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Plasma *Trans* Palmitoleic Acid is Associated with Cardio-metabolic Risk Factors in Youth with Type 1 Diabetes

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The adverse effects of industrially produced *trans* fatty acids on health outcomes are well-documented, with increased dietary intake of *trans* fatty acids associated with coronary heart disease, abnormal lipid profiles, and glucose dysregulation [1]. However, fewer studies have examined the effects of *trans* fatty acids produced from bio-hydrogenation by bacteria in ruminant gut, such as *trans*-palmitoleic acid (TPA; *trans* 16:1n-7) primarily found in dietary sources such as unprocessed red meats and full-fat dairy foods [2]. TPA may reduce mortality from cardiovascular causes [3], but epidemiologic findings have been inconsistent.

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Appendix supplementary material

Supplementary materials (Table S1) associated with this article can be found at http://www.scincedirect.com at doi . . .

Given that youth with diabetes are at greater risk for cardiovascular disease [4], the identification of modifiable factors that improve glucose and fat metabolism in this population may be critical for improving long-term health outcomes. The aim of the current study is to examine the association of TPA with cardio-metabolic risk factors in youth with diabetes, using both cross-sectional and longitudinal data.

The SEARCH for Diabetes in Youth Study is an ongoing multi-centre epidemiologic study of physician-diagnosed diabetes among youth < 20 years at diagnosis [5]. The SEARCH Nutrition Ancillary Study (SNAS) was derived from the SEARCH study, with the aim to examine the associations of nutritional factors with diabetes-related health outcomes among youth with type 1 diabetes (T1D). Both SEARCH and SNAS were reviewed and approved annually by the appropriate institutional review boards. Written informed consent and assent were obtained from parents of participants < 18 years and from participants aged ≥ 18 years at the time of data collection.

Fasting blood samples were obtained under conditions of metabolic stability, defined as no episode of diabetic ketoacidosis during the previous month. All samples were processed at the local site and then shipped within 24 hours to the central laboratory where specimens were assayed for Hb_{A1c}, total cholesterol (TC), triglycerides, and high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol. Fasting blood samples at the baseline SEARCH visit were analysed for two diabetes autoantibodies: glutamic acid decarboxylase-65 and insulinoma-associated-2. In addition, Human Leukocyte Antigen class II genotyping was performed. Height, weight, waist circumference and blood pressure (BP) were measured according to standardized protocol by trained and certified staff. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2) and converted to an age and gender-specific BMI z-score. Plasma fatty acids were measured by using frozen fasting blood samples that were collected at the time of the SEARCH examinations. Plasma TPA was expressed as percentage (by weight) of total fatty acids detected. The percent coefficient of variation [%CV] in the quality control pool samples for TPA was 20%. The standardized measurement and assay protocol for the SEARCH and SNAS study was previously described [5, 6].

Data on sociodemographic characteristics (age, gender, race/ethnicity, highest level of parent education in the household, household income) and diabetes related factors (disease duration, insulin regiment type, unites of insulin/kg, clinic site) were obtained by interviewing parents (for participants < 18 y) or from participants (for participants ≥ 18 y of age).

These analyses include data from the 2002–2005 incident cohorts of SEARCH, consisting of participants who were aged 3–20 years when diagnosed. Cross-sectional analyses included 826 youth with physician-diagnosed T1D, who additionally had a positive test for at least one diabetes autoantibody and had both fatty acids and fasting blood lipids measured at the baseline visit. For a repeated measures analyses, participants from the cross-sectional sample who had fasting blood lipids and fatty acid data at the 12-month follow-up visits were included (n = 379).

Though all eligible participants had a fatty acid panel, at baseline only 652 had a detectable value for their TPA measurement; the remaining participants had no TPA value reported but had measurements for other fatty acids. A missing fatty acid percentage denotes that there was no peak detected, which is likely indicative of a low percentage of that particular fatty acid [7]. Omission of these participants can yield biased results [8], as it excludes the participants with the lowest TPA. Rather than ignore cases with missing values, missing TPA values were set to 0.01 (n = 174 at baseline, n = 107 at 12-month follow-up), which was lower than all observed values (lowest = 0.018). Simple imputation is appropriate here because the missing value is known to come from a very narrow range relative to the rest of the data. As a sensitivity analysis, we repeated the baseline analyses using a maximum likelihood method for left-censored (i.e. missing below an upper limit) data, and the results were virtually identical to those with the simple imputation presented here.

Descriptive analyses were conducted to determine the distribution of demographic measures and baseline cardio-metabolic factors across quartiles of TPA. Chi-square tests and generalized linear models were used to compare the quartiles for categorical and continuous data, respectively.

Separate, multiple linear regression was used to examine baseline cross-sectional and longitudinal associations between TPA and each cardio-metabolic factor. The distribution of TPA was skewed right, and thus was log-transformed for regression models. Models were adjusted for demographic and diabetes-related variables. In addition, to determine how TPA is associated with the distribution of fat across all weight categories, models examining waist circumference as an outcome were adjusted for BMI z-score. For the participants with follow-up data, we used linear mixed regression models for each outcome, which account for the correlation between observations within a participant, and also adjusted for the baseline value of the outcome and time since baseline.

Baseline characteristics of the study population by quartile of TPA are presented in Table S1 (see supplementary material associated with this article online). Cross-sectional models showed that there was a positive association of TPA with LDL-cholesterol, total cholesterol, diastolic BP and HDL-cholesterol. TPA was not associated with IS score, log-triglycerides, Hb_{A1c}, BMI z-score, systolic BP, or waist circumference (Table 1**)**. Results from the longitudinal analysis mirrored the cross-sectional findings, except that TPA was also inversely associated with Hb_{A1c}.

The few existing studies of the relationship between TPA and cardio-metabolic risk factors have also shown complex results. In a multi-ethnic study of U.S. adults, circulating plasma TPA was associated with higher LDL-cholesterol but also with lower triglycerides, fasting insulin, blood pressure, and incident diabetes [9]. No relationships were observed for HDL-cholesterol, total: HDL-cholesterol ratio, or fasting glucose [9]. A study of elderly adults found no association between circulating plasma TPA and LDL-cholesterol, but observed a positive association with HDL-cholesterol, and inverse associations with triglycerides, total: HDL-cholesterol ratio, and insulin resistance [10]. Separate analyses from a small subset of the Nurses' Health Study (NHS) also reported a positive association between plasma TPA with HDL-cholesterol, inverse associations with total: HDL-cholesterol ratio and HbA_{1c}

[10]; however, high concentrations of erythrocyte TPA were not associated with any lipid markers in a European cohort [11]. The inconsistent findings across the few existing epidemiologic studies of TPA may be attributed to differences in study population, methods to measure fatty acids, and covariates included in the analyses.

TPA exists in low levels in plasma phospholipids, and thus is not often measured in studies that include other fatty acid biomarkers or food frequency questionnaires. Thus, the mechanisms by which TPA influences cardio-metabolic risk factors are not well understood. Some have hypothesized that the potentially beneficial effects of TPA may be a result of TPA mirroring exogenous sources of palmitoleate, the cis isomer of TPA (cis 16:1n-7c) [10]. In vivo animal models showed that adipose produced palmitoleate improves insulin sensitivity through enhanced muscle and liver signalling and down-regulates *de novo* hepatic lipogenesis [12]. However, our data in youth with T1D do not fully support this hypothesis. It is possible that in our sample, TPA may be a marker for other fatty acids such as saturated fat, which is consumed in larger quantities and has been shown to increase both LDL-cholesterol and HDL-cholesterol; but, the inverse association between TPA and HbA_{1c} observed in our study is not consistent with this explanation. It may be that TPA does not mechanistically act similarly to its geometric isomer or that the TPA measured in our participants was not necessarily principally derived from ruminant sources, but from the partial hydrogenation of vegetable oils [9]. Alternatively, the metabolism of fatty acids in individuals with diabetes may differ from healthy individuals [13]. Further delineating the mechanisms by which TPA affects cardio-metabolic health in humans is an area of great research potential.

There are notable strengths of this study. First, our study utilized data from SNAS, a large multi-centre prospective cohort of youth with T1D. Previous studies examining the relationship of TPA on cardio-metabolic risk factors have been conducted in older-populations [9, 10] precluding generalizability to a population of youth with T1D who are at high risk of developing cardiovascular disease. Additionally, the existing studies have primarily utilized a cross-sectional design. In the current study, we included a repeated measures analysis that provides increased precision and power over existing cross-sectional analyses [14]. Second, our study used plasma TPA, which are not subject to the recall-based measurement errors of dietary surveys, and may be more suitable to capture this unique, understudied dietary exposure that is found in relatively low amounts in foods [2]. Third, the large sample size and use of data from SEARCH allowed us to control for several desired potential confounders.

Limitations of the study should be noted. First, it is possible there may have been measurement error of our TPA data. Plasma phospholipid levels of TPA were relatively low (mean 0.174%), but these levels are consistent with other epidemiologic studies of TPA [9, 10]. Further, there were several participants with missing TPA data. These data were not missing at random, so to minimize loss of precision and bias [8], we set missing TPA values to approximately half the lowest observed value, which is consistent with methods for missing data below detection limits [15]. Second, although we adjusted for numerous confounders, residual confounding may be present as we did not utilize estimates of other dietary factors. The food frequency questionnaire used in SNAS obtained dietary

information among participants ages ≥ 10 years and the use of available dietary measures would have decreased sample size substantially.

The present study provides novel findings regarding longitudinal relationship between TPA and cardio-metabolic risk factors in youth with type 1 diabetes. Our findings suggest both cardio-protective influences (HDL-cholesterol and HbA_{1c}) and potentially detrimental influences (LDL-cholesterol, total cholesterol, and DBP). Given our limited understanding of TPA, future studies are needed to clarify whether observed associations are related to long-term health outcomes in youth with T1D.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations:

BMI	body mass index
CVD	cardiovascular disease
DBP	diastolic blood pressure

HbA_{1c}	glycated haemoglobin
HDL	high density lipoprotein
HLA	human leukocyte antigen
IS	insulin sensitivity
LDL	low density lipoprotein
SBP	systolic blood pressure
SEARCH	SEARCH for Diabetes in Youth Study
SNAS	SEARCH Nutrition Ancillary Study
T1D	type 1 diabetes
TPA	*trans* palmitoleic acid

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TABLE 1

Multiple regression analysis examining the association between TPA (log-transformed) and cardiometabolic factors at baseline in youth with type 1 diabetes[1]

Cardiometabolic Factor	β coefficient ± SE	
	Cross-Sectional[2]	Longitudinal[3]
IS Score	−0.0038 ± 0.0075	0.007 ± 0.006
log-Triglycerides, log-mg/dL	0.0102 ± 0.0116	−0.005 ± 0.01
LDL-cholesterol, mg/dL	2.1094 ± 0.7514**	1.611 ± 0.619**
HDL-cholesterol, mg/dL	0.8448 ± 0.3458*	0.831 ± 0.285**
Total Cholesterol, mg/dL	3.129 ± 0.896**	2.371 ± 0.736**
HbA1c, %	−0.0609 ± 0.0407	−0.083 ± 0.033*
BMI z-score	0.0527 ± 0.0271	0.028 ± 0.022
SBP, mmHg	0.0234 ± 0.2755	0.075 ± 0.222
DBP, mmHg	0.8672 ± 0.2768**	0.575 ± 0.225*
Waist Circumference, cm[4]	−0.04 ± 0.1773	−0.112 ± 0.141

[1] Values are β coefficients ± SE,

* $P < 0.05$,

** $P < 0.01$

[2] Cross-sectional model was adjusted for demographic and diabetes-related variables

[3] Longitudinal model was adjusted for demographic and diabetes-related variables

[4] Adjusted for BMI z-score