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Link between plasma *trans*-fatty acid and fatty liver is moderated by adiposity☆

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ABSTRACT

Background: The prevalence of non-alcoholic fatty liver disease (NAFLD) is rising. This increase may be associated with obesity. It has been suggested that *trans*-fatty acids (TFAs) play an important role in non-communicable diseases.
Aim: We examined the link between liver tests, fatty liver index (FLI) and plasma TFAs. Furthermore, we evaluated the impact of adiposity on this link.
Methods: The National Health and Nutrition Examination Survey (NHANES) was used to obtain the data on TFAs and liver function biomarkers. We took account of complex NHANES data, masked variance and weighting methodology.
Results: Of the 4252 participants, 46.4% were men. The mean age was 50.6 years overall; 51.3 years for men and 49.8 years for women ($p = 0.206$). In a fully adjusted model (demographic and clinical factors), FLI increased as *trans*-9-hexadecenoic acid and *trans*-11-octadecenoic acid levels increased; FLI was 38.1 and 42.3 for the first quarter (Q1) of *trans*-9-hexadecenoic acid and *trans*-11-octadecenoic acid, respectively, reaching 65.1 and 69.3 for the highest quarters (Q4) ($p < 0.001$ for all comparisons). Multivariable logistic regression showed for all four studied TFAs, the likelihood of NAFLD (determined by FLI) increased with increasing TFAs levels (quartiles) in a stepwise manner ($p < 0.001$ for all comparisons). Based on moderation analysis, a strong impact of body mass index (BMI) on the link between FLI and TFAs was observed.
Conclusions: Our results suggest a direct significant association between plasma TFAs, liver tests and NAFLD (assessed by FLI). Furthermore, BMI was shown to mediate this relationship. These findings highlight the importance of avoiding TFAs consumption in order to minimize cardiometabolic risk.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has attracted increasing attention given its high prevalence, estimated at 20–44% in Western countries and 5–38% in Asia [1, 2]. The disease is characterized by the accumulation of fat (>5%) in hepatic cells among individuals without excessive consumption of alcohol, use of steatogenic drugs or hereditary diseases [1, 2]. Diet may affect liver fat accumulation in humans. A study involving patients with NAFLD showed that a diet containing more carbohydrates and less fat, but equal amounts of protein and energy, was associated with a greater histological severity of NAFLD [3]. However, reports in the literature on the association of NAFLD with dietary patterns are scarce [4].

Trans-fatty acids (TFAs) are unsaturated fatty acids that are uncommon in nature, but commonly produced industrially (starting in the 1950s) from vegetable fats for use in margarines, snack foods, packaged baked goods and fried foods [5, 6]. According to epidemiological investigations, TFAs consumption is associated with an increased risk of cardiovascular disease and metabolic disorders [7]. In this context, accumulating evidence suggests that TFAs are involved in obesity and insulin resistance [7], but the underlying mechanisms are not yet fully understood. In this context, we have recently reported a strong link between plasma TFAs and inflammation [8].

Recent studies have reported that a high fat diet and high TFAs levels could induce NAFLD by oxidative stress [9]. NAFLD could progress to non-alcoholic steatohepatitis (NASH) with hepatic fibrosis and, eventually, to cirrhosis and hepatocellular carcinoma [10]. Of note, NAFLD has

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been associated with several cardiovascular risk factors including dyslipidemia, obesity, insulin resistance (IR) and diabetes [11]. Furthermore, NAFLD/NASH patients have an increased cardiovascular morbidity and mortality [12]. With regard to NAFLD treatment, there is a lack of widely accepted recommendations. However, a recent expert panel suggested the implementation of lifestyle measures (healthy diet and exercise) and the use of pioglitazone and statins in order to improve both biochemical and histological NAFLD features [13]. In this context, in a trial involving patients with coronary heart disease (CHD), NAFLD (based on liver tests) was associated with 22 (10%) cardiovascular events in 227 patients on a statin (3.2 events/100 patient-years) and 63 (30%) in 210 patients who did not receive a statin (10.0 events/100 patient-years; 68% relative risk reduction, $p < 0.0001$). In participants with normal liver tests, there were 90 [14%] events in 653 patients on a statin [4.6/100 patient-years] vs 117 [23%] in 510 patients not on a statin [7.6/100 patient-years]; 39% relative risk reduction, ($p < 0.0001$). Therefore, among patients not taking a statin, the risk of an event was greater in those with NAFLD compared with those without NAFLD (10.0 vs 7.6/100 patient-years). However, this was a post hoc analysis, the number of patients was small and NAFLD was not confirmed with a liver biopsy (the "gold" standard for diagnosis) [14].

Most studies recommend a decrease in the consumption of TFAs and an increase in the intake of poly- and mono-unsaturated fatty acids in NAFLD patients [15].

There is a need for additional evidence of direct evidence and studies on the molecular mechanism of action of TFAs in lipid disorders as well as in liver steatosis [16]. In this context, a previous animal study reported that a high fat diet could induce hepatic IR and steatosis [17]. Another study in non-human primates suggested that TFAs might stimulate visceral fat deposition irrespective of weight gain when consumed in considerable amounts over a long period of time [18]. Studies in rats found that a high intake of TFAs may lead to increased deposition of fat in the liver [19]. In humans, it has yet to be established whether TFAs intake affects liver fat deposition. In several rodent studies, TFAs feeding increased liver fat by >100% compared with animals fed *cis* fatty acids [19] although this was not a consistent finding [20].

Given the lack of human studies with regard to the impact of plasma TFAs on liver tests, we evaluated the link between 4 different TFAs [i.e. *trans*-9-hexadecenoic acid (palmitelaidic acid, C16:1n-7t), *trans*-9-octadecenoic acid (elaidic acid, C18:1n-9t), *trans*-11-octadecenoic acid (vaccenic acid, C18:1n-7t) and *trans*-9, *trans*-12-octadecadienoic acid (linolelaidic acid, C18:2n-6t, 9t)] and the presence of NAFLD (assessed by liver tests and fatty liver index, FLI). We also examined the impact of body mass index (BMI) on the above associations by studying a large, representative, cross-sectional study of American adults, i.e. the National Health and Nutrition Examination Survey (NHANES). We hypothesized that individuals with a higher intake of TFAs would also have abnormal liver tests and a higher prevalence of NAFLD.

2. Methods

2.1. Population

The NHANES are ongoing repeated cross-sectional surveys conducted by the US National Center for Health Statistics (NCHS). NHANES uses a multistage probability sampling strategy, which oversamples certain subgroups of the population, including blacks, Mexican-Americans, and those of lower socioeconomic status. The NCHS Research Ethics Review Board approved the NHANES protocol and consent was obtained from all participants. Data collection on demographic, dietary and behavioural information collected through in-home administered questionnaires, while anthropometric and biomarkers data are collected by trained staff using mobile exam units. More detailed information on the NHANES protocol is available elsewhere [21]. A blood specimen is drawn from the participant's antecubital vein according to a standardized protocol. Laboratory test details are described in the NHANES Laboratory/Medical Technologists Procedures Manual [21]. The activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) in U/L were measured spectrophotometrically using their respective kinetic enzymatic methods.

The current study was based on the analysis of data from the 1999–2000 and 2009–2010 NHANES cycles. Analyses were restricted to participants aged 20 years and older. Hypertension (HTN) was diagnosed in the presence of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, or current use of anti-hypertension medication. Diabetes (DM) was based on self-reported history of DM or fasting plasma glucose ≥126 mg/dL. C-reactive protein (CRP) and white blood cells counts (WBC) were measured as previously described [22]. For the calculation of the inflammatory score, *Z*-scores from biomarkers (CRP and WBC) were summed up.

2.2. Fatty liver index (FLI)

FLI was calculated according to the United States fatty liver index (USFLI) [23]. The USFLI has been validated and shown to correlate well with the presence of NAFLD diagnosed by ultrasound [Receiver Operating Characteristic (ROC) curve of 0.80; 95% CI = 0.77 ± 0.83] [23]. Using the recommended values, a USFLI score ≥ 30 was selected to rule in NAFLD [23].

2.3. Plasma TFAs

Total (free and esterified) content of selected *TFAs* in the plasma were measured [21]. Fatty acids in the plasma are converted into free fatty acids by subsequent acidic and alkaline hydrolysis. The free fatty acids are extracted from the sample solution using liquid-liquid extraction and derivatized with pentafluorobenzylbromide (PFB-Br). The derivatized fatty acids are separated by capillary gas chromatography and detected by mass spectrometry using negative chemical ionization. Fatty acids are then identified based on their chromatographic retention time and on the specific mass to charge ratio of the ion formed in the ion source. Retention times are compared against those obtained with known standards [21]. Quantitation is performed with standard solution using stable isotope-labelled fatty acids as internal standards. These fatty acids cover >95% of all fatty acids in the plasma [24]. This method determines the following four TFAs: *trans*-9-hexadecenoic acid (palmitelaidic acid, C16:1n-7t), *trans*-9-octadecenoic acid (elaidic acid, C18:1n-9t), *trans*-11-octadecenoic acid (vaccenic acid, C18:1n-7t), *trans*-9, *trans*-12-octadecadienoic acid (linolelaidic acid, C18:2n-6t, 9t).

2.4. Statistical analysis

Analyses were conducted according to the Centers for Disease Control and Prevention (CDC) guidelines for analysis of complex NHANES data, accounting for the masked variance and using the proposed weighting methodology [21]. We computed adjusted (model 1: age, sex and race, model 2: age, sex, race, alcohol intake, energy intake, smoking and physical activity, model 3: age, sex, race, alcohol intake, energy intake, smoking, physical activity, HTN, high density lipoprotein cholesterol (HDL-C) and DM) mean of measures of liver tests (USFLI, ALT, AST) by quartile of TFAs using analysis of covariance (ANCOVA) with Bonferroni correction. To determine any association between plasma TFAs and liver tests, multivariable-adjusted linear regression models (model 1: age, sex and race, model 2: age, sex, race, alcohol intake, energy intake, smoking and physical activity, model 3: age, sex, race, alcohol intake, energy intake, smoking, physical activity, HTN, HDL-C and DM) were used.

Furthermore, logistic regression analysis was performed to determine the likelihood of NAFLD (based on the different quartiles of TFAs) in a fully adjusted model (adjusted for age, sex, race, alcohol intake, energy intake, smoking, physical activity, HTN, HDL-C and DM). Adjusted (adjusted for age, sex, race, alcohol intake, energy intake, smoking, physical activity, HTN and HDL-C) linear regression was applied to evaluate the magnitude of the link between TFAs and USFLI with inflammation (magnitude expressed as standardized β coefficient (β)). We also quantified the impact of adiposity (assessed by BMI) on the link between TFAs with USFLI by applying the moderation model using the SPSS Macro developed by Preacher and Hayes [25]. This statistical model can simultaneously examine the moderator impact of the variable of interest, i.e. adiposity, adjusting for confounding factors. Moreover, this approach allowed visualisation of the impact of each standard deviation change in the potential moderators on the relationship between independent and dependent variables. We tested for the presence of an effect of adiposity adjusted model (adjusted for age, sex, race, alcohol intake, energy intake, smoking, physical activity, HTN, HDL-C and DM). Groups were compared using analysis of variance and Chi-square tests. Multi-collinearity for the multiple linear regressions was assessed with variance inflation factors (VIF) at each step [26]. Multi-collinearity was considered high when the VIF was >10 [26].

All tests were two sided and $p < 0.05$ was the level of significance. Results were analysed using SPSS complex sample module version 22.0 (IBM Corp, Armonk, NY). Sample weights were applied to account for unequal probabilities of selection, nonresponse bias, and oversampling.

3. Results

Of the 4252 participants, 46.4% were men. Clinical and anthropometrical information relating to our sample are shown in Table 1. The mean age was 50.6 years overall; 51.3 years for men and 49.8 years for women ($p = 0.206$). With regard to education level, 19.2% of the participants had attended tertiary education, 42.2% had

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