 for trans fats (95% CI, 1.27–2.31, test for trend, $P = 0.0003$), 0.77 for EPA (95% CI, 0.59–1.00, test for trend, $P = 0.048$), and 0.89 for DHA (95% CI, 0.68–1.18, test for trend, $P = 0.354$). Similar patterns were observed for CVD events. Trans fats presented the largest area under the receiver operator characteristic curve (0.740, 95% CI, 0.716–0.766) for the prediction of all-cause death. A mutually adjusted two-marker model indicated that saturated fats and trans fats were significant predictors of all-cause death and CVD; however, the other fatty acids were not. In addition, trans fats presented the greatest improvement in net reclassification for all-cause death (7.7%, $P = 0.003$), followed by EPA (3.8%, $P = 0.033$). Saturated fats presented the greatest improvement in net reclassification for CVD events (5.6%, $P = 0.039$).

Conclusions: Our data provides strong evidence to support that plasma saturated fats and trans fats can predict all-cause death and CVD more effectively than other fatty acid markers.

1. Introduction

A wide range of fatty acids, including saturated fatty acids, trans fats and n-3 fatty acids, have been linked to the risk of cardiovascular disease (CVD) in Caucasian and Japanese populations [1–3]. Recent clinical trials have further demonstrated that n-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), influence cardiovascular outcomes [4,5]. Serum biomarkers of fatty acids have been validated as surrogates for the dietary intake of fatty acids, applicable to large-scale community-based cohort studies [6,7]. However, very little data which differentiates and evaluates the influence of specific fatty acids on the risk of CVD is available. To the best of our knowledge, no previous study has provided a direct comparison of the predictive capacity of fatty acid biomarkers in Asian populations. Therefore, we conducted the prospective study to examine the roles of various fatty acids in the

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prediction of all-cause death and the incidence of CVD among ethnic Chinese in Taiwan.

2. Methods

2.1. Study design and study population

Details regarding the design of this cohort study have previously been published [8–10]. Briefly, the study began in 1990, with the recruitment of 1703 male and 1899 female subjects. All participants were of homogeneous Chinese descent, older than 35 years of age, and living in Chin-Shan Township, 30 km north of metropolitan Taipei, Taiwan. Information related to anthropometry, lifestyle, and medical conditions was obtained through interview questionnaires in 2-year cycles. The validity and reproducibility of the collected data and measurements have also previously been reported in detail [11]. The study protocol was approved by the Committee Review Board of the National Taiwan University Hospital.

2.2. Clinical variables and biochemical measurement

We performed the biochemical measurements and fatty acid profiles once in the baseline. The procedures involved in blood sample collection were previously reported [11]. Briefly, all venous blood samples were drawn after a 12-hour overnight fast, immediately refrigerated, and transported within 6 h to the National Taiwan University Hospital. Serum samples were stored at −70 °C before conducting batch assays to determine the levels of total cholesterol, triglycerides, and high density lipoprotein cholesterol (HDL-C). To ascertain these levels, standard enzymatic tests for serum cholesterol and triglycerides were employed (Merck 14354 and 14366, Germany, respectively). The supernatants were measured for HDL-C levels following the precipitation of specimens using a reagent of magnesium chloride phosphotungstate (Merck 14993). Low density lipoprotein concentrations were calculated as total cholesterol minus cholesterol in the supernatant obtained through precipitation (Merck 14992) [12].

2.3. Fatty acid determination using gas chromatography

The procedures employed to measure fatty acids have previously been described [13]. In brief, 10-mL tubes of EDTA-anticoagulated blood were collected, refrigerated on-site, and forwarded to the core laboratory of the National Taiwan University Hospital within 3 h. The blood was centrifuged at 800× *g* for 10 min, whereupon plasma was separated, dispensed into aliquots, and frozen at −70 °C. All analyses of fatty acid content were performed by the same technician. After thawing the plasma, 0.5 mL samples were extracted and combined with 0.5 mL methanol followed by 1.0 mL chloroform under a nitrogen atmosphere. The lipid extract was then filtered to remove proteins and methyl esters were separated and measured using a 5890 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a 30 m-FFAT WCOT glass capillary column (J & W Scientific, Folsom, CA) and a flame-ionization detector. A total of 29 individual fatty acids were identified by comparing the retention times of peaks to the retention times of synthetic FA standards with known compositions (Supelco 37 Comp. FAME Mix, 47885-U; Bellefonte, PA, USA). The relative quantity of each FA (% of total FAs) was determined by integrating the area beneath the peak, and dividing the result by the total area for all FAs. The concentrations of saturated fatty acids, trans fat, monounsaturated fat, DHA, and EPA were specified for further analysis.

2.4. Ascertainment of outcomes

Cause of death was obtained from official death certificates and verified by house-to-house visits [8,9]. Incident CVD events included coronary disease and stroke. Incident coronary heart disease included cases of nonfatal myocardial infarction, fatal coronary heart disease, and hospitalization due to percutaneous coronary intervention or coronary bypass surgery. Fatalities were attributed to coronary disease when hospital records listed myocardial infarction as the cause of death, when the death certificate listed coronary heart disease as the cause of death, or when coronary disease was the most plausible cause of death. Cases of stroke were ascertained by a sudden neurological deficit of vascular origin lasting longer than 24 h and supporting evidence from medical imaging devices. Data related to non-fatal ischemic events and cases of stroke were obtained from annual questionnaires and all cases were confirmed by neurologists and internists. Until 2008, the response rate for annual questionnaires was 85%.

2.5. Statistical analysis

Participants were categorized on the basis of saturated fatty acid quartiles, and continuous variables are presented by mean and standard deviation, with ANOVA to test the difference among quartiles; categorical data are presented in contingency tables, with the chi-square test to test the difference. The incidence rates of all-cause death and CVD events were calculated by dividing the number of cases by the number of person-years of the follow-up for each quartile of a given fatty acid. Relative risk of all-cause death and CVD events was calculated by dividing with the incidence rate of each quartile. The multivariate Cox proportional hazard model was used to estimate the relative risks and respective 95% confidence intervals. Model 1 was adjusted for age groups (35–44, 45–54, 55–64, 65–74, >=75 years old) and gender only. Model 2 included additional confounding factors: adjusted for the following baseline covariates: body mass index (<18, 18–20.9, 21–22.9, 23–24.9, >=25 kg/m^2), smoking (yes/no or abstinence), current alcohol consumption habits (regular/none), marital status (single, married and living with spouse, separated, or divorced), education level (less than 9 years, at least 9 years), occupation (not employed, manual labor, or office job), and regular exercise (yes/no). Model 2 included additional clinical variables: baseline hypertension (yes/no), diabetes (yes/no), continuous LDL cholesterol, and HDL cholesterol values. We also compared the mutually adjusted effects of pairs of fatty acids on outcomes in order to determine the role of specific fatty acids in the multivariate Cox models. To test for linear trends across categories of lipid markers, we selected the median fatty acid levels within quartiles as a continuous variable. The assumption of proportionality and the fit of the proportional hazard model were verified using Grambsch and Therneau statistics [14]. In addition, the goodness of fit of the model was verified for binary outcomes using the Hosmer and Lemeshow test [15] ($P > 0.05$). The analyses were repeated according to gender status, and the effect modification significance level was examined by incorporated with three interaction terms and tested by the likelihood ratio values.

To facilitate the comparison of predictive capacity between models with and without specific fatty acid values, we evaluated various measures of performance, including the area under the receiver operating characteristic curve (AUC), and net reclassification improvement (NRI). In brief, the AUC was adopted as a global performance indicator for prognostic factors [16,17]. The curve is plotted on a graph of sensitivity versus 1-specificity (or false-positive rate) for various cutoff definitions of positive diagnostic test results [16]. Statistical differences in AUC values were compared using the method outlined by DeLong et al. [18] Nonetheless, the